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*National Cancer Institute (U.S.)
Annual report.*

Division of

CANCER TREATMENT

Volume II

October 1, 1982-September 30, 1983

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1982 through September 30, 1983

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1982 - September 30, 1983

The Clinical Oncology Program is the intramural treatment research arm of the National Cancer Institute. At the present time there are six independent Branches and a statistical support Section within the Program.

During the past year significant advances have been made in the treatment of several malignancies including ovarian cancer, soft tissue sarcomas, acute leukemia of childhood, and small cell carcinoma of the lung. In addition, significant research advances have occurred in the development of monoclonal antibodies to small cell carcinoma of the lung; in the clarification of mechanisms of hormone resistance in human breast cancer; in describing cytogenetic abnormalities associated with cancer; in the cloning and proliferation of cytotoxic human T-cells, in analyzing the role of immune response genes; in the characterization of important new metabolites of methotrexate; in evaluating drug-resistance mechanisms of tumor cells; in understanding the mechanisms of free radical mechanisms of tissue injury; in understanding pharmacokinetic parameters needed to treat meningeal cancer; and in assessing the relationship between oncogenes and human tumors.

During the past year, the Program has undertaken many new initiatives which will be summarized under specific Branch reports. However, two research areas receive special supervision and coordination through the Office of the Associate Director:

1. Acquired immunodeficiency syndrome/Kaposi's (AIDS/KS) sarcoma and
2. HTLV-associated lymphomas.

1. During the past year, the DCT has formed a special task force to coordinate intramural AIDS/KS research and clinical coordination of this responsibility resides with the Program Associate Director's office. The COP currently has three major treatment protocols for AIDS/KS. The first involves the use of interferon. The second involves a trial of potentially non-cross-resistant combination chemotherapy regimens. The third involves the use of total skin electron beam therapy. These protocols are discussed in further detail below but are underscored here to reflect the very high priority assigned to this disease in the NCI.

2. The Program has continued its special interest in HTLV-associated neoplasms. During the past year a clearer understanding of the spectrum of clinical features of disease caused by HTLV (human T-cell leukemia virus) has emerged.

The Program has played an important role in defining that HTLV is, in fact, endemic in the southeastern United States, affecting young blacks in disproportionate numbers. Clinical features include an aggressive course with rapid onset of disseminated skin lesions, hypercalcemia, and other metabolic disturbances. An acquired immunodeficiency state with opportunistic infections is commonly seen. Leptomeningeal leukemia is also commonly seen. A paraneoplastic syndrome characterized by increased bone turnover, abnormal bone scans, and hypercalcemia is almost always present. Intensive chemotherapy can produce short-term remissions, but most patients do not show durable disease-free survival.

It has recently been possible to generate a long-term line of cytotoxic immune T cells with specificity for HTLV from a patient in remission suggesting new avenues of research. It has also been possible to show that virions of HTLV isolated from patients with lymphoma are associated with the receptor for T-cell growth factor (interleukin 2), suggesting new possibilities for further research.

The Program's interest in HTLV-associated diseases is heightened by the observation of Gallo and co-workers that patients with AIDS may be at risk of infection with HTLV and various elements of the Program are collaborating with Dr. Gallo's lab in this area.

Program Accomplishments

Clinical Pharmacology Branch: Dr. Charles E. Myers, Chief

Over the past year this Branch has undergone a major restructuring. As a group, a common focus has been developed. The goal of this Branch can be said to be to analyze the reasons for success and failure in the application of chemotherapy to human neoplasia. While this sounds global, as a group specific projects were carefully selected on the basis of their probable eventual clinical impact. The target areas thus identified include:

1. Adriamycin Mechanism of Action:

This drug class is one of the three most successful in cancer treatment. They have focused on the free radical generating potential of these agents because evidence suggests this may play a role in toxicity but not antitumor effect. We are focusing on structure activity studies of adriamycin analogs in order to identify means of preserving antitumor effect and eliminating radical based toxicity. Over the past year, this group has focused on the interaction of adriamycin with metal ions. These results reveal a very special chemistry associated with this interaction which endows adriamycin with the capacity to punch holes in membranes and cleave DNA via a free radical mechanism. They have defined the structural basis for this chemistry and can now predict which drug chromophores will possess this capacity.

2. Methotrexate Polyglutamate Formation:

Conversion of methotrexate to its polyglutamates has potentially profound implications for use of this agent in high dose treatment protocols and

understanding of how these drugs work. This Branch has developed the best HPLC assay for this metabolic event and this has allowed the analysis of polyglutamate formation in human tumor cells. The surprising result was that two human tumor cell lines have been identified which appear to be resistant to MTX by virtue of deletion of the capacity to make MTX polyglutamates. These results suggest that MTX may, in fact, be a prodrug requiring metabolic activation for cytotoxicity.

3. Mechanisms of Drug Resistance:

As a general area, this is of major importance in developing intelligent treatment strategies. There are three major projects in this area. First, Dr. Cowan is examining the genetic bases of MTX resistance. In addition to the previously discussed work on polyglutamate formation, Dr. Cowan has been involved in cloning the DHFR gene. This has now finished and he will be using this cloned gene to study regulation of that gene expression. This is important because early results suggest that this gene obtained from breast cancer cells is regulated by estrogen.

The second area of interest is in the phenomenon of pleiotropic drug resistance. Dr. Curt has obtained CHO cells from Dr. Victor Ling which possess this resistance and he is in the process of comparing the glycoprotein associated with this resistance with comparably resistant human cells. The hope is that this will show whether a similar mechanism is at work in man associated with this project. Dr. Curt is attempting to reverse adriamycin resistance via calcium channel blockers which are thought to work by altering the transmembrane movement of this agent.

The third area of work is that of Dr. Batist. Dr. Batist has discovered a glutathione peroxidase bound to the nuclear envelope. Interest in this enzyme was initially provoked by the observation that tumor promoters like the phorbol esters appear to work via oxygen radical production and this enzyme could potentially modulate this process. Additionally they have shown this enzyme is induced by the anticarcinogen; cis retinoic acid. It is also controlled by estrogen and a variety of other stimuli and it appears that modulation of this enzyme might explain many of the empirical observations in carcinogenesis. As part of this work, the substrate specificity of this enzyme has been examined and found that it would use irradiated DNA as a substrate. It turns out that radiation damage to DNA causes formation of base hydroperoxides and that this enzyme repairs this damage. The enzyme is thus a potential mechanism of DNA repair after free radical injury. We are studying the role of this enzyme in resistance to radiation and free radical forming drugs. This research will have the highest priority in the Branch over the coming year.

4. Pharmacokinetics:

There are many practical questions about drug usage which can be answered with the tools of clinical pharmacokinetics. Over the past year, Dr. Collins has made major contributions in the use of 6MP and cis-DDP via analysis of their pharmacokinetics. In the case of 6MP, in collaboration with Dr. Poplack in the Pediatric Branch, they have been able to show that 6MP cannot reliably be given orally. In the case of cis-DDP, in collaboration with Dr. Ozols, Dr. Collins has been able to show that changing the saline loading technique effectively allows a doubling in the tolerated cis-DDP dose. Progress in this area,

especially since Dr. Collins only became an official member of this Branch in the latter half of the fiscal year, has been remarkable.

Plans for the coming year include the establishment of an NMR facility in the Branch focusing on the application of NMR to a range of clinical and research pharmacologic questions.

Medicine Branch: Dr. Robert C. Young, Chief

In 1982-1983 the Medicine Branch staff published 85 papers, articles, or book chapters and has accepted or has in press 22 additional publications. This is the largest number of scientific publications in the history of the Branch and represents a 17% increase over last year. Forty-one active protocols are maintained primarily by the Medicine Branch. Details of the clinical and laboratory studies are presented in detail in the annual report document.

1. Advanced Diffuse Aggressive Lymphoma:

The success of the ProMACE-MOPP regimen highlighted the fact that 74% of patients enter complete remission. The median survival of the entire group of patients has not yet been reached, but 62% of all patients remain continuously disease-free after therapy. These results are the best yet reported by any investigators for the treatment of advanced diffuse lymphoma. ProMACE-MOPP appears to double the cure rate of this disease. This study has been followed by a prospective randomized comparison of ProMACE-MOPP day 1 and 8 vs. ProMACE-CytaBOM. These two regimens have been designed to reduce toxicity, convert to outpatient administration, and ideally increase the complete remission rate. The first two of these goals have been accomplished. Fifty-three patients have been randomized. The overall complete remission rates are 75% and 81%, respectively. Essentially all of the therapy is given on an outpatient basis and the severe infectious complications have been cut in half. The success of either or both of these regimens will greatly simplify this treatment and allow wider national use in larger numbers of patients.

2. AIDS and Kaposi's Sarcoma:

This year the Medicine Branch has initiated an intramural clinical and laboratory research effort in AIDS with particular emphasis on patients with concomitant Kaposi's sarcoma. Both clinical trials and laboratory studies are underway. Fourteen patients have been entered into two clinical trials, one studying human lymphoblastoid interferon and the other studying two non-crossresistant combinations. Initial results indicate 2/13 objective responses with interferon and 3/5 clinical responses (2 complete and 1 partial) with the combination chemotherapy approach. Laboratory studies involve search for retrovirus or human parvovirus in AIDS, the characterization of the T cell defect in AIDS, and the identification of the HTLV-I genome in 2 patient lymphocytes out of 135 specimens examined from individuals with the AIDS syndrome. Molecular clones of one of the isolates have been made and a detailed characterization is underway to identify differences between this strain and the HTLV-I isolates from leukemic individuals. Details of these studies can be found in the sections entitled, Genetic Regulation and the Immune Response and Human Retroviruses and Onc Genes in Human Malignancy and Immunodeficiency.

3. Hodgkin's Disease Cell Line in Culture:

A neoplastic Reed-Sternberg cell line from a patient with advanced Hodgkin's disease has been established, and the origin and immunologic function of the Reed-Sternberg cells have been partially characterized. In regard to immunologic function and cell surface characteristics, these cells resemble the dendritic cell. Mouse monoclonal antibodies have been prepared against the tumor cell line and react with 18/20 of the Reed-Sternberg cells from tissue sections obtained from patients. The specificity of these monoclonal antibodies is now being determined and the characterization of the antigen being recognized by these antibodies is in progress.

4. HTLV-II:

During the investigations of the involvement of human retrovirus in human malignancy, viral genes of 2 strains (I and II of the human T cell leukemia virus) HTLV have been cloned. The HTLV-II has thus far been associated with only one case of chronic T cell leukemia. A molecular survey of other leukemia cases is underway to try to determine the specific disease spectrum associated with this newly described retrovirus.

5. Treatment of Advanced Testicular Cancer:

A new 4-drug combination, PVBV, composed of high dose cis-platinum (40 mg/m² qd x 5), velban, bleomycin, and VP-16, produces an extremely high (89%) complete remission rate in patients with poor prognosis, advanced non-seminomatous testicular cancer. Standard regimens in the past have been able to produce only a 40-60% CR rate in this group of patients. A randomized trial is underway comparing this new regimen with the standard PVB. Initial complete response rate to PVBV is 86% with 79% of patients still NED after therapy. For patients treated with PVB there is a 70% CR rate with only 57% of patients remaining NED. No patient on PVBV who has achieved a complete remission has relapsed. These preliminary results indicate that a high sustained complete remission rate can be achieved in even poor prognosis testicular cancer patients.

6. High-Dose Cis-Platinum:

Studies completed this year indicate that the nephrotoxicity associated with cis-platinum can be completely prevented even when the drug is administered at high dose (40 mg/m² qd x 5) by hypertonic saline administration and a sustained chloruresis. Studies this year with 60 cycles of therapy on 21 patients have established no significant renal toxicity using a program of vigorous hydration, hypertonic and normal saline, as well as furosemide. This new approach to therapy allows higher doses of cis-platinum to be safely used in both testicular and ovarian cancer.

7. Thymic Role in Self-Non-Self Recognition:

Studies indicate that the thymic cell is critical to thymic determination of T cell self and antigen recognition. The thymus determines the expression of the antigen receptors by developing T cells. Since certain thymic cells (epithelium derived from the 3rd pharyngeal pouch) and others (dendritic or antigen presenting cells derived from the bone marrow) it is possible to create an animal whose thymus gland is chimeric, bearing epithelial cells of one genotype and dendritic

cells of another. In general, T cells emerging from such a chimeric thymus gland appear to express this self-restriction in antigen-specific receptor repertoire of the bone marrow-derived dendritic cell rather than the epithelial cells. These studies have important implications in the future of organ transplantation.

8. Ovarian Cancer Cell Lines:

Three new ovarian cancer cell lines have been established, including a line which has steroid hormone receptors. These lines have human cytogenetic characteristics, form tumors in nude mice, clone in soft agar, and allow study of mechanisms of drug resistance. Drug resistant variants which are 6-10 times more resistant to melphalan, adriamycin, and cis-platinum have been developed and demonstrated that malphalan resistance is linked to glutathione levels. Furthermore, using techniques to alter the levels of glutathione or to change the permeability of cell membranes, they have been able to restore drug sensitivity in melphalan and adriamycin resistant lines respectively.

9. Early Hodgkin's Disease: MOPP vs. Radiation Therapy:

Twenty-four patients have been randomized to radiation therapy alone and 20 to MOPP. 24/24 patients on radiation achieved CR, however, 9 have relapsed and 4 are dead. 18/20 patients on MOPP have achieved CR and only 2 have relapsed. Only 1 patient in this arm of the study has died and that death was not related to Hodgkin's disease (myocardial infarction). This is the only randomized trial of these two modalities as primary therapy in early Hodgkin's disease in the world.

10. Hormone Dependence in Human Breast Cancer:

Study of the mechanisms whereby steroid and polypeptoid hormones stimulate growth and specific protein synthesis in human breast cancer is under study. The intercellular pharmacokinetics of estrogen and antiestrogen metabolism and efflux from human breast cancer cells using perfusion systems have been completed. These studies have led to new insights into hormone receptor interactions with the genome. Specifically, it has been discovered that internuclear estrogen receptors are changed over time to a less easily extractable form associated with the onset of steroid effects. This processed receptor appears tightly bound to DNA, is extractable by nuclease digestion, and may be the proximate receptor form involved in gene regulation. In addition, soft agar cloning techniques have been developed which has permitted the development of clones of antiestrogen resistant variants (putative mutant) cell lines derived from hormone-dependent wild type cells. These variant cells are currently being analyzed biochemically and via somatic cell hybridization.

11. In Situ Hybridization Studies:

A variety of in situ hybridization studies are underway including localization of oncogenes (myc, sis) in Burkitt's lymphoma patients having various chromosomal translocations - two patients have been studied who have AIDS syndrome and Burkitt's lymphoma; the localization of HTLV gene in patients with HTLV positive diseases; localization of genes for β , ϵ and λ hemoglobin in two variants of CML tissue culture cell line K562; and localization of the genes for dihydrofolate

reductase in various HSRs and double minute bearing human tissue culture cell lines.

12. Early Ovarian Cancer:

Preliminary results on an early ovarian cancer trial were presented this year. One hundred sixty-one patients have now been randomized to two separate trials; the first for patients with stage Ia and Ib disease compares adjuvant melphalan to no additional therapy after comprehensive initial surgery, the second for patients with minimal residual disease compared melphalan to i.p. ^{32p}. Initial conclusions are that (a) accurate staging in ovarian cancer is a crucial prerequisite to decisions regarding appropriate adjuvant therapy, (b) carefully staged patients with stage I_{A1} and I_{B1} disease with well or moderately well differentiated histology have an extremely good prognosis and may not require any adjuvant therapy of any kind, (c) other patients with stage Ia and IIc disease, even after careful surgical staging, experience a 20% recurrence and 12% death rate the first two years after surgery. Such patients are appropriate for adjuvant therapy after surgery. This early ovarian cancer trial is being performed in conjunction with the ovarian cancer study group and the GOG and is the only trial in early ovarian cancer currently active in the United States.

These 12 highlighted studies represent only a small portion of the clinical and laboratory investigations completed or underway during this report year. Further details can be obtained on these and other aspects of the Medicine Branch program by reviewing the attached annual report.

NCI-Navy Medical Oncology Branch: Dr. John D. Minna, Chief

The NCI-Navy Medical Oncology Branch continued its efforts to integrate into the clinical cancer treatment program of the Naval Hospital Bethesda and to institute clinical research protocols of the NCI at the Naval Hospital. Approximately 125 patients will be entered onto NCI protocols this fiscal year. The integration of the NCI and the Navy program in oncology/hematology is complete with an integrated senior staff and fellowship program with regard to patient care. The outpatient clinic, consultative service, and inpatient ward are fully integrated. Via the Radiation Oncology Branch fellowship program, there is considerable integration with radiotherapy at the Naval Hospital. Two of the NCI senior staff (Drs. Gazdar and Matthews) provide excellent integration with Anatomic Pathology.

Currently the combined NCI and Navy oncology and hematology program is following some 1300 active patients, and the program sees over 370 new patients without prior chemotherapy or radiotherapy each year. If a large portion of these patients can be entered onto NCI protocols, and if the many other patients with newly diagnosed cancer seen at the Naval Hospital, can be subsequently seen in the oncology program and then entered onto NCI studies, these integrative efforts would be of significant benefit to the NCI. For all these reasons the effort to identify patients, obtain protocol approval, protocol entry and patient data collection are continuing.

The major clinical studies are in lung cancer and in adult T-cell lymphomas. These involved randomized trials of combined modality therapy and are notable for integrating clinical with laboratory studies. In addition, considerable

effort was expended in reviewing clinical oncology protocols developed by other Clinical Oncology Program Branches and getting them in shape for submission and approval by the Naval Hospital. These include protocols in lymphomas, testicular cancer, breast cancer, and phase I and phase II studies of investigational agents.

Integration of the clinic and laboratory involves studies of tumor cell biology on tumor samples taken from patients under treatment protocols. Studies of this material have revealed new ways to grow lung cancer and T-cell lymphoma using serum free hormone supplemented medium. These have led to the discovery of autocrine growth factors (notably, the peptide hormone bombesin for small cell lung cancer, plus other as yet uncharacterized peptides for lung cancer and T-cell lymphomas). Studies with monoclonal antibodies against hormones such as bombesin have shown that tumor growth can be dramatically suppressed by neuroendocrine manipulation of tumor cells suggesting a new form of cancer treatment.

Clinical studies integrated with the laboratory work of Dr. Gallo's group has led to the identification of the human T cell lymphoma (retrovirus) associated syndrome. In addition, studies of cutaneous T cell lymphoma patients have led to the identification of histologic conversion, clone formation, and more malignant behavior (large cell histologic variants) associated with this type of disease. This should provide the basis for molecular genetic studies.

Biochemical and immunologic markers of lung cancer have been developed that will ultimately be used in routine clinical work to type lung cancer into prognostic groups and help in selection of therapy. The most prominent of these are creatine kinase BB isoenzyme, neuron specific enolase, the hormone bombesin, HLA and beta 2 microglobulin, a panel of monoclonal antibodies directed against the cell surface membrane of lung cancer cells, and a cytogenetic abnormality (deletion of the short arm of chromosome 3).

Studies of the cell biology of lung cancer have revealed considerable evidence for a common pathway of differentiation for lung cancer cells of different histologic types and the identification of a new class of lung cancer cells ("variant" cells of small cell lung cancer). This variant class is associated with very malignant behavior and poor prognosis in patients.

Drug and radiation sensitivity testing of lung cancer cell lines have revealed that the cell lines mirror in vitro their behavior in vivo in patients. This has led to the development of new protocols for treatment of small cell and non-small cell lung cancers based on in vitro selection of therapy (either as phase II trials using the panel of existing cell lines or as selection of therapy for individual patients). The current new agent selected by the panel is tiazofurin. The technology of growing the cell lines plays an important part in developing these trials. For this reason a major new effort is being devoted to work out the details for growing non-small cell lung cancer. These studies provide a foundation for studying drug sensitivity and resistance of lung cancer cells.

Radiobiologic studies in collaboration with the Radiation Oncology Branch (Drs. Mitchell and Kinsella) have revealed heterogeneity between tumors of radiobiologic response and the identification of a radiation repair process. These studies provide the foundation for studying radiation sensitivity and resistance of lung cancer cells.

A panel of monoclonal antibodies against lung cancer cells has been developed. Some of these distinguish non-small cell from small cell lung cancer. Others when added to cloning assays inhibit the clonal growth of lung cancer cells in vitro and the growth in nude mouse xenografts. These should provide new diagnostic and therapeutic reagents.

A major new direction in the laboratory research program has been the establishment of a molecular genetics laboratory. This involved the recruitment of Drs. W.M. Kuehl, I. Kirsch, and G. Hollis. They are beginning studies of the molecular genetics of B lymphoma development, chromosomal translocations, and gene movement in tumor cells. In addition, this allowed the molecular genetic studies of oncogenes in human lung cancer cells. The most visible early result of this work is the discovery of dramatic amplification of the c-myc oncogene in highly malignant, undifferentiated variants of small cell lung cancer. This oncogene is expressed at high levels and could explain the behavior of the undifferentiated state and the radiobiologic resistance of these variant cells which appear to be associated with unfavorable prognosis and tumor progression in patients.

Pediatric Branch: Dr. Philip A. Pizzo, Chief

1. Clinical Studies:

In the study of acute lymphoblastic leukemia this Branch has investigated the efficacy of high-dose protracted intravenous methotrexate as an alternative to the conventional administration of cranial radiation plus intrathecal methotrexate to achieve central nervous system prophylaxis. An additional aim of this study has been to improve the systemic treatment for patients with poor prognostic factors. The hypothesis being tested is that CNS preventive therapy using a methotrexate infusion alone is equally effective and less toxic than the current standard form of CNS prophylaxis. To date, 177 patients have been randomized on this study: 59 to cranial radiation plus intrathecal methotrexate (standard therapy); 118 patients to high-dose intravenous methotrexate infusion (randomizations weighted on 2:1). The overall remission rate is 98% with a continuous remission rate of 80% at two years for the entire study group. With a median duration on study of 18 months, there is no significant difference in the CNS relapse rate for either treatment group. Long-term followup evaluation of neurotoxicity (by CT scan, neuroendocrine evaluation and psychometric testing) is underway. Should these results hold up, it appears that the use of combined cranial radiation and intrathecal therapy can be avoided in nearly 60% of children with ALL, thus reducing the potential long-term neurotoxicity associated with combined therapy. The systemic efficacy of this regimen in average and high risk ALL patients appears to be better than other known regimens at this time.

Relapse during maintenance therapy remains a major reason for failure in children with ALL. It has been demonstrated that orally administered 6-Mercaptopurine (6-MP), one of the most important maintenance drugs in ALL, has a bioavailability of only 16%. The peak level of 6-MP achieved by most patients following oral therapy is approximately a log lower than the levels shown to be optimal for cytotoxic efficacy in in vitro systems. The variations observed from patient to patient in their absorption of oral 6-MP is notable and raises the question of whether oral maintenance chemotherapy is optimal. These observations will

form the basis for a new primary ALL protocol which will attempt to correlate the results of prospective periodic pharmacokinetic bioavailability studies with relapse rate and remission duration.

Previously it has been demonstrated that T-lymphoblasts have higher adenosine deaminase (ADA levels) than non-B, non-T lymphoblasts. This group has attempted to utilize these observations clinically by studying the role of an adenosine deaminase inhibitor, 2'-deoxycoformycin (2'-DCF). Studies performed to date have evaluated 2'DCF as a single agent and in combination with adenosine arabinoside.

Utilizing previously described subhuman primate model for studying CSF pharmacokinetics, they have investigated a recently developed aziridiny] benzoquinone (AZQ) and have determined that its CSF half-life is extremely short (approximately 32 minutes). This rate of AZQ clearance (0.2 ml/min) exceeds that of CSF bulk flow, and indicates that metabolism and/or transcapillary passage may be important clearance mechanisms for this drug. However, in spite of its rapid clearance, substantial levels of AZQ can be achieved in the lumbar CSF following intraventricular injection. These studies are leading to the development of a phase I-II trial of intraventricular AZQ in man.

A study for patients with undifferentiated lymphomas (both Burkitt's and lymphoblastic types) employs alternating cycles of a high-dose methotrexate infusion with CHOP, administered on approximately 10-day intervals without delays for neutropenia. Our analysis of the first 65 patients entered into this protocol permits the following conclusions: (a) the overall long-term survival is approximately 60%; (b) bone marrow infiltration appears to be among the most important prognostic variables, since 11 of 12 patients with bone marrow involvement at the time of diagnosis have relapsed, whereas the disease-free survival for patients without bone marrow involvement is 70%. Furthermore, all 10 patients with lymphoblastic lymphoma who did not have bone marrow involvement are disease-free, as are 13 of the 14 patients who presented with resectable abdominal disease; (c) there was no difference in outcome between patients classified as having Burkitt's versus undifferentiated non-Burkitt's lymphoma. We have also demonstrated that nearly 20% of microscopically normal bone marrows in patients with undifferentiated lymphomas contain occult tumor cells. In patients with extensive undifferentiated lymphomas, monoclonal immunoglobulin bands can be found in the serum of nearly 60% of these patients, raising the potential that they might serve as a biological marker.

An intensive treatment program for patients with high risk pediatric sarcomas designed to overcome both resistance to initial induction therapy and relapse following successful induction therapy has been initiated. This protocol combines high dose chemotherapy during induction (emphasizing intensive adriamycin) in combination with cyclophosphamide, vincristine and actinomycin D. Following induction, patients undergo high dose total body irradiation (800 rads) in conjunction with autologous bone marrow reconstitution. To date, 25 patients have been enrolled in this protocol and the results suggest that early intensive therapy is well tolerated and highly effective in achieving a successful induction. Evaluation of the bone marrow transplant component of this protocol is currently underway.

To assess whether synergistic combinations of antibiotics are necessary for febrile granulocytopenic patients if a single antibiotic has a very broad

spectrum of activity (particularly against gram negative bacteria) and achieves high serum levels, patients were randomized to either conventional combination of cephalothin, gentamicin, carbenicillin (KGC) versus a new third generation cephalosporin, ceftazidime (CTZ). To date, 204 granulocytopenic patients have been randomized to either antibiotic regimen when they become febrile. The initial response during the first 72 hours was 99% for KGC versus 100% for CTZ. So far, the overall results for patients who remain neutropenic or who had documented infections and who were treated with either KGC versus CTZ are exactly comparable.

In an attempt to prevent infections in patients who become granulocytopenic after chemotherapy, a double blind randomized trial was conducted in which antibiotic prophylaxis with trimethoprim/sulfamethoazole plus erythromycin was compared to a placebo. Analysis of the 150 patients entered into this study indicates that the antibiotic prophylaxis exerts a significant benefit for patients with leukemia in reducing the incidence of fever and infections. However, this result was dependent upon the degree of patient compliance in taking the antibiotic regimen. Moreover, patient compliance appeared to be both a dependent as well as an independent variable in affecting outcome and emphasizes the importance of other patient related factors in influencing the putative efficacy of a therapeutic modality.

2. Preclinical Studies:

This group has previously demonstrated the role for steroidal sex hormones in leukemogenesis. To evaluate the status of sex hormone receptors in ALL, estrogen (estradiol, E₂) and androgen (dihydrotestosterone, DHT) receptors were studied in human leukemic lymphoblasts. Scatchard analysis of binding data revealed saturable, high affinity E₂ binding in approximately 20% of the leukemia cells studied. No DHT receptors were detected. These findings suggest that a subset of patients with ALL may respond to anti-estrogen therapy.

In the subhuman primate model for CSF pharmacokinetic studies, this group recently evaluated tiazofurin (TCAR) a C-nucleoside which produces guanine nucleotide depletion by the inhibition of inosine monophosphate dehydrogenase. They demonstrated excellent penetration of TCAR into the CSF following IV administration; the CSF-plasma ratio is approximately 25%. Lethargy has been the only side effect observed in the animal studies. These observations suggest that TCAR may have potential benefit in the treatment of central nervous system malignancies. Presently, a phase I clinical trial is being conducted.

This group has also evaluated the CSF pharmacokinetics of Ara-C in the subhuman primate model. With an intraventricular administration of Ara-C (30 mg), extremely high CSF levels can be obtained but with undetectable plasma levels. These therapeutic CSF levels are maintained for a 24-hour period. The clearance of Ara-C from the CSF of .42 ml per minute suggests that the drug is primarily cleared by CSF bulk flow. Current studies are evaluating intraventricular Ara-C in humans.

The influence of protein-carbohydrate variations on cyclophosphamide-induced myelosuppression in a murine model was studied. Peripheral white blood counts as well as bone marrow CFUc's were measured serially in animals fed diets low and high in protein:calorie ratios. The time to white cell recovery was significantly faster for the group fed the high protein:calorie ratio,

suggesting that a high protein diet can decrease the white blood count nadir and accelerate granulocyte recovery.

To improve the utility of granulocyte transfusions in clinical practice, monoclonal and polyclonal antibodies have been attached against a variety of bacteria to human granulocytes. Using in vitro bactericidal assays, they have demonstrated that "armed" granulocytes were significantly more efficient in bacterial killing when compared to unarmed granulocytes, and the degree of killing correlates with the specificity of the antibody attached to the granulocytes. Further studies are exploring both the mechanism of action, the effects on granulocyte function, and the utility of this procedure in in vivo animal models.

Two cell lines have been derived from patients with the acquired immunodeficiency syndrome. One of these cell lines has an 8;22 translocation and the other has an 8;14 translocation. Since very little information is currently available regarding the lymphomas which occur in AIDS patients, the cell lines should prove invaluable in further studying the biology of this disease.

This group has shown that phorbol esters (TPA) will induce plasmacytoid differentiation in most of the cell lines they have derived with undifferentiated lymphomas. In one of these cell lines, TPA also induces a marked increase in IgM secretion. This is associated with an increase in the synthesis of new messenger RNA as well as with an increase in the c-myc RNA. Thus, expression of this oncogene and the immunoglobulin gene appears to be concordant, thus being consistent with the hypothesis that in cells with a translocation, c-myc is under the influence of sequences which enhance immunoglobulin gene expression.

They have shown that the C3d receptor on the Raji cell line differs from the C3b receptors on normal lymphoblastoid cell lines and from those derived from patients with infectious mononucleosis. In particular, the Raji receptor is capable of interacting with soluble but not cell bound C4b and EAC3b rosette formation was severely limited by increasing ionic strength, whereas binding of EAC3bi was only moderately decreased at physiologic ionic strengths. In contrast, EAC3bi binding to monocytes, polymorphs, and human erythrocytes are markedly reduced with increases in ionic strength whereas EAC3b binding to these cells was less sensitive to changes in ionic strength.

In collaboration with Dr. Carlo Croce (Wistar Institute), it appears that the lymphoma derived cell lines have break points on both the 8th chromosome and the immunoglobulin chromosomes which may vary within a given chromosomal region such that reciprocally exchanged material may differ quite markedly from one cell line to another. These findings raise questions about the proposed hierarchy of immunoglobulin gene rearrangements as well as whether chromosomal translocations may take place unassociated with VG joining.

To evaluate the additional viral encoded information required for tumorigenicity, they constructed a polyoma viral mutant which encodes a functional middle T antigen, a full-sized small T antigen, but only 20 amino acids of the unique region of the polyoma large T antigen. DNA prepared from this mutant viral genome is tumorigenic in newborn hamsters. This suggests that a very short region of the large key antigen (20 amino acids) is critical for virus-induced tumorigenicity.

They have sought to characterize the physiological activity of the polyoma virus oncogene product, the polyoma middle T antigen. In vitro studies have identified a polyoma middle T antigen associated with tyrosyl kinase activity, and have sought to characterize this enzymatic activity by constructing monoclonal antibodies which inhibit the associated tyrosyl kinase activity. We have also identified group specific reagents which inhibit this kinase activity.

They have studied the ability of a variety of biological modifiers to induce the differentiation of neuroblastoma cell lines in culture. Retinoic acid, under certain growth conditions, may induce the terminal differentiation of neuroblastoma cell lines. This is characterized by growth arrest, complete loss of detectable clonogenicity in soft agar, the induction of physiological levels of neurotransmitters, and the outgrowth of characteristic neurites. They are presently studying the alterations in gene expression associated with this biological differentiation.

Radiation Oncology Program: Dr. Eli Glatstein, Chief

The Radiation Oncology Branch (ROB) of the National Cancer Institute continues in transition. In the summer of 1982, the Branch moved into its new clinical facility. Further space on 1B is in the process of being renovated so that the physics and laboratory sections can move to 1B before renovations of B3 take place. The renovations on B3 are pivotal before the final staffing situation can be completed. Space has been at a premium in this Department throughout the last 5 1/2 years, since Dr. Glatstein became Chief of the Branch.

The three main goals of the Radiation Oncology Branch continue unchanged:

1. Major emphasis on clinical trials of combined modality nature, predominantly collaborative with other clinical Branches.
2. Strong radiation biology program with heavy emphasis on basic science and clinical questions of relevance.
3. A training program in radiation therapy, equivalent in stature to the programs in medical, surgical, and pediatric Branches within the National Cancer Institute.

The in vitro laboratory program has studied radiation and experimental chemotherapy, radiation modifying agents, and hyperthermia as well. Eventually, it is hoped to experiment in the area of photo sensitivity. Much of the present work has been centered on human CFUC and human tumor cell lines in collaboration with other Branches.

As far as the training program is concerned, approval has been obtained from the AMA of Residency Review Committee, for the Uniformed Services University of Health Sciences, working through Walter Reed Army Medical Center and the National Naval Medical Center in Bethesda as well as the National Cancer Institute to have a three-year training program under the direction of Dr. Eli Glatstein. Half of this will be spent within the National Cancer Institute and half within the military structure. A need for this integrated program reflects the complementary nature of the clinical material of the various hospitals, with gynecologic, head and neck, and genitourinary cancers in abundance at the

military hospitals and almost completely lacking within the Cancer Institute base. Two of the four positions for training come from the military.

The clinical program within the Radiation Oncology Branch is centered on combined modality studies. Many of these are collaborative with other Branches, the most important of which are small cell carcinoma of the lung and mycosis fungoides, both in collaboration with the NCI-Navy Medical Oncology Branch. Ongoing studies suggest benefits of combined modality treatment for these two diseases. However, additional patients are required before final conclusions can be made since they represent prospective randomized studies. There are also collaborative ventures with the Surgery Branch in soft tissue sarcomas and with the Pediatric Branch in Ewing's sarcoma and rhabdomyosarcoma. Pilot studies have begun on ovarian cancer in conjunction with the Medicine Branch as well as the studies of lymphomas with the Medicine Branch.

Primary Radiation Oncology Branch studies center around intraoperative radiation therapy. The new facility is not yet completed as far as the intraoperative suite is concerned. Thus, patients are still transported through the hallways under anesthesia when such surgery and intraoperative therapy is planned. Large single doses of electron beam treatment are applied intraoperatively to the tumor bed with critical normal viscera moved out of the way. This has been done in conjunction with misonidazole. These have been extremely difficult management problems constituting primary pancreatic and gastric cancer and retroperitoneal soft tissue sarcomas. At the present time the treatment definitely appears to be safe. The actual efficacy is still unclear. The studies are considered first steps to later studies which will incorporate chemotherapy as well.

Intravenous misonidazole was studied in some detail and the pharmacology was delineated. The intravenous compound was studied in a randomized fashion with carcinoma of the esophagus and was shown not to be effective. In a one arm study for locally unresectable osteogenic sarcoma and chondrosarcoma there are a few patients who have received misonidazole and have had impressive responses to treatment despite the fact that these tumors are generally considered "radio-resistant." Indeed, one patient has been followed for over four years without any evidence of growth of the tumor mass.

Another major Radiation Oncology Branch study revolves around stage I and II breast cancer. In this randomized study radical surgery is compared to definitive irradiation with the preservation of the breast. This study, in conjunction with the Surgery Branch, has accrued over 115 patients in the first 3 1/2 years. This is considered a major accomplishment in view of the fact that no prior patient base has been recruited for such a study here at the National Cancer Institute and the difference of the two arms therapeutically makes for difficult randomization. It is far too soon to make any conclusion, but at the moment there is no obvious superiority of either arm suggesting that the long-term results of treatment may well be comparable.

Under the direction of Jan van de Geijn, CT scanning has been fully incorporated in radiotherapy treatment planning. Virtually all patients who are treated with curative intent are now scanned in the treatment position and computerized treatment plans are routinely generated, superimposed on CT cross sections. Dr. van de Geijn has developed a program which allows for adequate dose calculations, even when blocks are placed in the field. It is also possible

to account for tissue inhomogeneity as well. The treatment plans now generated from within the Radiation Oncology Branch are extraordinarily sophisticated compared to what can be done in other medical centers with commercial units. On the other hand, the down time of the CT scanner itself has been a major limitation, along with the relatively small aperture available on the EMI 5005. This unit will soon be surplus.

In the long-range plans, the intraoperative program is considered high priority because it offers a unique opportunity in which to combine sensitizing drugs, and even hyperthermia, in the treatment of abdominal neoplasms. It offers precise localization of the tumor and the ability to eliminate critical normal tissues, or at least protect them from high doses of irradiation. We believe it will ultimately prove to be an ideal approach for retroperitoneal nodes as well as pelvic neoplasms.

New areas of investigation include a study with the Medicine Branch on advanced cervix cancer using radiation therapy and adjuvant chemotherapy. In addition, studies of electron beam therapy for AIDS patients who have Kaposi's sarcoma limited to the skin are also under way. In this investigation, emphasis is placed on identifying a minimal dose-response curve from which time-dose-fractionation schedules for whole skin electron beam treatment will evolve. In addition, before the end of this fiscal year, the Radiation Oncology Branch expects to have a study going in bladder cancer in conjunction with the Surgery and Medicine Branches. The main thrust of the Radiation Oncology Branch role will be to investigate the role of interstitial implantation in dealing with bladder cancer, particularly to take advanced lesions and implant I-125 labelled suture material.

Surgery Branch: Dr. Steven A. Rosenberg, Chief

Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Prospective randomized protocols in the treatment of soft tissue sarcomas have demonstrated the effectiveness of adjuvant chemotherapy with adriamycin, cytoxan, and high dose methotrexate in the treatment of patients with high grade soft tissue sarcomas of the extremities. Disease-free and overall survival rates appear to be doubled in patients receiving chemotherapy. Another prospective randomized trial has demonstrated that limb-sparing surgery is as effective as amputation in the local management of most of these patients. Preliminary results from a third prospective randomized trial indicates that a short course of adriamycin/cytosin is as effective as a long course of treatment with these drugs in preventing recurrences in patients with high grade soft tissue sarcomas of the extremities. Similar protocols are in progress evaluating the role of adjuvant chemotherapy for the treatment of soft tissue sarcomas of the trunk and head and neck.

Another prospective randomized trial in the Surgery Branch is evaluating the use of intraperitoneal 5FU as an adjuvant in the treatment of poor prognosis patients with colorectal cancers. A prospective randomized trial is in progress in the treatment of esophageal cancer that is evaluating whether pre- and post-operative chemotherapy can improve the prognosis of these patients. A prospective randomized trial is in progress evaluating the use of an implantable continuous infusion pump compared to standard therapy for the treatment of patients with hepatic metastases. Over 100 patients have been randomized to a prospective

randomized protocol comparing breast conservation surgery compared to modified radical mastectomy in the treatment of patients with breast cancer. Other Surgery Branch trials are evaluating nutritional support in cancer patients, intraoperative radiation therapy for the treatment of pancreatic and gastric cancer and the resection of hepatic metastases.

Laboratory efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients. Techniques have been developed for isolating cloned lymphoid cells that are capable of curing mice of selected syngeneic solid and disseminated tumors. Preliminary clinical trials have been performed studying the use of activated killer cells for the treatment of human cancers. Studies are in progress to isolate lymphoid cell lines and long-term lymphoid cloned cell lines capable of reacting against human tumors that may be effective for the adoptive immunotherapy of cancer. An IND has been obtained for the use of interleukin 2 in vivo in cancer patients and in patients with acquired immunodeficiency syndrome, and these trials are about to begin. In experimental animals, the in vivo use of IL-2 has been shown to enhance immune responses. Other Surgery Branch laboratory efforts are devoted to studying methods for selectively delivering chemotherapeutic agents to hepatic metastases. Extensive studies are in progress to evaluate tumor host metabolic interactions and to evaluate basic metabolic effects of total parenteral nutrition on the tumor host relationship. Other Surgery Branch laboratory studies involve the study of immunosuppressive factors produced by tumors and the development of monoclonal antibodies against human sarcomas and pancreatic cancer.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06309-01 CO

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Association of Human T-cell Leukemia/Lymphoma Virus (HTLV) with the Tac Antigen

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Samuel Broder, M.D., Associate Director, Clinical Oncology Program, DCT, NCI

COOPERATING UNITS (if any)

Metabolism Branch, DCBD, NCI
Laboratory of Tumor Cell Biology, DCT, NCI

LAB/BRANCH

Office of the Associate Director, Clinical Oncology Program, DCT, NCI

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:

4

PROFESSIONAL:

3

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neoplastic cells from patients infected with HTLV generally express receptors for T-cell growth factor (TCGF) (Interleukin-II) and do not require prior activation with antigens or lectins to undergo TCGF-induced proliferation. Furthermore, neoplastic T-cell lines originating from such patients may constitutively produce TCGF, TCGF receptors, and HTLV virions. HTLV is transmissible from cell to cell, and the infection of human T cells in vitro is associated with expression of TCGF receptors, which can be identified by the monoclonal antibody termed anti-Tac. In our experience to date, T-cell populations that produce HTLV also express epitopes found on TCGF receptors without exception. Recognition of an association between HTLV virions and the Tac antigen would have clinical and theoretical implications. We present evidence that during the replication of release of HTLV the virion becomes preferentially associated with the Tac antigen.

As previously reported, the 55,000-dalton glycoprotein detected by the monoclonal antibody anti-Tac is a normal T-cell activation marker that is identical or very closely linked to the receptor for TCGF. We have been able to detect the Tac antigen in preparations of the neoplastic T-cell line HUT-102-B2 derived from a T-cell lymphoma patient C.R. and in pelleted virus (HTLV_{CR}), which is released by this line, using anti-Tac containing ascites and an enzyme-linked immunosorbent assays (ELISA). The anti-Tac antibody readily bound to the purified (double-banded) HTLV preparation over a range of dilutions, while equivalent concentrations of control murine ascites did not. A similar pattern of binding was observed when a preparation of HUT-102-B2 cells was assayed. By contrast, the anti-Tac antibody did not bind to an Epstein-Barr virus transformed B-cell line (CR-B) derived from the same patient, or to the T-cell line HUT-78, which did not release HTLV.

Zeev Lando, Clinical Oncology Program, DCT, NCI
Prem Sarin, Laboratory of Tumor Cell Biology, DCT, NCI
Mary Megson, Clinical Oncology Program, DCT, NCI
Warner C. Green, M.D., Metabolism Branch, DCBD, NCI
Thomas A. Waldmann, M.D., Metabolism Branch, DCBD, NCI
Robert C. Gallo, M.D., Laboratory of Tumor Cell Biology, DCT, NCI

We next asked whether TCGF-containing culture media could block the binding of anti-Tac to preparations of HTLV_{CR} and HUT-102-B2, as well as to phytohaemagglutinin-stimulated peripheral blood lymphocytes from a normal donor. The results show a comparable blocking effect in all three cases. Moreover, a highly purified preparation of TCGF blocked the binding of anti-Tac antibody to HTLV_{CR}. The data, taken together, are compatible with the view that the Tac marker detected in association with the HTLV preparations represents a functional receptor for TCGF. These results prompted us to compare the relative enrichment of other known T-cell differentiation antigens, which can be expressed by cells infected with HTLV.

The binding of several monoclonal antibodies served as a probe for assessing the relative enrichment of T-cell surface differentiation antigens on standardized preparations of HTLV and HTLV-producing cells. These studies relied upon an internal standard of reference, in which the expression of an antigen in the HTLV preparation was compared to the expression of the same antigen in the HUT-102-B2 preparation under the same conditions. We found that the Tac antigen was preferentially expressed in the viral preparation in comparison to OKT4 (a marker on the inducer T-cell subset), LyT3 (the sheep erythrocyte rosette receptor), and OKT9 (the transferrin receptor). The same results were obtained using another HTLV_{CR} preparation purified independently of the one tested. Comparable results were also obtained using another virus preparation (HTLV_{C10/MJ-2}) which was derived from a different patient with an HTLV-associated T-cell lymphoma, then transmitted to normal cord blood cells, and propagated in secondary cultures before paired assays of virus and virus-producing cord cells were done.

To further analyze the preferential association of the Tac antigen with this human retrovirus, we utilized a competitive binding assay and referred to two additional normal differentiation antigens (HLA-DR and Leu-1), which may be detected on HTLV-infected T-cells in culture. HLA-DR, found on normal B cells, macrophages, and activated T-cells, is a class II histocompatibility antigen. The marker detected by Leu-1 (also referred to as T101 and OK-T11) represents a p67-antigen found on the majority of circulating human T-cells. Using the ELISA technique, we tested the capacity of solubilized HTLV_{CR} and HUT-102-B2 starting-cell preparations to competitively block the binding of monoclonal antibodies reactive against Tac, HLA-DR, and Leu-1 to their respective ligands on HUT-102-B2 target cells on plastic. The enrichment of a given antigen in the viral preparation versus the cellular preparation can be estimated by the position of the solubilized-HTLV competition curve relative to the solubilized HUT-102-B2 cell curve. When there is sufficient enrichment of an antigen on the virus compared to the starting cell which gave rise to the virus, the competition curve with the virus will be to the left of that with solubilized HUT-102-B2 cells. The competitive blocking assays illustrated

the enrichment of Tac antigen in the viral preparation by comparison to the HLA-DR and Leu-1 antigens. The amount of solubilized viral preparation needed to cause 50% inhibition of the binding of anti-Tac to its ligand was about one-sixth that of the solubilized HUT-102-B2 starting-cell preparation needed for a comparable blocking effect (1.2 ug/ml and 7 ug/ml, respectively), indicating that the Tac antigen was present in relatively greater amounts in the HTLV preparation than in the preparation of cells which secreted the retrovirus. On the other hand, in comparison to the HUT-102-B2 preparation, 3 and 10 times as much solubilized HTLV preparation were required in the HLA-DR and Leu-1 systems, respectively, to achieve a 50% inhibition of binding by the appropriate monoclonal antibody. This indicates that these latter antigens were expressed in proportionately lesser amounts in the preparation of HTLV than in the starting cell preparation. It is important to stress that these studies allow conclusions regarding only the relative enrichment of an antigen on the HTLV preparation versus the preparation of cells which released the virus.

It is theoretically possible that these results could reflect a process of selective proteolysis during the purification of HTLV. However, as expected, the competitive binding assay revealed that the viral preparation was enriched with respect to the p19 structural protein encoded by HTLV. We believe this militates against selective proteolysis (sparing Tac but affecting other markers tested) as the explanation for the association of the Tac antigen with HTLV described in the present studies.

In animals, enveloped viruses can incorporate or become associated with many cell surface components sometimes in very high concentrations. For example, Scheinberg and Strand have described a gp55 rat cell membrane glycoprotein, which is found in concentrations up to 100-fold higher in rodent retroviruses than in cells. To our knowledge, the preferential association between a retrovirus and a marker of a cell receptor for a growth factor has not been reported. The observations presented here lead to the conclusion that Tac antigen, which is very closely linked or identical to the receptor for TCGF, is preferentially expressed on HTLV in comparison to other markers of T-cell differentiation. One may speculate upon the biologic significance of the association of this marker for a T-cell trophic factor receptor with the virus. Perhaps the Tac antigen is topographically restricted to regions of the cell membrane which are involved in the budding process by which infected cells release the retrovirus. It is clear from several viral systems that the budding process is not a random event but rather the complex integration of specific virally encoded polypeptides and nucleic acids into a structural unit leading to the release of extracellular virions. The transition of viral-specific glycoprotein precursors in the intracellular membrane system to a plasma membrane maturation-complex involves many post-translational alterations to produce polypeptides ready for incorporation into the budding site. The Tac antigen might be linked to these events.

It is also possible that the association of the Tac antigen with HTLV is important for the primary induction of the transformed state in T-lymphocytes. Interaction between a cell and a virus particle at the surface level is, of course, one of the essential initial steps in viral infection of cells.

Human cells susceptible to infection by retroviruses may have a class of surface receptors that mediates the adsorption and penetration of the virus particle. The Tac antigen might belong to this class, and it is conceivable that HTLV infection expands a small subset of T cells that express the Tac antigen at the time of initial contact with the virus. In keeping with this, enveloped viruses in animals have specific receptors on cells that are susceptible to infection by these viruses. Specific binding sites of enveloped viruses on cell surfaces have been visualized by electron microscopy frequently near the sites of virus budding. Binding sites specific for thymotropic murine leukemia viruses were found in high concentration on thymic lymphoma cell lines induced by this class of virus but were hardly detectable on several non-T leukemias, plasmacytomas and normal thymocytes or spleen cells. Of interest in regard to the present studies, Cogniaux et al. analyzed a number of monoclonal antibodies produced by murine hybridomas after immunization with semipurified baboon endogenous virus (BaEV). Two monoclonal antibodies tested appeared to react with cellular epitopes localized on or near the receptors for BaEV on human cells. Finally, there is the possibility that HTLV has evolved the gene(s) encoding antigenic determinants which cross-react with the Tac antigen, thereby conferring an as yet undefined selective advantage to the virus in its replication and transmission within human beings. These will be topics for further research.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06310-01 CO

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Immunity Against Human T-cell Leukemia/Lymphoma Virus (HTLV)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Samuel Broder, M.D., Associate Director, Clinical Oncology Program, DCT, NCI

COOPERATING UNITS (if any)

Medicine Branch, COP, DCT, NCI
 Laboratory of Human Carcinogenesis, DCCT, NCI
 Laboratory of Tumor Cell Biology, DCT, NCI

LAB/BRANCH

Office of the Associate Director, Clinical Oncology Program

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:

4

PROFESSIONAL:

3

OTHER:

1

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The human type-C retrovirus known as human T-cell leukemia/lymphoma virus (HTLV)* was first isolated from neoplastic cells derived from patients with adult T-cell malignancies. In general, the HTLV isolates studied thus far are very similar to one another, and they represent a family of acquired viruses with apparent T-cell tropism. In co-culture studies, cells producing HTLV can infect and transform suitable target cells. HTLV can be readily distinguished from the known animal retroviruses by nucleic acid hybridization studies, antigenic profiles of structural proteins, and analysis of reverse transcriptase. In the West Indies and in southern Japan, infection with HTLV is an endemic phenomenon, which is associated with the development of a T-cell lymphoma, often made up of neoplastic cells with suppressor immunoregulatory function and OKT4+ phenotype. It has been reported that 6% to 37% of adults in endemic areas are asymptomatic carriers of the virus, and in a few instances it has even been possible to establish cytotoxic T-cell lines reactive against autologous virus-bearing cells from donors who otherwise appeared well.

*The strains of HTLV discussed in this report are in the subgroup HTLV-I.

Associate Investigators:

Hiroaki Mitsuya, Clinical Oncology Program, DCT, NCI
 Lou Matis, M.D., Medicine Branch, COP, DCT, NCI
 Mary Megson, Clinical Oncology Program, DCT, NCI
 Dean Mann, M.D., Laboratory of Human Carcinogenesis, DCCP, NCI

As discussed in the Program summary, the T-cell neoplasms associated with this virus carry an exceedingly grave prognosis because durable remissions with chemotherapeutic regimens are difficult to achieve in most patients; moreover, there is a tendency to develop life-threatening infections with Pneumocystis carinii and other opportunistic organisms. In the United States and Europe, HTLV infection should be suspected in adults who have fulminant T-cell lymphoproliferative disorders, particularly if hypercalcemia is present. The full spectrum of diseases associated with HTLV is not known, and as mentioned earlier, there are data supporting the hypothesis that homosexual patients with the recently defined acquired immunodeficiency disease syndrome (AIDS) have an increased risk of infection with viruses in the HTLV-family.

Relatively little is known about those lymphocytes that may participate in a tumor-specific cellular immune response in patients infected with HTLV. In this project, we attempted to generate long-term cultures of HTLV/tumor-specific T cells from 6 patients with HTLV-associated lymphomas. At the time of study, one of these patients had an unusually long disease-free survival following therapy for his malignancy. We were able to generate a long-term line of T cells capable of mediating specific in vitro cellular immune reactions by producing T-cell growth factor (interleukin 2) and proliferating in response to autologous tumor cells, and also carrying out an HLA-restricted cytotoxic activity against HTLV-infected target cells.

Patients

The patients studied ranged from 26 to 62 years of age and had HTLV-associated T-cell lymphomas. Infection with HTLV was confirmed by detection of the HTLV-genome in neoplastic cells and circulating antibodies to the p19 and p24 viral proteins. Four of the patients have died despite combination chemotherapy. A fifth patient (WA) had experienced one relapse of lymphoma before these studies began and was initially studied during a period of chemotherapy-induced remission; however, his tumor relapsed during the course of these studies.

Patient MJ is a 53 year old white male Merchant Marine Seaman who first noted a "psoriatic" rash in 1969. The skin lesions gradually progressed and evolved into a generalized erythroderma. In 1977, a skin biopsy was consistent with cutaneous T-cell lymphoma (mycosis fungoides). At that time, circulating neoplastic cells with cytogenetic abnormalities were noted and a diagnosis of cutaneous T-cell lymphoma was made. Clinical staging was otherwise negative (stage III). He was treated with whole body electron beam irradiation and a regimen of chemotherapy including vinblastine, adriamycin, bleomycin, cyclophosphamide, methotrexate, prednisone. His disease cleared gradually,

and he became a complete responder with negative skin biopsy and peripheral blood examination in 1979. He received no treatment through June 1981, when he experienced a recurrence in his skin which resolved after topical nitrogen mustard therapy. The patient's disease was considered to be in clinical remission at the time these studies were initiated in January 1983. His tumor remains in remission at the time of this annual report was written.

Materials and Methods

Cell separation and establishment of cultured T-cells reactive with HTLV-infected autologous tumor cells.

Peripheral blood mononuclear cells (PBM) were prepared from heparinized blood by Ficoll-Isopaque gradient centrifugation, and a long-term T-cell line was generated from MJ, the only patient in durable remission of the six patients studied, as previously described. Briefly, 10^7 PBM from patient MJ were co-cultured with 10^6 irradiated (12,000 rads) MJ-tumor cells, then at day six and beyond, these cells were exposed to T-cell growth factor (TCGF), also referred to as interleukin 2. The TCGF was a lectin-free preparation. The culture was repeatedly stimulated with irradiated MJ-tumor cells at 7-14 day intervals. The resultant TCGF-dependent cultured T-cell lymphocyte population is referred to as MJ-CTL. The MJ-CTL population expressed a normal, male karyotype. Evaluation of the MJ-CTL population by fluorescence-activated cell sorter analysis revealed an OKT3⁺, OKT8⁺, HLA-DR⁺, and Tac-antigen⁺ phenotype.

Antigen-Driven Proliferative Assays.

One hundred thousand MJ-CTL were cultured for 3 days with various numbers of irradiated (12,000 rads) autologous MJ-tumor stimulator cells in the absence of exogenous TCGF in 200 μ l of RPMI 1640 media supplemented with 10% heat-inactivated fetal calf serum (FCS), 4mM l-glutamine, 50 U/ml penicillin, and 50 μ g/ml streptomycin at 37°C in 5% CO₂-containing humidified air. At the end of 3 days in culture, the cells were pulsed for 5 hour with 0.5 μ Ci of ³H-thymidine, harvested onto glass fibers, and assessed for the incorporation of isotope as an indicator of proliferation in response to antigen. The results are expressed as the mean counts per minute (cpm) ⁺ one standard deviation for triplicate cultures. In some experiments, 10^5 MJ-CTL were cultured with 5×10^4 irradiated MJ-tumor cells in the presence of a monoclonal antibody (anti-Tac) that reacts with an epitope on or very near the receptor for TCGF.

Assay for the production of TCGF

Seven hundred thousand MJ-CTL were cultured with or without an equal number of irradiated MJ-tumor cells in the absence of exogenous TCGF in 1.4 ml of media for 12 hours. The supernatants were collected after centrifugation and stored at -20°C until assays were performed. A TCGF-dependent mouse HT-2

cell line served as an indicator for the presence of TCGF in a standard assay: 2×10^3 HT-2 cells in 100 ul of media were cultured with an equal amount of supernatant undergoing testing for 21 hours, and pulsed with 1.0 uCi of ^3H -thymidine for another 4 hours before harvesting. The incorporation of ^3H -thymidine was used as an indicator of a response to TCGF in the supernatant.

Cytotoxic Effector Function

Standard 4 hour ^{51}Cr -release and cold-target inhibition assays were used to assess the specific cytotoxic activity of cultured T cells as previously described. The percentage specific release of ^{51}Cr was determined by the following formula: $100 \times (\text{release in test-spontaneous release}/\text{maximum release-spontaneous release})$. Spontaneous release was always less than 20% of maximum release for all target cells reported.

Stimulator and Target Cells

MJ-tumor is an HTLV-producing neoplastic T-cell line that was derived from the peripheral blood of patient MJ and propagated in the absence of TCGF. MJ-B is an Epstein-Barr virus (EBV)-transformed B-cell line derived from patient MJ. We also used phytohemagglutinin(PHA)-induced, TCGF-dependent T-cell blast line from patient MJ, generated after his tumor was in remission. GT-B and MM-B, derived from normal individuals, represent EBV-transformed B-cell lines that were partially HLA-matched with the patient MJ. We also tested a panel of cell lines that were generated by the infection of unrelated normal human cord cells with HTLV, as well as a panel of standard cells used as controls for cytolytic assays.

Histocompatibility Antigen (HLA) Typing.

Cells used in these experiments were analyzed for the expression of HLA-A, HLA-B, HLA-C, and HLA-DR- alloantigens by the microcytotoxicity technique.

Results

Generation of a specific T-cell Line which proliferates in response to HTLV-infected autologous tumor cells.

The capacity to recognize an antigen and respond by proliferating in the absence of exogenously added T-cell growth factor is one of the hallmarks of immune T cells. We attempted to generate tissue culture lines of HTLV/tumor-specific immune T cells from 4 patients whose HTLV-associated lymphomas were not in remission at the time of study. We also attempted to generate such a line from a patient whose tumor had already recurred following combination chemotherapy, but whose disease was in temporary clinical remission at the time of study. We were unable to establish antigen-specific T cell lines in

any of these cases. By contrast, we were readily able to establish a long-term T-cell line reactive with autologous cultured tumor cells from patient MJ by periodically stimulating with irradiated tumor cells from the patient and expanding the cultures with lectin-free T-cell growth factor.

This long-term T-cell line (MJ-CTL) recognized autologous HTLV-infected target cells. When co-cultured with a variety of HTLV-infected and uninfected target cells in the absence of exogenous TCGF, the MJ-CTL proliferated in response only to the autologous HTLV-infected tumor cells. Moreover, phytohemagglutinin-induced autologous blast cells from the patient's peripheral blood and Epstein-Barr virus transformed B cells from the same patient did not induce a proliferative response in the MJ-CTL.

We then tested the possibility that the mechanism for the proliferative response observed was the production of endogenous T-cell growth factor by the MJ-CTL population after stimulation with autologous tumor cells. We observed that MJ-CTL co-cultured with irradiated autologous tumor cells for 12-hours secreted TCGF into the media. Neither MJ-CTL nor the tumor cell population cultured alone produced detectable TCGF. These observations were extended by testing whether the antigen-driven proliferative response of MJ-CTL could be inhibited by the presence of a monoclonal antibody (anti-Tac) which reacts with an epitope identical or closely linked to the cellular receptor for TCGF. The anti-Tac preparation inhibited the proliferation of MJ-CTL in response to stimulation by autologous tumor cells. A control mouse ascites preparation (RPC5) failed to inhibit this *in vitro* proliferative response. Taken together, these data indicate that MJ-CTL can recognize autologous tumor cells and undergo a specific proliferative response mediated by the production of T-cell growth factor in the responding population.

Cytotoxic Activity of the Cultured T-cell Line from patient MJ

During the early phases of the culture the MJ-CTL population mediated a non-specific form of cytotoxicity; however, after repetitive cycles of stimulation with autologous tumor cells and expansion with lectin-free TCGF this line mediated substantial cytotoxicity against autologous tumor target cells and very little non-specific cytotoxicity. The specific T-cell mediated lysis of virally-infected target cells requires a simultaneous associative recognition of histocompatibility gene-encoded antigens in a variety of systems. These observations are encompassed within a "virally-modified self theory" of recognition, in which it is a general rule of immune reactions cytotoxic T cells recognize viral antigens only in the context of the appropriate histocompatibility antigens. Therefore, in the current studies, we explored which HLA-antigens could serve as restriction elements in the killing of HTLV-infected target cells by MJ-CTL. We observed that MJ-CTL could kill autologous HTLV-infected tumor cells, and the autologous tumor cells could inhibit the cytotoxic activity in cold-target inhibition studies. We observed that MJ-CTL failed to kill HTLV-infected targets that did not share HLA antigens expressed by MJ-CTL. However, they were cytotoxic against target cells that were infected with HTLV and shared at least one HLA antigen (HLA-A1). Therefore, the cytotoxicity of MJ-CTL seems to be directed against HTLV-associated antigens, but is at the same time restricted by at least one

HLA antigen. The contribution of other HLA-antigens to this restricted form of cytotoxicity will require further research. Of note, in these studies, there appeared to be more specificity in the induction of proliferation by antigen in this line (only autologous tumor cells could specifically stimulate proliferation), than in the cytotoxic effector function mediated by the line (since other HTLV-infected target cells could be lysed if they expressed the appropriate HLA-antigens).

Application in the Detection of Early Tumor Relapse in a Histocompatible Patient Using HTLV-Reactive Cytotoxic T Cells

In the course of our studies, we came upon an unexpected application of the immune reactivity of MJ-CTL against HTLV-transformed cells in the context of an appropriate HLA-background. At the beginning of these studies, patient WA was in clinical remission having received re-induction chemotherapy following his first relapse. It is important to emphasize that patient WA shared several histocompatibility alleles with patient MJ; moreover an HTLV-producing neoplastic cell-line established at the time of initial presentation shared several HLA-antigens with the MJ-CTL described above, and was susceptible to the cytotoxic activity of MJ-CTL. In a routine examination of the patient, an otherwise normal hemogram was noted to contain 17% "abnormal lymphocytes" (although these were not reported in a follow-up hemogram and the patient's clinical evaluation was negative). Circulating lymphocytes from the patient were placed in culture in the absence of exogenous T-cell growth factor for 18 days, and the resulting cell outgrowth from this patient was used as a target population for the HTLV-specific cytolytic activity mediated by MJ-CTL. Previous attempts to obtain such outgrowths earlier in the course of the remission were unsuccessful. At effector to target ratios of 1:1 or more, a significant level ($P < .001$) of cytotoxicity was observed. This provided evidence that the patient, in fact, had a relapse of his lymphoma, with HTLV-bearing cells in his circulation. The patient subsequently developed a clinical relapse.

Since these studies were initiated, we have obtained a panel of cloned interleukin-2 dependent T cells from the parent MJ-CTL line. These generally mediate a comparable cytotoxic activity, and in some cases mediate both proliferative and cytotoxic reactions to HTLV.

We have also established a unique clone (called K7) which can spontaneously replicate without exogenous interleukin 2, yet retains antigen-specific cytotoxicity against HTLV. This clone has been shown to have one copy of HTLV in the genome; however, to date it appears that the clone fails to express HTLV. Thus, it appears possible to generate "transformed" functional T cells from patients who have had HTLV. To our knowledge, this is a unique development.

Perspective

While it has been possible to extensively analyze the molecular biology, serology, and epidemiology of viruses belonging to the HTLV family, a great deal remains to be learned about tumor-specific cellular immune functions in patients who have HTLV-associated neoplasms. Progress in elucidating the relevant mechanisms of T-cell activation has been hampered by the difficulty of isolating and characterizing antigen-specific T cells from these patients. Indeed, to date it has been difficult in such patients to obtain direct evidence for cellular immunity against transformed-cells bearing HTLV in any context.

We have turned our attention to this problem by attempting to propagate antigen-specific T cells from 6 patients who developed lymphomas in the setting of HTLV-infection. We were unsuccessful in 4 patients whose survival despite therapy was short. We were also unsuccessful in a fifth patient whose tumor has relapsed twice following regimens of intensive chemotherapy. On the other hand, we were able to successfully propagate a T-cell line (MJ-CTL) with specific reactivity against autologous HTLV-producing tumor cells from a patient who had an unusually long survival following therapy for an HTLV-associated T-cell lymphoma.

This antigen-specific T-cell line (MJ-CTL) has been maintained in culture for >160 days. The cells in this line produce T-cell growth factor (interleukin 2) following exposure to autologous HTLV-infected tumor cells, and presumably this property confers the ability to proliferate in the absence of exogenous T-cell growth factor when exposed to autologous irradiated tumor cells. This view is supported by the inhibition of antigen-specific proliferation brought about by the addition of anti-Tac, a monoclonal antibody reactive with the receptor for T-cell growth factor. Finally, MJ-CTL mediates the lysis of HTLV-infected target cells (including autologous cultured tumor cells), but the cytotoxic effect appears restricted by antigens encoded by the human major histocompatibility locus. The data suggest that HLA-A1 can serve as one of the possible restriction elements.

One perspective from which the current results may be viewed relates to the role of the host cell-mediated immune response in the control tumors associated with retroviruses. The immune response to retrovirus-induced tumors has been studied in a number of systems in animals. It has been shown that the development of specific cell-mediated immunity in the setting of a regressing tumor can bring about resistance to subsequent challenge with the same tumor. Such immunity appears to be mediated by antigen-specific T cells and can be adoptively transferred to unimmunized syngeneic hosts. Moreover, there are well-defined murine systems in which fresh or cultured immune T cells specific for retrovirus-induced tumors cause complete regressions of tumors following adoptive transfer to genetically compatible animals bearing otherwise lethal tumor burdens. Sometimes such anti-tumor effects are seen to best advantage when both adoptive cell transfer and chemotherapy are employed. Interestingly, there are data in the Friend virus leukemia system of mice that certain histocompatibility genes influence the rapidity with which Friend-virus-specific T-lymphocyte proliferative responses can develop, and a delay in the development of such immune T-cell proliferative responses has a deleterious

effect on the recovery from leukemias induced by this retrovirus. Taken together, these observations in animal systems suggest that the results presented in this report may eventually have prognostic or therapeutic applications in diseases associated with human retroviruses. This will be a high priority of the Program.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06308-12 BRB

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biometric Research Branch

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Richard M. Simon Chief, Biometric Research Branch, COP, DCT, NCI

COOPERATING UNITS (if any)

Clinical Oncology Program, DCT,NCI; Developmental Therapeutics Program, DCT,NCI; Cancer Therapy Evaluation Program, DCT,NCI; Radiation Research Program,DCT,NCI; Biological Response Modifiers Program, DCT,NCI; Clinical Branches of Division of Cancer Biology and Diagnosis, NCI.

LAB/BRANCH

Biometric Research Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.0

PROFESSIONAL:

5.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The branch is the statistical component of the Division of Cancer Treatment, and provides statistical leadership for major activities of the Division. The branch designs, conducts and analyzes intramural and national clinical trials of experimental treatments, conducts studies to identify important prognostic and treatment selection factors, evaluates diagnostic and surveillance procedures, and develops improved classification systems. The branch collaborates with the Cancer Therapy Evaluation Program in the planning, review and coordination of NCI supported extramural clinical therapeutic research. The branch collaborates with the Developmental Therapeutics Program in the conduct of a national evaluation of the clonogenic assay for pre-clinical screening of new compounds, in a major evaluation of the tumor panel and P388 pre-screen, and in the evaluation of new methods for toxicology testing. The branch develops new statistical designs and biometric methods related to the development and evaluation of new cancer treatments. The branch provides statistical consultation and collaboration for laboratory research and maintains computerized data collection systems.

Associate Investigators

S. S. Ellenberg, Ph.D., Staff Fellow, BRB, COP, DCT, NCI
 R. W. Makuch, Ph.D., Senior Investigator, BRB, COP, DCT, NCI
 M. N. Wesley, Ph.D., Staff Fellow, BRB, COP, DCT, NCI
 R. A. Wesley, Ph.D., Expert, BRB, COP, DCT, NCI

1. Collaborative Intramural Clinical Research

Members of the Biometric Research Branch (BRB) participate extensively in the development of new protocols and the interim monitoring of ongoing studies. The following is a list of studies for which major analyses have been performed in the past year and which are complete enough to be reported at meetings or in the literature. In addition to these completed studies, there are numerous ongoing collaborations and consultations. Substantial effort also continues to be devoted to improved methods of data collection and protocol management for the intramural clinical trials.

- (1) Evaluation of presenting features and prognosis for patients with non-Hodgkin's lymphoma of the skin.
- (2) Influence of drug dose and timing on response for patients with advanced Hodgkin's disease.
- (3) A randomized evaluation of chemotherapy with hormonal synchronization for patients with metastatic breast cancer.
- (4) Evaluation of prophylactic cranial irradiation in small cell lung cancer.
- (5) Evaluation of doxorubicin, cytoxan and VP16 in the treatment of small cell lung cancer.
- (6) Long-term cardiovascular evaluation of Hodgkin's disease patients treated by thoracic mantle radiation therapy.
- (7) Evaluation of prognostic factors for osteogenic and soft-tissue sarcoma patients receiving resection of pulmonary metastases.
- (8) Review of the treatment of acute lymphocytic leukemia in adults.
- (9) Analysis of Cannula dysfunction with radial artery catheterization.
- (10) Treatment of terminal transferase negative acute leukemia: study of effects of chemotherapy, immunotherapy and splenectomy.
- (11) Evaluation of ototoxicity in granulocytopenic cancer patients with pharmacokinetically dosed Amikacin.
- (12) Evaluation of prognostic factors for patients with nodular mixed lymphomas.
- (13) Long-term inhibition of quantitative sebum production with Isotretinoin.
- (14) Evaluation of histological prognostic factors for patients with soft-tissue sarcomas.
- (15) Evaluation of cognitive function in patients with lupus erythematosus.
- (16) Evaluation of adjuvant parenteral nutrition for sarcoma patients.
- (17) Evaluation of ProMACE/MOPP chemotherapy for patients with diffuse lymphomas.
- (18) Evaluation of bone scans in the detection of recurrence for patients with Ewing's sarcoma.
- (19) Evaluation of liver function tests, liver scans and abdominal CT scans as predictors of liver involvement for patients with small cell lung cancer (SCLC).
- (20) Evaluation of whether increasing intensity of induction chemotherapy improves prognosis of extensive SCLC patients.
- (21) Evaluation of whether late intensification therapy with autologous marrow infusion improves prognosis of SCLC patients.
- (22) Evaluation of combined modality treatment for metastatic Ewing's sarcoma patients.

- (23) Evaluation of factors predicting the development of second malignancies for Hodgkin's disease patients.
- (24) Evaluation of body weight and height as predictors of prognosis for patients with breast cancer.
- (25) Evaluation of associations between tumor cell S fraction and anuploidy on clinical course and response to treatment for patients with non-Hodgkin's lymphoma.
- (26) Evaluation of ethiodized oil emulsion as a contrast agent for CT scans in the detection of liver metastases.
- (27) A re-evaluation of histologic type as a prognostic factor in Hodgkin's disease.
- (28) Evaluation of the effect of in-vivo interferon on natural killer cell activity.
- (29) Evaluation of whether there is an association between long term use of vitamin A derivatives and the development of vertebral abnormalities.
- (30) Evaluation of the effects of vitamin A derivatives on sperm counts and other indicators of sexual function.

2. Multi-Institution Clinical Studies

The BRB serves as statistical center for multi-institutional clinical trials of head and neck cancer, early ovarian cancer, multiple myeloma, and therapeutic clinical trials of Diet Nutrition and Cancer Program. The branch also serves as statistical center for a randomized study comparing intrathecal methotrexate prophylaxis to systemic high dose methotrexate for children with acute leukemia. This is a Pediatric Branch protocol being participated in by other institutions. The BRB also participates with the working groups of the Radiotherapy Research Program. There are working groups for hyperthermia, intraoperative radiotherapy, photoradiotherapy and nuclear magnetic resonance imaging. Each working group consists of several contract institutions and data is collected centrally at NCI. Further analysis and reporting of results for the international non-Hodgkins lymphoma classification project is also being conducted.

3. Preclinical Drug Discovery and Development

The BRB works closely with the Developmental Therapeutics Program on projects related to drug discovery and development. A major area of collaboration is the clonogenic assay screening project. The pilot phase has been completed and extensive analysis of the data collected led to a suggested protocol for future screening (e.g. how many tumors to test per compound, types of tumors to be included, criteria for activity, presence of positive controls, types of dose-response and mini-toxicology studies, etc.). The results of the pilot study were evaluated with regard to the feasibility, validity and potential of the assay for discovering useful new drugs. The analysis demonstrated sensitivity to most standard agents, discrimination of non-toxic compounds, and preliminary evidence of ability to identify compounds negative in the P388 screen.

Other collaborations with the pre-clinical drug development program include evaluations assessing redundancy in the eight tumor panel systems, preliminary assessment of whether to include the ovarian M5076 tumor in the new panel, and ongoing consultations to deal with the statistical issues encountered in running a large scale screening program.

4. Extramural Clinical Program

The BRB works closely with the Cancer Therapy Evaluation Program (CTEP) in the administration of the extramural clinical program. All major proposed clinical protocols funded by the NCI are reviewed by the BRB and members of the BRB serve on the CTEP protocol review committee and Clinical Oncology Review Committee. The BRB participates in the development of national, international and inter-group studies. During the current year, inter-group studies have been developed for head and neck, soft tissue sarcoma, melanoma and pancreatic cancer patients. The BRB serves as NCI liaison to the extramural statistical centers, represents the NCI on site visits, and makes recommendations concerning the funding of statistical and data management activities of the extramural groups. The BRB works with the statistical centers to resolve problems and to ensure that the clinical trials are planned, conducted, terminated, analyzed and reported in a sound statistical manner. During the past year the BRB has been involved in the planning and conduct of two major workshops involving the extramural community: one on utilization of developments in computer technology within the cancer cooperative groups, and the other on randomized and non-randomized methodology for the evaluation of treatments. The BRB has collaborated with members of the Environmental Epidemiology Branch in evaluating the incidence of leukemias and pre-leukemic syndromes in patients receiving MeCCNU as adjuvant therapy for gastrointestinal tumors. The BRB also participated in decision-making concerning the disposition of clinical trials involving MeCCNU. The BRB has participated in meetings with the Food and Drug Administration concerning the use of historical controls and other issues related to development of new guidelines for the approval of oncologic drugs. The BRB has consulted on numerous statistical issues relating to the extramural clinical trials program, and has prepared comments for the DCT Board of Scientific Counsellors and Congressional Office of Technology Assessment with regard to such issues. The BRB is collaborating with the Investigational Drug Branch and the Clinical Investigations Branch on an extensive re-evaluation of many aspects of the clinical drug development and clinical trials program.

5. Other Research

(1) Development of clinical trial designs incorporating early termination criteria when initial results do not appear promising for the experimental treatment. Such early termination decisions are common, but effective statistical criteria have not been previously published. One major paper on this topic has been published by a member of the BRB in this year, and the development is continuing.

(2) Development of nonparametric methods for the graphical representation of the association between time dependent covariates (e.g. tumor response) and prognosis. The method developed has broad applicability in biostatistics, epidemiology and demography. An invited paper on this topic has been presented and a manuscript submitted.

(3) An evaluation of the use of randomized consent designs (where consent is sought before randomization) for clinical trials. These designs are being used with increasing frequency though their properties are not well understood. An invited paper on these results was presented at the 1983 spring Biometric Society meeting, and a manuscript has been submitted for publication.

- (4) Development of a software system (DERSYS) for the Dec 10 computer which allows full screen data entry, correction, review and retrieval.
- (5) Identification of methodologic issues in assessing the value of the clonogenic assay for selecting treatment. The predictive value of the assay is substantially effected by overall probability of response, even when the sensitivity and specificity are high.
- (6) Development of a more efficient version of the Bonferroni multiple comparison correction for comparing treatments within subsets.
- (7) Development of statistical methods for planning crossover clinical trials to evaluate anti-emetics and anti-pain medications.
- (8) Development of mathematical models for treatment planning in the face of tumor cell heterogeneity and evolution to resistance.

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SUMMARY OF CLINICAL PHARMACOLOGY BRANCH
CLINICAL ONCOLOGY PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

October 1, 1982 - September 30, 1983

Over the past year this Branch has undergone a major restructuring. As a group, we have developed a common focus. The goal of the Branch is to analyze the reasons for success and failure in the application of chemotherapy to human neoplasia. While this sounds global, as a group we have carefully selected the specific projects on the basis of their probable eventual clinical impact. The target areas thus identified include:

1. Adriamycin Mechanism of Action:

This drug class is one of the three most successful in cancer treatment. We have focused on the free radical generating potential of these agents because evidence suggests this may play a role in toxicity but not antitumor effect. We are focusing on structure activity studies of adriamycin analogs in order to identify means of preserving antitumor effect and eliminating radical based toxicity. Over the past year, we have focused on the interaction of adriamycin with metal ions. These results reveal a very special chemistry associated with this interaction which endows adriamycin with the capacity to punch holes in membranes and cleave DNA via a free radical mechanism. We have defined the structural basis for this chemistry and can now predict which drug chromophores will possess this capacity.

2. Methotrexate Polyglutamate Formation:

Conversion of methotrexate to its polyglutamates has potentially profound implications for the use of this agent in high dose treatment protocols and for our understanding of how these drugs work. This Branch has developed the best HPLC assay for this metabolic event and this has allowed the analysis of polyglutamate formation in human tumor cells. The surprising result was that two human tumor cell lines have been identified which appear to be resistant to MTX by virtue of deletion of the capacity to make MTX polyglutamates. These results suggest that MTX may, in fact, be a pro-drug requiring metabolic activation for cytotoxicity.

3. Mechanisms of Drug Resistance:

As a general area, this is of major importance in developing intelligent treatment strategies. There are three major projects in this area. First, Dr. Cowan is examining the genetic bases of MTX resistance. In addition to the previously discussed work on polyglutamate formation, Dr. Cowan has been involved in cloning the DHFR gene. This has now finished and he will be using this cloned gene to study regulation of that gene expression. This is important because early results suggest that this gene obtained from breast cancer cells is regulated by estrogen.

The second area of interest is in the phenomenon of pleiotropic drug resistance. Dr. Curt has obtained CHO cells from Dr. Victor Ling which possess this resistance and he is in the process of comparing the glycoprotein associated with this resistance with comparably resistant human cells. The hope is that this will show whether a similar mechanism is at work in man. Associated with this project, Dr. Curt is attempting to reverse adriamycin resistance via calcium channel blockers which are thought to work by altering the transmembrane movement of this agent.

The third area of work is that of Dr. Batist in my laboratory. Dr. Batist has discovered a glutathione peroxidase bound to the nuclear envelope. Our interest in this enzyme was initially provoked by the observation that tumor promoters like the phorbol esters appear to work via oxygen radical production and this enzyme could potentially modulate this process. We have been able to show that this enzyme is induced by the anticarcinogen; cis retinoic acid. It is also controlled by estrogen and a variety of other stimuli and it appears that modulation of this enzyme might explain many of the empirical observations in carcinogenesis. As part of this work, we have examined the substrate specificity of this enzyme and found that it would use irradiated DNA as a substrate. It turns out that radiation damage to DNA causes formation of base hydroperoxides and that this enzyme repairs that damage. The enzyme is thus a potential mechanism of DNA repair after free radical injury. We are studying the role of this enzyme in resistance to radiation and free radical forming drugs. This research will have the highest priority in the Branch over the coming year.

4. Pharmacokinetics:

There are many practical questions about drug usage which can be answered with the tools of clinical pharmacokinetics. Over the past year, Dr. Collins has made major contributions in the use of 6MP and cis-DDP via analysis of their pharmacokinetics. In the case of 6MP, in collaboration with Dr. Poplack in Pediatrics, they have been able to show that 6MP can not reliably be given orally. In the case of Cis-DDP, in collaboration with Dr. Ozols, Dr. Collins has been able to show that changing the saline loading technique effectively allows a doubling in the tolerated cis-DDP dose. We have been very pleased about the progress in this area, especially since Dr. Collins only became an official member of our Branch in the latter half of the fiscal year.

Our plans for the coming year include the establishment of an NMR facility in the Branch focusing on the application of NMR to a range of clinical and research pharmacologic questions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-6402-13-CP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tumor Growth Kinetics and Chemotherapy

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Stanley E. Shackney, M.D.

COOPERATING UNITS (if any)

Laboratory of Applied Clinical Engineering Branch
 Medicine Branch

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Cellular Kinetics Section

INSTITUTE AND LOCATION

NCI, National Cancer Institute, Bethesda, MD 20205

TOTAL MANYEARS:

5

PROFESSIONAL:

3

OTHER:

3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have focused on: a) scheduling considerations for tubulin binding agents, specifically vincristine. The results favor infusions over pulse doses, and indicate schedule-dependent synergism with hydroxyurea and adriamycin, respectively. b) Defining the relationship between cell proliferation and the quantitative expression of specialized gene products. An inverse relationship was found between the rate of cell proliferation and the quantitative expression of immunologic surface markers in an early B cell line; similarly, an inverse relationship was observed between cell proliferation and cell content of estrogen receptor. c) Studying the mechanisms underlying clonal evolution as they may relate to clinical and histologic transformation of the lymphomas and as they may relate to the development of drug resistance. Flow cytometry studies in the non-Hodgkin's lymphomas suggest that clonal evolution is fueled by a sequence of i) cell tetraploidization ii) long term cytogenetic instability of the tetraploid line, iii) progressive chromosome from the tetraploid line, and iv) overgrowth of aneuploid cell lines that are produced by this process.

Other Professional Personnel

Hamza Mujagic, M.D.	CPB, COP, DCT, NCI
William Schuette	ACES, BEIB, NIH
Marc Lippman, M.D.	MB, COP, DCT, NCI
Raymond Jakesz, M.D.	MB, COP, DCT, NCI
Jon Minford, M.D.	LMPH, COP, DCT, NCI
Leonard Zwelling, M.D.	LMPH, COP, DCT, NCI
Richard Fisher, M.D.	MB, COP, DCT, NCI
Susan Bates, M.D.	MB, COP, DCT, NCI

1. Theoretical and Methodological Studies

1.1 DNA histogram analysis

In collaboration with William Schuette, of the Applied Clinical Engineering Section, studies were completed on a computer based mathematical method for increasing the resolution of closely spaced aneuploid populations in DNA histograms. The results of these studies have recently been published in Cytometry.

A graphical technique has also been developed for determining S fractions in DNA histograms that contain aneuploidy. A paper describing and validating this technique is in press.

A count-dependent filter for smoothing DNA histograms containing small numbers of cells in the S region has also been developed. The results are being prepared for publication.

2. Experimental Studies

2.1 Vincristine studies in Sarcoma 180 In Vitro

Studies on schedule dependent effects of vincristine in sarcoma 180 were completed this year. Cells that were partially synchronized with hydroxyurea were found to be most susceptible to the effects of vincristine as they progressed through the latter half of the cell cycle. Cells closest to cell division were the most sensitive to drug. Two papers describing the effects of VCR have been accepted for publication in Cancer Research.

Studies have been undertaken to explore the schedule dependence of adriamycin and vincristine combinations. Preliminary data indicate that there is schedule dependent synergism between these two drugs.

2.2 Proliferation and maturation in a murine early-B cell lymphoma line

Studies have been completed on the relation between cell proliferation and quantitative surface marker expression in WEHI-231 cells. These studies have shown that maximal surface marker expression occurs during late log early plateau phase growth. This would suggest that rapidly proliferating cells might be relatively resistant to the effects of specific antibody therapy.

2.3 Cell proliferation and estrogen receptor in MCF-7 cells.

Collaborative studies with Raymond Jakesz and Marc Lippman have demonstrated that the growth characteristics of MCF-7 cells are inoculum-size dependent. The expression of estrogen receptor was found to be maximal in slowly proliferating cells. Two papers describing this work have been submitted for publication.

2.4 Intercalator-induced cytotoxicity and cell proliferation.

Collaborative studies with Jon Minford and Leonard Zwelling have demonstrated enhancement of AMSA induced cytotoxicity and protein-associated DNA breaks by ara-C or hydroxyurea. Cells in S were maximally affected. These studies are being prepared for publication.

2.5 The mechanism of steroid resistance in a Hodgkin's cell line

Studies being carried out in collaboration with Richard Fisher and Susan Bates indicate that steroid resistant clone sublines contain predominantly tetraploid cell populations. It would appear that those tetraploid lines have a proliferation advantage over their diploid counterparts. These studies are continuing.

3. Clinical Studies

3.1 Flow cytometry studies in the non-Hodgkin's lymphomas

Collaborative studies with the Medicine Branch, hematopathology section, and the University of Southern California, on the biology of the non-Hodgkin's lymphomas have been completed. Multiparameter flow cytometry studies suggest that the clinical and histological transformation may reflect cell tetraploidization, cytogenetic instability of the tetraploid with progressive chromosome loss, and the overgrowth of aneuploid clones that are formed from this process. A paper describing this work has been submitted for publication.

3.2 Image analysis studies

Image analysis studies of 5 cases of non-Hodgkin's lymphoma were completed and analyzed this year. The data demonstrate that high S fractions are associated with high grain counts, indicating that the former reliably reflect the rate of cell proliferation. The data also show that the small lymphoma cells have lower grain counts in mid-S than the larger cells, indicating that the small cells in any given tumor form a kinetically distinct, slowly proliferating population.

PUBLICATIONS

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Shackney, S.E.: Principles of Cell Kinetics. In Medical Oncology, Calabresi, P. and Schein, R. (Eds.) W.B. Saunders and Co., Philadelphia, PA. In press.

Jakesz, R., Smith, C.A., Huff, K., Aitken, S., Schuette, W., Shackney, S.E. and Lippman, M.E.: Influence of cell proliferation and cell cycle phase on expression of estrogen receptor in MCF-7 cells. Cancer Res. In Press.

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Pharmacology of Antitumor Agents

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Bruce A. Chabner, M.D., Director, DCT

DCT, NCI

COOPERATING UNITS (if any)

Navy-NCI Medical Oncology Branch

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:

6

PROFESSIONAL:

4.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year we have continued to examine the role of polyglutamate formation in the cytotoxic action of methotrexate. We have examined various aspects of the formation, retention, and binding of polyglutamates in human breast cancer cell lines. The major findings have been 1) that higher polyglutamates, particularly those with 4 or 5 glutamyl groups, are preferentially retained by human breast cancer cell lines, 2) that these longer polyglutamates have slower rates of dissociation from the enzyme as determined in whole cells, 3) studies with isolate dihydrofolate reductase have shown that methotrexate and its polyglutamates have roughly equivalent dissociation constants, implying that unidentified factors, such as association of DHFR with thymidylate synthetase in intact cells, may account for the slower off rate seen in the whole cell, and 4) methotrexate polyglutamates have additional sites of inhibitory action, including potent inhibition of thymidylate synthetase and AICAR transformylase. We have also identified and described gene amplification in a human small-cell carcinoma line derived from a patient treated with high-dose methotrexate, and have shown that the amplification is unstable in culture, likely because of the failure of the double-minute chromosomes to segregate equally during cell division. The loss of double-minute chromosomes in this cell line during continuous passages in tissue culture was associated with a fall in intracellular DHFR and a return to sensitive state. We have initiated studies on pleiotropic drug resistance in human and murine tumor cell lines. Through continuous exposure to colchicine in tissue culture we have derived 10- to 20-fold resistant cell lines which display cross-resistance to adriamycin and vincristine. In murine cell lines this resistance is reversible with verapamil, while in human breast cancer cell lines the resistance is only minimally reversible. In addition, the human breast lines show a cytotoxic response to verapamil alone. We plan to define the calcium channels present on these cells and will study the relationship of channels to drug resistance. We have also initiated studies attempting to transfer drug resistance through DNA-mediated transfection to sensitive receptor cell lines.

OTHER PROFESSIONAL PERSONNEL

Jacques Jolivet, M.D., Cancer Expert	CPB, DCT, NCI
Gregory A. Curt, M.D., Clinical Associate	CPB, DCT, NCI
Neil J. Clendeninn, M.D., Ph.D., Pharmacology Res. Fellow	CPB, DCT, NCI
Grace Yeh, Ph.D., Cancer Expert	CPB, DCT, NCI
Kenneth H. Cowan, M.D., Ph.D., Senior Investigator	CPB, DCT, NCI
Desmond Carney, M.D., Visiting Scientist	Navy-NCI Medical Oncology Branch, DCT, NCI
Carmen Allegra, M.D., Clinical Associate	CPB, DCT, NCI
Robert Fine, M.D., Clinical Associate	CPB, DCT, NCI
James C. Drake, Biologist	CPB, DCT, NCI
Brenda D. Bailey, Biologist	CPB, DCT, NCI

PUBLICATIONS

Erlichman, C., Donehower, R.C., Speyer, J.L., Klecker, R., and Chabner, B.A.: A phase I-II trial of N-phosphonacetyl-L-aspartic acid and 5-fluorouracil given by bolus injection. J. Natl. Cancer Inst. 68: 227-231, 1982.

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Ellims, P.H., Kao, A.Y., and Chabner, B.A.: Kinetic behaviour and allosteric regulation of human deoxycytidylate deaminase derived from leukemic cells. Molec. Cell Biochem., in press.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06515-04-CP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adriamycin Free Radical Chemistry

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Charles E. Myers, M.D., Chief

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biochemical Pharmacology Branch

INSTITUTE AND LOCATION

NCI, National Institutes of Health, Bethesda, MD 20205

TOTAL MANYEARS:

5.2

PROFESSIONAL:

4.2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is broken down into two broad areas: adriamycin free radical generation and enzymatic mechanisms of free radical defense. Over the past year our work on adriamycin free radical generation has been dominated by our interest in the adriamycin-iron complex. Our work in free radical defenses has focused on isolation and characterization of a new family of enzymes-the membrane bound glutathione peroxidases.

Other Professional Personnel:

Luca Gianni, M.D., Visiting Fellow	CPB, COP, DCT, NCI
Helen Eliot, Biologist	CPB, COP, DCT, NCI
Brian Corden, M.D., Clinical Associate	CPB, COP, DCT, NCI
Josephia Muindi, M.D., Visiting Fellow	CPB, COP, DCT, NCI
Jerry Batist, M.D., Clinical Association	CPB, COP, DCT, NCI

Adriamycin free radical generation

In the past year, we have been following up on our observation that adriamycin-iron complexes are able to destroy erythrocyte ghost, *Biochemistry* 21: 1707, 1982 (November 82). We quickly found ourselves immersed in the complexities of iron chemistry. Nevertheless, we have been able to discover several interesting properties of the adriamycin iron complex. First, it binds very tightly to DNA via a mechanism which is distinct from intercalation. The DNA bound drug can then catalyze the oxidative destruction of the DNA in the presence of glutathione. We have shown that the DNA cleavage is a two stage process. The first step is the reduction of oxygen to hydrogen peroxide. In the second step, the hydrogen peroxide is used to cleave the DNA. One of the more interesting aspects of this DNA damage is that it is not random. The first step cuts the SV40 DNA we use as a target exactly in half yielding two 2,300 base pair fragments. From this point, general random cleavage begins. This work has been submitted for publication.

The second interesting aspect of the drug-metal complex is its reaction with oxygen. We have been able to show that after adriamycin binds Fe(III) there is an electron transfer which occurs. The new complex is composed of a one electron oxidized adriamycin and Fe(II). This species reacts avidly with oxygen to form a purple colored complex. This purple complex is then able to cleave DNA or damage cell membranes. In the coming year we hope to use a variety of physicochemical techniques to determine the chemical details of this reaction sequence.

Third, we have been able to establish the structure of the adriamycin-iron complex. The iron binds to the oxygens attached to C₁₁ and C₁₂ of the drug chromophore. In addition, we have done analog studies which have allowed us to determine the structural features which affect this binding and now have a clear understanding what drugs should or should not bind iron. This should prove valuable when we begin to look at the biologic activity of the adriamycin-iron complex. This material is being written up now.

Fourth, we have used cyclic voltammography to investigate the electrochemistry of adriamycin. This study is a comparison of a series of quinones starting from very simple structures and proceeding to adriamycin. The surprising result is that adriamycin and its analogs have unique electrochemical properties not found in other compounds of very similar structure. These results also show a surprising correlation with biologic activity and give strong support to the notion that the electrochemistry of these compounds plays a role in determining biologic affect. A manuscript on this is in second revision.

Finally, there are a series of minor projects which are spin-offs of this work that were completed and published over the past year. These will be found in the attached bibliography.

PUBLICATIONS:

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- Kerr, I.G., Lippman, M.E., Jenkins, J. and Myers, C.E.: The Pharmacology of 13-Cis-Retinoic Acid in Man. Cancer Res. 42: 2069-2073, 1982.
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- Myers, C.E.: The Biochemical Basis for Selective Free Radical Injury. Rational Basis for Chemotherapy, Chabner, B.A. (Ed.): Alan R. Liss, Inc. N.Y., pp. 423-426, 1983.
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06516-02-CP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Drug Resistance in Human Tumor Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Kenneth H. Cowan, M.D., Ph.D., Sr. Staff Fellow

COOPERATING UNITS (if any)

Medicine Branch, COP, DCR, NCI

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, National Institutes of Health, Bethesda, MD 20205

TOTAL MANYEARS:

3

PROFESSIONAL:

3

OTHER:

1/2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our laboratory is working in the area of drug resistance in human tumor cells and in particular, in the genetic mechanisms involved in the development of resistant human breast cancer cell lines. One, has increased levels of DHFR and amplified DHFR genes. We are using this gene amplified cell line to study the regulation of DHFR gene expression. We have also cloned the human DHFR gene and are planning to study the structural features which regulate this gene. Another human MTX resistant cell line is of particular interest, since it is defective in MTX polyglutamate formation. Studies in this cell line raise important questions regulating the mechanism of action of this drug and strongly suggest that MTX is in fact a pro-drug that needs to be metabolically converted to a more active form in order to exert its cytotoxicity. Both of these cell lines are being tested against a wide variety of antifolate analogues. Indeed the different mechanisms of resistance (DHFR overproduction and defective polyglutamate formation), result in marked differences in cross resistance and collateral sensitivity to other antifolate agents. We have also in collaboration studied the mechanisms of resistance in tumor specimens obtained from patients who have failed therapy with MTX and have shown that DHFR gene amplification does occur in vivo.

Other Professional Personnel

Merrill E. Goldsmith, Ph.D., Staff Fellow	CPB, COP, DCT, NCI
Elizabeth Rubalcalba, B.A., Chemist	CPB, COP, DCT, NCI
Richard Levine, M.D., Clinical Associate	MB, COP, DCT, NCI
Jacques Jolivet, M.D., Cancer Expert	CPB, COP, DCT, NCI

1. MTX^R Resistance in Human Tumor Cell Lines 1.1 Development and characterization of resistant cells

MTX^R human breast cancer (MCF7 cells) have been selected which are 5000 fold less sensitive to this drug than the parental cell line. MTX^R MCF7 cells contain 50 fold higher levels of DHFR than drug sensitive cells. Human DHFR from

WT and MTX^R cells have been purified in collaboration with Drs. Neil Clendeninn, B. Chabner, and B. Kaufman. The DHFR from MTX^R and Wild Type (W.T.) MCF7 cell is essentially identical with respect to molecular weight (20.5K Daltons), isoelectric point, Km (DHF), Km (NADPH) and KD (MTX). Additional studies have examined the interaction of MTX polyglutamates with the purified enzyme and in particular both the affinity and the dissociation rate of MTX and each its polyglutamate derivatives from purified human DHFR.

1.2 Cytogenetic Analyses

Cytogenetic Analysis has been done in collaboration with Drs. Pete Douglass, Shan Chen, and J. Whang-Pang. MTX^R MCF7 cells contain HSRS which appear on chromosome 7, 9, 12, and 21. In Situ hybridization studies are being done to identify the precise location of the amplified DHFR genes. A MTX-resistant human small cell lung cancer cell line 249P contain amplified DHFR gene but instead of an HSRS, this cell line contains numerous double minute chromosomes. Cytogenetic analysis and in situ hybridization will be done prospectively in patients with leukemia and lymphoma before treatment and following relapse on combination chemotherapy which include MTX in order to evaluate the overall frequency of DHFR gene amplification in vivo.

1.3 Molecular Genetic Studies

Human DHFR gene initial Southern blot hybridization studies had indicated that the DHFR gene was amplified in the MTX^R MCF7 cells. Dr. Goldsmith has cloned most of the human DHFR gene and two DHFR pseudogenes. The human gene is being subcloned, mapped, and sequenced. Recombinant vectors are being constructed using the 5' flanking sequences (promotor) and the 3' polyadenylation sites of the genomic DHFR gene linked to the coding regions of the human DHFR cDNA. In this way vectors much smaller than the 35Kb size of the genomic DHFR gene will be used to study the regulatory regions of the DHFR genes. These vectors will be introduced into mutant Chinese hamster ovary which are minus DHFR. We are also isolating DHFR minus mutants of human MCF7 cells. Using these DHFR⁻ cell lines we should be able to identify the sequences of the DHFR gene which are necessary for transfection and subsequent transcription. The regulatory sites which are required for the stimulatory response seen following incubation with serum and estrogen should be identified in this manner.

1.4 DHFR gene Regulation

In collaboration with Drs. R. Levine and M. Lippman (MB/DCT) we are examining the hormonal regulation of amplified DHFR genes. MTX^R MCF7 cells contain estrogen receptors which are quantitatively and qualitatively similar to the receptor proteins in the parental MCF7 cells. Following incubation with E₂ there is an induction of progesterone receptors an increase in cell growth, and increases in DNA, RNA and protein synthesis in the MTX^R cells, which are similar to that observed that observed in the MTX sensitive cell line. Furthermore, although the MTX^R cells contain over a 50 fold increase in DHFR levels, there is still a two to four fold increase in the level of this enzyme following the addition of E₂. The increase begins 15 to 20 hours after incubation with E₂ and the rise in enzyme levels parallels the increase in thymidine incorporation induced by E₂. However inhibition of cell DNA synthesis by Ara C does not block the increase in DHFR levels by E₂. Radiolabeling studies followed by MTX affinity column chromatography have demonstrated that the induction in DHFR levels by E₂ is the result of an increased synthesis of this enzyme. Pulse and pulsechase experiments demonstrate that estrogen increase the specific rate of DHFR synthesis but has little if any effect on enzyme stability.

Northern blot hybridization indicates the presence of 3 species of DHFR mRNA (2.8, 1.1, 0.9, kb). All three species are increased following incubation with E₂. ³²P labelling reveals that this increase in DHFR mRNA concentration may be due to increased synthesis of DHFR mRNA.

In collaboration with Drs. J. Jolivet and R. Levine we have been studying the increase in DHFR by MTX. The MTX induction of DHFR is physiologically important particularly in gene amplified cell lines. Since the transport of MTX is slow, a 3 to 4 fold increase in DHFR levels in a cell line which already controls a 25 fold increase in DHFR results in a condition in which the enzyme is 75 to 100 fold higher than control. Since saturation of DHFR by MTX is a requirement for MTX cytotoxicity the incubation of MTX^R MCF7 cells with MTX whereas results in an increase level of MTX binding due to the increase in DHFR levels in the resistant cells but no accumulation of free MTX. We are now studying the precise mechanism of this condition using amino acid and RNA labelling studies. We are also investigating combined hormonal (E₂ or tamoxifen) and MTX therapy in these breast cancer cell lines to determine whether lowering the level of DHFR by treatment with tamoxifen will result in synergistic cytotoxicity with MTX.

1.5 New Class of MTX Resistant Cells: Defective MTX-Polyglutamate Formation

We have also isolated another MTX resistant human breast cancer cell line. Although the selection procedure of gradual stepwise passage in MTX was the same as that employed in the isolation of the DHFR gene amplified MTX^R MCF7 cells, these MTX^R 7R75 cells contain normal levels of DHFR. The enzyme has the same K_d and K_i for MTX. While transport of MTX is 3 fold slower into the resistant cells, this only partially accounts for the resistance to MTX.

W.T. 7R75 cells form significant quantities of MTX polyglutamates. In contrast the resistant cells fail to form significant amounts of these derivatives even when exposed to very high levels of MTX (200 μ M) do not even in the presence of 200 μ M MTX. This is the first example of an in vitro derived MTX^R cell line in which a defect lies in the disturbance in MTX polyglutamate formation. Furthermore, since MTX levels which apparently saturate the enzyme are insufficient to cause cell death in these resistant cells, these studies raise important questions regarding the role of MTX polyglutamates in the determination of cytotoxicity. We have also measured polyglutamamyl synthetase levels in both cells lines and there is no difference. Thus, the actual mechanism of the defect in MTX polyglutamate formation in these cells remains to be identified.

2. Additional Studies on Drug Resistance

MCF7 cells resistant to azauridine and pyrazofuran have been selected. These agents inhibit OMP decarboxylase, the last enzyme in the de novo pyrimidine biosynthetic pathway. The enzymes orotate phosphoribosyltransferase and OMP decarboxylase are both present on a single protein molecule (protein U). Pyr^R MCF7 cells are over 1000 fold less sensitive to both Pyr and Aza U than the parental cell line and contain a 40 fold increase in protein. However, these resistant cells display a collateral sensitivity to 5FU. Since one of the pathways of activation of 5FU is via the enzyme orotate phosphoribosyltransferase (5FU----> 5FUMP), this observation is consistent with an over production of complex U in the Pyr^R cells. We are now studying the regulation of expression of this gene. If this enzyme complex is inducible by estrogen and inhibited by TAM it might result in a strategy for combined chemotherapy and hormonal therapy (Pyr^R and TAM) (E₂ and Fu). In fact 5FU is more actively converted to 5FU and in these cells and more 5FU is incorporated into RNA in these cells which is consistent with the collateral sensitivity which is observed.

PUBLICATIONS:

Cowan, K.H., and Lippman, M.: Steroid Hormone Receptors in Cancer. In Rothfeld, B., (Ed): In Nuclear Medicine In Vitro, Lippincott, pp. 421-431, 1983.

Cowan, K.H., Levine, R., Aitken, S.C. Douglass, P., Goldsmith, M.E., Clendeninn, N., Neinhuis, A.W. and Lippman, M.E.: Dihydrofolate Reductase Gene Amplification and Possible Rearrangement in Methotrexate-Resistant Estrogen Responsive Human Breast Cancer Cells. J. Biological Chem. 257: 15079-15086.

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Curt, G., Carney, D.N., Cowan, K.H., Jolivet, J., Bailey, R.S., Drake, J.C., Kao-Shan, C.S., Minna, J.D. and Chabner, B.A.: Unstable methotrexate resistance in human small cell carcinoma associated with double minute chromosomes. New England J. of Med., 308: 199-202, 1982.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06518-02-CP

PERIOD COVERED
October 1, 1982 to September 30, 1983TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
PharmacokineticsPRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)
(Name, title, laboratory, and institute affiliation)
Jerry M. Collins, PH.D., Pharmacologist,COOPERATING UNITS (if any)
NCI/DCI/COP: MB, SB, PB, ROB
Non-NCI: BEIB/DRS/NIHLAB/BRANCH
Clinical Pharmacology BranchSECTION
Pharmacokinetics SectionINSTITUTE AND LOCATION
NCI, National Institutes of Health, Bethesda, MD 20205TOTAL MANYEARS:
5.0PROFESSIONAL:
5.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary function of this group has been to apply the principles of pharmacokinetics to questions of relevance to the clinical oncology service. Studies completed or active include:

1. Intraperitoneal 5FU as adjuvant treatment for colon carcinoma- Surgery Branch
2. Halogenated pyrimidine radiosensitizers-- Radiation Oncology Branch
3. Intraventricular chemotherapy -- Pediatric Branch
4. Phase I trials of new agents (Tiazofuran, Carboplatinum, Dihydroazacytidine) -- Medicine Branch
5. Pharmacokinetic evaluation of established agents-- Adriamycin, 6-mercaptopurine, cisplatin -- Medicine and Pediatric Branches

In addition to direct clinical pharmacokinetic projects, this group has ongoing projects on the relationship between preclinical and human pharmacokinetic studies. Both experimental studies (rodents) and theoretical aspects.

More detailed pharmacokinetic modeling has been jointly developed in collaboration with the Biomedical Engineering and Instrumentation Branch, DRS/NIH. Pharmacokinetic consultation is also provided to the Surgical Neurology Branch, NINCDS, for the treatment of CNS neoplasms.

Other Professional Personnel

Raymond Klecker, Chemist	COP, CPB, DCT, NCI
Charles Myers, M.D.	COP, CPB, DCT, NCI
Solomon Zimm, M.D.	COP, PO, DCT, NCI
Jean Jenkins, R.N.	COP, CPB, DCT, NCI
Gregory Curt, M.D.	COP, CPB, DCT, NCI
John Grygiel, M.D.	COP, CPB, DCT, NCI
Brian Corden, M.D.	COP, CPB, DCT, NCI

PUBLICATIONS:

- Bachur, N.B., Collins, J.M., Kelley, J.A., Van Echo, D.A., Kaplan, R.S. and Whitacre, M.: Diaziquone, 2,5-diaziridinyl-3,6-biscarboethoxyamino-1,4 benzoquinone, plasma and cerebrospinal fluid kinetics. Clin. Pharmacol. Ther. 31: 650-655, 1982.
- Dedrick, R.L., Flessner, M.F., Collins, J.M. and Shultz, J.S.: Is the peritoneum a membrane? ASAIO J. 5:1-8, 1982.
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- Zimm, S., Collins, J.M., Riccardi, R., O'Neill, D., Narang, P.K., Chabner, B.A., and Poplack, D.G.: Variable bioavailability of oral mercaptopurine: Is maintenance chemotherapy in acute lymphoblastic leukemia being optimally delivered? New Engl. J. Med. 308: 1005-1009, 1983.
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- Curt, G.A., Grygiel, J.J. Corden, B.A., Ozols, R.F., Weiss, R.B., Tell, D., Myers, C.E., and Collins, J.M.: A phase I and pharmacokinetic study of carboplatinum (CBDCA) NSC 241240. Cancer Res. In press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 03403-18 M

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Trials and Miscellaneous Clinical Investigations

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: Robert C. Young Chief M NCI

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI; Navy-MOB, NCI; Clinical Pharmacology Branch NCI; Biometric Research Branch, NCI; Surgery Branch, NCI; Immunology Branch, NCI; Laboratory of Molecular Pharmacology, Environmental Epidemiology Branch, NCI.

LAB/BRANCH

Medicine Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

30

PROFESSIONAL:

21.5

OTHER:

8.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Medicine Branch is a major clinical facility of the NCI. Its activities are divided between clinical therapeutic trials in cancer patients and related laboratory research. Clinical trials of cancer treatment are currently underway in breast cancer, ovarian cancer, Hodgkin's disease, non-Hodgkin's lymphomas, testicular tumors, Kaposi's sarcoma in AIDS, soft tissue sarcomas, cervical carcinoma, and brain tumors. Phase I-II clinical trials have been completed this year on the following new experimental agents: CBDCA, AZQ, Interferon. Phase II trials continue on CBDCA, AZQ, intraperitoneal chemotherapy of adriamycin and Interferon. New Phase I studies include dihydro-5-azacytidine (DHAC) and tiazofuran. Additional summaries of clinical studies are summarized under reports entitled "Clinical Program in Breast Carcinoma." Laboratory research of the Branch is summarized under reports entitled, "Mechanisms of Drug Resistance, Cytogenetic Studies, Immunologic Aspects of Cancer, Mechanisms of Hormone Dependence of Human Malignancy, Genetic Regulation of the Immune Response, and Human Retroviruses and Onc Genes in Human Malignancy and Immuno-deficiency".

Major Accomplishments in 1982-1983General:

In 1982-1983 the Medicine Branch staff published 85 papers, articles, or book chapters and has accepted or has in press 22 additional publications. This is the largest number of scientific publications in the history of the Branch and represents a 17% increase over last year. Forty-one active protocols are maintained primarily by the Medicine Branch. Details of the clinical and laboratory studies will be reviewed in the subsequent sections.

Other:	Bruce Chabner	Director	DCT	NCI
	Charles Myers	Chief	CP	NCI
	Richard Fisher	Sr Investigator	M	NCI
	Marc Lippman	Sr Investigator	M	NCI
	Edward Gelmann	Sr Staff Fellow	M	NCI
	Dan Longo	Sr Investigator	M	NCI
	Louis Matis	Sr Staff Fellow	M	NCI
	Robert Ozols	Sr Investigator	M	NCI
	Jacqueline Whang-Peng	Sr Investigator	M	NCI
	Vincent T. DeVita, Jr.	Director		NCI
	Richard Simon	Chief	BR	NCI
	Steven Rosenberg	Chief	S	NCI
	Eli Glatstein	Chief	RO	NCI
	Allen Lichter	Sr Investigator	RO	NCI
	Paul Bunn	Sr Investigator	Navy-MOB	NCI
	Elaine Jaffe	Sr Investigator	LP	NCI
	Gregory Curt	Clinical Associate	CP	NCI
	Leonard Zwelling	Sr Investigator	LMP	NCI
	Susan Hubbard	Chief	SI	NCI
	Ami Ostchega	Chemo Res Nurse	M	NCI
	Joan Jacob	Chemo Res Nurse	M	NCI
	Jane Cassidy	Chemo Res Nurse	M	NCI
	First and Second Year Associates		M	NCI

Non-Hodgkin's Lymphoma: Established:

- 1) Advanced Diffuse Aggressive Lymphoma: The success of the ProMACE-MOPP regimen highlighted last year and published this year has been followed by a prospective randomized comparison of ProMACE-MOPP day 1 & 8 vs. ProMACE-CytaBOM. These two regimens have been designed to reduce toxicity, convert to outpatient administration and ideally increase the complete remission rate. The first two of these goals has been accomplished. Fifty-three patients have been randomized. The overall complete remission rates are 75% and 81%, respectively. Essentially all of the therapy is given on an outpatient basis and the severe infectious complications have been cut in half (10% septic deaths in the first study vs. 4.3% in the present trial). The success of either or both of these regimens will greatly simplify this treatment and allow wider national use in larger numbers of patients.
- 2) Stage I Aggressive DHL: Using modified ProMACE-MOPP and involved field radiation therapy, it has been possible to induce high frequencies of complete remissions without the necessity of staging laparotomy. Of the first 14 patients on trial, nine are evaluable and 8/9 achieved a CR. None have relapsed and there have been no infectious deaths related to this outpatient therapy.

- 3) Nodular Lymphoma: Watch and Wait vs. Aggressive Chemotherapy. Fifty-two patients have been entered on trial. In patients treated with aggressive combination chemotherapy, there has been an 83% complete response rate and no relapses to date. For those randomized to watch and wait (47%) remain untreated with maximum follow up now 34+ months. This study when completed will clarify 1) whether sustained CR's are possible with aggressive therapy; 2) how frequently does histologic conversion occur in untreated patients; 3) how many patients require no initial therapy and for how long, and finally, 4) will CR's be more easily achieved in patients who convert after initial prior treatment. This study is the only one of its kind underway in the United States.
- 4) Characterization of the clinical manifestations of HTLV-related lymphoma. Three of the initial 10 patients who were used to characterize the clinical manifestations of HTLV-lymphoma in the U.S. (i.e. aggressive disease, bone marrow involvement, lytic bone lesions, hypercalcemia, and opportunistic infections) were hospitalized, diagnosed and treated on the Medicine Branch inpatient service.

Non-Hodgkin's Lymphoma: Published:

- 1) The successful use of ProMACE-MOPP induction therapy in advanced diffuse large cell lymphoma has been published this year. 74 patients with advanced disease have been treated. The complete remission after restaging is 74%. Only 18% of the patients have relapsed with follow-up now in excess of 31 months. The median survival of the entire group of patients has not yet been reached but 62% of all patients remain continuously disease-free after therapy. These results are the best yet reported by any investigators for the treatment of advanced diffuse lymphoma. Continuous disease-free survivals from all previous studies have been approximately 30-35%. ProMACE-MOPP appears to double the cure rate in this disease.
- 2) Second malignancies in non-Hodgkin's lymphoma after treatment. The comprehensive analysis of 515 patients treated at the NCI has been published this year. Results show a significant increase in second malignancies, specifically acute leukemia in patients requiring long term therapy to control their disease. The risk appears in the "indolent" histologies (NPDL or DWDL) where continuous therapy is required. Paradoxically, those patients with aggressive lymphomas (DHL & DML) who are cured with short-term chemotherapy are not at increased risk.
- 3) Comprehensive reviews of the histology, staging, prognostic factors, and response to treatment of 473 patients at the NCI. These large review studies form the basis upon which all subsequent clinical trials at the NCI will be based and compared.
- 4) Studies on the use of a new EOE-13 radiologic enhancing material for the detection of hepatosplenic lymphoma.

Testicular Carcinoma: Established

- 1) A new 4-drug combination (PV_EBV) composed of high dose cis-platinum (40 mg/M² qd x 5), velban, bleomycin and VP-16 appears to have an extremely high (89%) complete remission (CR) rate in patients with poor prognosis advanced non-seminomatous testicular carcinoma. Standard regimens in the past had been able to produce only a 40-60% CR rate in this group of patients. This improved CR rate has been accomplished without significant renal toxicity by using hypertonic saline as a vehicle for the cis-platin infusions. Based upon the initial good results with this regimen a randomized trial was initiated and has accrued 26 patients. Initial CR rate to PVBV is 86% with 79% of patients still NED. For patients treated with PBV there is a 70% CR rate with only 57% of patients remaining NED. No patient on PVBV who achieved a CR has relapsed. These preliminary results indicate that high sustained complete remission rates can be achieved in even poor prognosis testicular cancer patients.
- 2) Prevention of nephrotoxicity from high dose cis-platinum with hypertonic saline, and sustained chloruresis. Doses of cis-platinum of greater than 100-120 mg/M² had been dose limiting because of nephrotoxicity. Studies completed this year with 60 cycles of therapy on 21 patients have established no significant renal toxicity using vigorous hydration, with hypertonic and normal saline as well as furosimide. This new approach to therapy allows higher doses of cis-platinum to be safely used in both testicular and ovarian carcinoma.

Testicular Carcinoma: Published

- 1) The treatment of poor prognosis non-seminomatous testicular cancer with a high dose platinum combination chemotherapy regimen (PVBV).
- 2) Persistent hypomagnesemia following cis-platinum therapy for testicular cancer. The original observation regarding the problem of hypomagnesemia was made by Medicine Branch clinicians. Now the long term follow up of such patients has defined the persistence of hypomagnesemia as a long term complication of such therapy.

AIDS and Kaposi's Sarcoma:

This year the Medicine Branch has initiated an intramural clinical and laboratory research effort in AIDS with particular emphasis on patients with concomitant Kaposi's sarcoma. Both clinical trials and laboratory studies are underway. Fourteen patients have entered two clinical trials. One studying human lymphoblastoid interferon and the other studying two non-cross resistant combinations (Adr, Vb1, Bleo-Actino, Vcr, DTIC). Initial results indicated 2/13 objective responses with interferon and 3/5 clinical responses (2 complete and 1 partial) with the combination chemotherapy approach. Laboratory studies involve search for retrovirus, or human parvovirus in AIDS, and characterization of the T cell defect in AIDS. Details of these studies can be found in the sections entitled Genetic Regulation of the Immune Response and Human Retroviruses and Onc Genes in Human Malignancy and Immunodeficiency.

Hodgkin's Disease: Established:

- 1) The randomized trial of MOPP vs. MOPP-SCAB in advanced Hodgkin's disease is the only trial of an alternating non-crossresistant combination other than MOPP-ABVD currently under study. Sixty-five patients have been randomized. Initial complete remission rates are 83% for MOPP and 84% for MOPP-SCAB. At 3 years follow up, 75% of both groups of patients remain alive and there is no significant difference between the two arms at this point. This alternating sequence regimen does not appear to produce better results than MOPP alone, although the results in both arms are as good as in the Bonadonna trial of MOPP-ABVD. Further follow up is required.
- 2) Early Hodgkin's Disease: MOPP vs. Radiation Therapy. Twenty-four patients have been randomized to radiation therapy alone and 20 to MOPP. 24/24 patients on radiation achieved CR, however, 9 have relapsed and 4 are dead. 18/20 patients on MOPP have achieved CR and only 2 have relapsed. Only one patient in this arm of the study has died and that death was not related to Hodgkin's disease (myocardial infarction). This is the only randomized trial of these two modalities as primary therapy in early Hodgkin's disease in the world.

Hodgkin's Disease: Published:

- 1) A comprehensive review of the remaining challenges in Hodgkin's disease therapy was published this year in order to highlight new research directions. Important research areas remain and include: a) the optimal management of early stage disease; b) the role of alternating sequence combination chemotherapy for advanced disease; c) the optimal approach to massive mediastinal Hodgkin's disease; d) the minimization of the long-term toxicities including sterility, second malignancies, and prolonged immunosuppression.
- 2) Several publications involving the persistence of the immunologic defect in Hodgkin's disease and the characterization of a Reed-Sternberg cell line in culture have been described in detail in the section entitled Immunologic Aspects of Cancer.

Ovarian Carcinoma: Established:

- 1) Preliminary results of an early ovarian cancer trial were presented this year. One hundred and sixty-one patients have now been randomized to two separate trials. The first for patients with Stage Ia and Ib disease compares adjuvant melphalan to no additional therapy after comprehensive initial surgery. The second, for patients with minimal residual disease, compared melphalan to I.P. P³². Initial conclusions are:
 - a) Accurate surgical staging in ovarian cancer is a crucial prerequisite to the decisions regarding appropriate adjuvant therapy.
 - b) Carefully staged patients with Stage I_{Aj} and I_{Bj} with well or moderately well differentiated histology have an extremely good prognosis and may not require any adjuvant therapy of any kind.

- c) Other patients with Stage Ia and IIc disease, even after careful surgical staging, experience a 20% recurrence and a 12% death rate in the first 2 years after surgery.
- d) Such patients are appropriate for adjuvant therapy after surgery. However, the preliminary data from the present trial does not yet define what adjuvant, if any, is preferred.

This early ovarian cancer trial is being performed in conjunction with the Ovarian Cancer Study Group and the GOG is the only trial in early ovarian cancer currently active in the United States.

- 2) A clinical trial in advanced disease has been established (CHIPS) in which alternating sequences of chemotherapy and radiation therapy are employed. Initial therapy with cyclophosphamide and hexamethylmelamine is followed by total abdominal irradiation, intraperitoneal misonidazole and systemic cis-platinum therapy. The latter three agents all show synergistic anti-tumor effects when used in animal systems. There are currently 25 patients on trial. Toxicity is acceptable compared to our previous therapies for advanced ovarian cancer. Overall response rate is 66% with 33% clinical complete remission. Median duration of survival exceeds 20 months. However, to increase the frequency of pathologically documented complete remission, we have increased the dose of platinum in the next series of patients.
- 3) The activity of high dose (40 mg/M² qd x 5) cis-platinum in refractory ovarian cancer. Six patients who had failed combination chemotherapy (containing conventional dose cis-platinum) were treated with the high dose regimen with saline diuresis and hypertonic saline (described under the section on testicular cancer). Three of the six experienced objective regression of tumor in spite of previous platinum treatment.

Ovarian Carcinoma: Published:

- 1) Intraperitoneal (I.P.) phase I & II trials have been completed and published and will be reviewed under sections of the Clinical Pharmacology Branch Annual Report. Essentially large volume intraperitoneal chemotherapy with several agents is feasible and produces a 25-300 fold excess of drug within the peritoneal space compared to plasma concentrations. In the adjuvant 5-FU study in patients rendered disease-free with intensive induction chemotherapy, 2 patients without intraperitoneal therapy have relapsed at 8 and 17 months. One patient in the I.P. 5-FU group has relapsed at 24 months. The phase II I.P. adriamycin trial continues. Ten patients have been entered at doses from 10-60 mg/2 liters. Three patients have thus far had objective regression of disease.
- 2) Extensive experience with the human ovarian cancer clonogenic cell assay has been published. Over 160 patients have been studied. Approximately 80% of samples from ascites, pleural fluid and peritoneal washings can be successfully cloned, and about 40% have >30 colonies per plate to allow some drug testing. However only about 15% have >100 colonies which would allow extensive drug testing. Clinical correlation with assay findings

exceeds 90% for inactive agents and exceeds 64% for those deemed active in the assay. A dose response relationship between adriamycin concentrations and ovarian cancer cells has been demonstrated which gives direct rationale to the intraperitoneal use of the drug in selected patients. Further improvement in the assay is required to allow more effective clinical application. However, focus in our laboratories is being redirected toward the study of mechanisms of drug resistance and the use of human ovarian cancer cell lines (see Section of Mechanisms of Drug Resistance).

- 3) The technique and effect on staging of comprehensive surgical staging in early ovarian cancer was published this year. Prior to referral only 25% of patients had a surgical incision which was adequate to evaluate the entire abdomen. Thirty-two percent of patients referred, apparently free of disease, had residual disease identified by careful staging. This study has established the need for careful surgical evaluation in "early" ovarian cancer and should alter significantly the future management of such patients. Other published papers include the use of peritoneoscopy in the management of ovarian cancer; reviews of the staging and treatment of ovarian cancer; strategies for effective management of early ovarian cancer; comprehensive reviews of the chemotherapy of gynecologic malignancies.

Breast Carcinoma:

Details of the clinical programs on breast cancer may be found with section entitled Clinical Program in Breast Cancer.

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1. Fisher, R.I., DeVita, V.T., Hubbard, S.M., Longo, D.L., Wesley, R., Chabner, B.A., and Young, R.C.: Diffuse aggressive lymphoma: Increased survival after alternating flexible sequences of ProMACE and MOPP chemotherapy. Ann. Int. Med. 98: 304-309, 1983.
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8. Blayney, D.W., Jaffe, E., Fisher, R.I., Schechter, G.P., Cossman, J., Robert-Guroff, M., Kalyanaraman, V.S., Blattner, W.A., and Gallo, R.C.: The human T-cell leukemia/lymphoma virus (HTLV), lymphoma, lytic bone lesions, and hypercalcemia. Ann. Int. Med. 98: 144-151, 1983.
9. Fisher, R.I.: Non-Hodgkin's lymphomas following treatment of Hodgkin's disease. Arch. Int. Med. 143: 427, 1983.

10. Longo, D.L., Young, R.C., and DeVita, V.T.: What is so good about the 'good prognosis' lymphomas? In Williams, C.F. and Whitehouse, J.M.A. (Eds.): Recent Advances in Clinical Oncology, Vol. 1, Edinburgh, Churchill-Livingstone, Ltd., 1982, pp. 223-231.
11. Longo, D.L. and DeVita, Jr., V.T.: Lymphomas. In Pinedo, H.M. (Ed.): Cancer Chemotherapy Annual 1982. Amsterdam, The Netherlands, Excerpta Medica, 1982.
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1. Schilsky, R.L., Barlock, A., and Ozols, R.F.: Persistent hypomagnesemia following cis-platin chemotherapy for testicular cancer. Cancer Treat. Rep. 66: 1767-1769, 1982.
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1. Young, R.C., Myers, C.E., Ozols, R.F. and Hogan, W.M.: Cancer of the ovary: Chemotherapy in advanced disease. Int. J. Rad. Oncol. Biol. Phys. 8: 889-902, 1982.
2. Ozols, R.F., Young, R.C., Speyer, J.L., Green, R., Jenkins, J. and Myers, C.E.: Phase I and pharmacologic studies of adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res. 42: 4265-4269, 1982.
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1. Longo, D.L., Young, R.C., and DeVita, V.T., Jr.: The chemotherapy of Hodgkin's disease. The remaining challenges. Cancer Treat. Rep. 66: 925-936, 1982.
2. Vanhaelen, C.P.J. and Fisher, R.I.: The increased sensitivity of T cells to regulation by normal suppressor cells persists in long term survivors with Hodgkin's disease. Am. J. Med. 72: 385-390, 1982.
3. Fisher, R.I.: Implications of persistent T cell abnormalities for the etiology of Hodgkin's disease. Cancer Treat. Rep. 66: 681-688, 1982.
4. Fisher, R.I. and Bostick-Bruton, F.: Depressed T cell proliferative responses in Hodgkin's disease: Role of monocyte-mediated suppression via prostaglandins and hydrogen peroxide. J. Immunol. 129: 1770-1774, 1982.
5. Fisher, R.I.: Non-Hodgkin's lymphomas following treatment of Hodgkin's disease. Arch. Int. Med. 143: 427, 1983.
6. Fisher, R.I., Bostick-Bruton, F., Sauder, D.N., Scala, G. and Diehl, V.: Neoplastic cells obtained from Hodgkin's disease are potent stimulators of human primary mixed lymphocyte cultures. J. Immunol. (In Press).
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Breast Cancer:

See Section entitled "Clinical Program in Breast Cancer"

Miscellaneous:

1. Longo, D., Howser, D., Wesley, M., Anderson, T., and Young, R.C.: Randomized blind crossover trial of scopolamine vs. placebo administered by transcutaneous patch for the control of chemotherapy-induced emesis. Cancer Treat. Rep. 66: 1975-1976, 1982.
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 CM 03404-12 M
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Aspects of Malignant Lymphomas		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) PI: Richard Fisher Sr Investigator M NCI		
COOPERATING UNITS (if any) Laboratory of Immunoregulation, NIAID; Laboratory of Pathology, DCBD, NCI		
LAB/BRANCH Medicine Branch		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 4.5	PROFESSIONAL: 3.5	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Ongoing studies are attempting to determine the origin and immunologic function of Reed-Sternberg cells in Hodgkin's disease by characterizing a neoplastic cell line obtained from a patient with advanced Hodgkin's disease. Initial studies demonstrated that the L-428 cell line is a potent stimulator of the human primary mixed lymphocyte response. The time course, dose response characteristics, nature of the responding cell, and the ability of the response to be blocked by monoclonal anti-I-A antibodies are all characteristic of mixed lymphocyte reactions. Of interest, the MLC response occurs without detectable interleukin I production in the cultures. This cell line is also capable of serving as an accessory cell for proliferative responses of purified T cells to mitogens. Purified T cells from patients with advanced stages of Hodgkin's disease have reduced proliferation in the presence of the L-428 accessory cell consistent with an inherent T cell deficit in patients with Hodgkin's disease. Studies have been initiated to determine the ability of the L-428 cells to present soluble antigens to T cell clones in a genetically restricted fashion. In regard to immunologic function and cell surface characteristics, the L-428 tumor cells resemble the dendritic cell.</p> <p>Mouse monoclonal antibodies have been prepared against the L-428 tumor cell and react with Reed-Sternberg cells in tissue sections obtained from patients. The specificity of these monoclonal antibodies is now being determined. The characterization of the antigen being recognized by the monoclonal antibodies is in progress.</p>		

Other:	Susan Bates	Clinical Associate	M	NCI
	Narendra Tuteja	Visiting Fellow	M	NCI
	Frieda Bostick-Bruton	Technician	M	NCI
	Toby Hecht	Cancer Expert	M	NCI
	Dan Longo	Sr Investigator	M	NCI
	Elaine Jaffe	Sr Investigator	LP	NCI
	Jeffrey Cossman	Sr Investigator	LP	NCI
	David Volkman	Sr Investigator	LIR	NIAID
	Marc Lippman	Sr Investigator	M	NCI

Growth of the L-428 cells is greatly suppressed by glucocorticoids, yet resistant cell populations develop within one month. Some resistant cells have reduced glucocorticoid receptors while others have normal levels of receptors. The mechanism of glucocorticoid resistance is being investigated.

Patients with untreated non-Hodgkin's lymphomas have diminished proliferative responses to pokeweed mitogen due to deficient helper T cell function. Monoclonal antibody studies reveal that the helper T cells are phenotypically present but functionally impaired. In addition, patients with non-Hodgkin's lymphoma have depressed antibody production as well. Of interest, both B and T cell non-Hodgkin's lymphomas can respond clinically to treatment with a non-specific anti-lymphocyte serum. The role of immunoregulatory abnormalities in the pathogenesis and biology of the non-Hodgkin's lymphoma is being investigated further.

Publications:

1. Vanhaelen, C.P.J., and Fisher, R.I.: The increased sensitivity of T cells to regulation to normal suppressor cells persists in long term survivors with Hodgkin's disease. Am. J. Med. 72: 385-390, 1982.
2. Fisher, R.I.: Implications of persistent T cell abnormalities for the etiology of Hodgkin's disease. Cancer Treat. Rep. 66: 681-688, 1982.
3. Fisher, R.I. and Bostick-Bruton, F.: Depressed T cell proliferative responses in Hodgkin's disease: role of monocyte-mediated suppression via prostaglandins and hydrogen peroxide. J. Immunol. 129: 1770-1774, 1982.
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06119-14 M

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cytogenetic Studies

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jacqueline Whang-Peng, Senior Investigator, Medicine Branch, NCI

COOPERATING UNITS (if any)

Pediatric Oncology Br., NCI; Clinical Pharm. Br., NCI; Clin. Hematol. Br., NHLBI; Medical Oncol. Br - NNMC, NCI; Lab. Chem. Biol, NIADDK; Radiat. Oncol., Br., NCI; Louisiana State University; Div. Virology, Bureau Biologics, FDA

LAB/BRANCH

Medicine Branch

SECTION

Cytogenetic Oncology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5

PROFESSIONAL:

3

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The areas of investigation:

1. Cytogenetic studies of human neoplastic, hematological, and congenital disease, with special emphasis on patients with acquired immune deficiency syndrome (AIDS) who develop leukemia, lymphoma, or Kaposi's sarcoma, and patients with adult T-cell lymphoma and leukemia.
2. In situ hybridization studies:
 - a. Localization of oncogenes (myc, sis, etc.) in Burkitt's lymphoma patients having various chromosome translocations; two patients who also may have had AIDS have been studied so far.
 - b. Localization of HTLV gene in patients with HTLV positive diseases; one patient with HTLV leukemia has been studied thus far.
 - c. Localization of genes for β , ϵ and γ hemoglobin in two variants of the CML tissue culture line K562.
 - d. Localization of the genes for DHFR in various HSR and double minute bearing tissue culture lines.

Other (cont'd):	Turid Knutsen	Med Tech.	MB	NCI
	Elaine Lee	Med Tech.	MB	NCI
	Chein-Song Kao-Shan	Visiting Assoc.	MB	NCI
	Yuan S. Kao	Path.	Louisiana State Univ.	
	John Minna	Branch Chief	MOB-NNMC	NCI
	Paul Bunn	Sr. Investigator	MOB-NNMC	NCI
	Kenneth Cowan	Sr. Staff Fellow	CPB	NCI
	Ian Magrath	Sr. Investigator	POB	NCI
	Susan Seiber	Deputy Director	DCCP	NCI
	Neal Young	Sr. Investigator	CHB	NHLBI
	Alan Schechter	Labor Chief	LCB	NIADDK
	Ann Dean	Chemist	LCB	NIADDK
	George Morstyn	Clinical Assoc.	ROB	NCI
	Gary Armstrong	Biologist	Div. Virol, BB	FDA

Areas of Investigation (cont'd):

3. In vitro cytogenetic studies of direct tumor material, tissue culture lines, and colony culture lines, and colony cultures derived or established from patients with Burkitt lymphoma, small cell carcinoma of the lung, ovarian cancer, and cutaneous T cell lymphoma.
4. Serial cytogenetic studies in long term survivors of Hodgkin's disease, non-Hodgkin's lymphoma, CLL, and small cell carcinoma of the lung to detect a possible correlation between chromosomal abnormalities and early detection of secondary leukemia.
5. Cytogenetic studies in refractory anemia or pancytopenia to determine whether or not there is a correlation between chromosomal abnormalities and the development of overt leukemia.
6. Serial studies of chromosomal clone formation in cutaneous T-cell lymphoma.
7. Study of steroid protection of spermatogenesis in chemotherapeutic trials using a mouse model.
8. In vivo and in vitro studies of sister chromatid exchanges following continuous intravenous effusion of BrdUrd as a radiosensitizer.
9. Serial cytogenetic studies in patients receiving azacytidine for sickle cell anemia to determine the drugs short-and long-term clastogenic effects in vivo.

Projects completed:

1. Cytogenetic studies in two Burkitt lymphoma patients with possible AIDS: Chromosomal abnormalities were consistent with those previously described but with an increased incidence of t(8;22). Unusual findings included production of kappa light chain immunoglobulins by cell lines derived from the tumor of one patient with t(8;22) and the occurrence of various sites of translocation of the duplicated portion of 1q in the other patient who had t(8;14). Manuscript is in preparation.

Projects completed in the past year:

2. Cytogenetic studies in lung cancer: successful banding analysis was obtained for 16 SCLC tissue culture lines, 3 short term tumor cultures, 54 direct bone marrow preparations (from SCLC patients, and 5 cell lines from non-small cell lung cancer. All 16 SCLC cell lines, 3 short term cultures, and 3 direct bone marrow had a common deletion of the short arm of chromosome #3, p(14-23). It was not found in the 5 non-SCLC cell line or the 2 lymphoblastoid lines and it was concluded that this deletion is a specific chromosomal marker for SCLC.
3. Cytogenetic studies of a human breast cancer line, MCF-7 and sublines derived from it: The MCF-7 cell lines demonstrated extensive aneuploidy with both numerical and structural abnormalities and a wide range of heteroploidy. Loss of marker chromosomes was a rare event in the various sublines while formation of additional structural abnormalities was very common. All chromosome pairs were involved in abnormalities and loss of a morphologically normal X chromosome may possibly be related to the loss of estrogen receptors. The inherent genetic instability in these MCF-7 cell lines may explain the heterogeneity found in this tumor and the continual chromosomal changes may reflect the ongoing process of selection and adaptation in these cell lines.
4. Cytogenetic studies of tissue culture lines derived from astrocytomas: Successful banding analysis was obtained of five astrocytoma lines. They showed different distributions of chromosome numbers, ranging from diploid to tetraploid and various chromosomal markers, none of which was common to all five lines or specific for astrocytoma.
5. Studies of the effect on sister chromatid exchanges in vivo administration of bromodeoxyuridine (BrdUrd) in humans: BrdUrd, a potentially radiosensitizing drug, was administered intravenously at doses of 650-1000 mg/m²/ day for up to two weeks and in vivo incorporation into the bone marrow was assayed by the sister-chromatid exchange technique. Virtually all mitoses analyzed showed BrdUrd incorporation but the staining was not uniform throughout each cell: the number of chromosomes showing BrdUrd incorporation varied from cell to cell and in some chromosomes only a portion of the chromatid appeared to have incorporated sufficient BrdUrd to stain in the characteristic manner. We concluded that in vivo administration at these concentrations causes significant amounts of BrdUrd to become incorporated into DNA.
6. Studies of sister chromatid exchanges in ataxia telangiectasia (AT): A study of the various aspects of lymphocyte chromosomal instability in three families comprised of five individuals affected with AT, their parents, and their unaffected sibs showed no significant differences in baseline sister chromatid exchanges or mitomycin-C induced increments in SCE's among family members or between AT heterozygotes or homozygotes.

Publications:

1. Whang-Peng, J., Kao-Shan, C.S., Lee, E.C., Bunn, P.A., Carney, D.N., Gazdar, A.F., Portlock, C., and Minna, J.D.: Deletion 3p(14-23), double minutes, and homogeneously staining regions in human small cell lung cancer. In Schmieke, R.T. (Ed.): Gene Amplification. Cold Spring Harbor Laboratory, Cold Spring, N.Y., 1982, pp. 107-113.
2. Whang-Peng, J., Bunn, P.A., Kao-Shan, C.S., Lee, E.C., Carney, D.N., Gazdar, A.F., and Minna, J.D.: A nonrandom chromosomal abnormality, del 3p(14-23), in human small cell cancer (SCLC). Cancer Genet. Cytogenet. 6: 119-134, 1982.
3. Bentley, S.C., Knutsen, T., and Whang-Peng, J.: The origin of the hematopoietic microenvironment in continuous bone marrow culture. Exp. Hematol. 10: 367, 1982.
4. Whang-Peng, J., Bunn, P.A., Knutsen, T., Matthews, M.J., Schechter, G., and Minna, J.D.: Clinical implications of cytogenetic studies in cutaneous T-cell lymphoma (CTCL). Cancer 50: 1539-1553, 1982.
5. Kohn, P.H., Whang-Peng, J., Levis, W.R.: Chromosomal instability in ataxia telangiectasia. Cancer Genet. Cytogenet. 6: 289-302, 1982.
6. Minna, J.D., Carney, D.N., Alvarez, R., Bunn, P.A., Cuttitta, F., Ihde, D.C., Matthews, M.J., Oie, H., Rosen, S., Whang-Peng, J., Gazdar, A.F.: Heterogeneity and homogeneity of human small cell lung cancer. In Owens, A.H., Coffey, D.S., and Baylin, S.B. (Eds.): Tumor Cell Heterogeneity. New York, Academic Press, 1982, pp. 29-52.
7. Kohn, P.H., Kraemer, K.H., and Buchanan, J.K.: Influence of ataxia telangiectasia gene dosage of bleomycin-induced chromosome breakage and inhibition of replication in human lymphoblastoid cell lines. Exp. Cell Res. 137: 387-395, 1982.
8. Monaco, M.E., Kohn, P.H., Kidwell, W.R., Strobl, J.S., and Lippman, M.E.: Vasopressin: Action on WRK-1 rat mammary tumor cells. JNCI 68: 267-270, 1982.
9. Douglass, E.C., Magrath, I.T., and Terebelo, H.: Burkitt cell leukemia without abnormalities of chromosomes no. 8 and 14. Cancer Genet. Cytogenet. 5: 181-185, 1982.
10. Cowan, K.H., Goldsmith, M.E., Levine, R.M., Aitken, S.C., Douglass, E., Clendeninn, N., Nienhuis, A.W., and Lippman, M.E.: Dihydrofolate reductase gene amplification and possible rearrangement in estrogen-responsive in estrogen-responsive methotrexate-resistant human breast cancer cells. J. Biol. Chem. 257: 15079-15086, 1982.

11. Curt, G.A., Carney, D.N., Cowan, K.H., Jolivet, J., Bailey, B.D., Drake, J.C., Kao-Shan, C.S., Minna, J.D., Chabner, B.A.: Unstable methotrexate resistance in human small-cell carcinoma associated with double minute chromosomes. N. Eng. J. Med. 308: 199-202, 1983.
12. Kao, Y.S., Whang-Peng, J., Lee, E.: A simple, rapid, high-resolution chromosome technic for lymphocytes. Am. J. Clin. Path. 79: 481-483, 1983.
13. Goffman, T.E., Mulvihill, J.J., Carney, D.N., Triche, T.J., Whang-Peng, J.: Fatal hypereosinophilia with chromosome 15q- in a patient with multiple primary and familial neoplasms. Cancer Genet. Cytogenet. 6: 197-202, 1983.
14. Shitara, N., McKeever, P.E., Whang-Peng, J., Knutsen, T., Smith, B.H. Kornblith, P.L.: Flowcytometric and cytogenetic analysis of human cultured cell lines derived from high- and low-grade astrocytomas. Acta Neuropath. (In Press).
15. Whang-Peng, J., Knutsen, T., Douglass, E.C., Chu, E., Ozols, R.F., Hogan, W.M., Young, R.C.: Cytogenetic studies in ovarian cancer. Cancer Genet. Cytogenet. (In Press).
16. Whang-Peng, J., Carney, D.N., Lee, E.C., Kao-Shan, C.S., Bunn, P.A., Gazdar, A., Minna, J.D.: A non-random chromosomal abnormality del(3)(p14-23) in small cell lung cancer (SCLC). In Crispen, R.G. (Ed.): Cancer: Etiology and Prevention. New York, Elsevier North Holland, (In Press).
17. Kao, Y.S., Whang-Peng, J., Lee, E.: A method to obtain longer chromosomes for karyotype. Mammalian Chromosome Newsletter. (In Press).
18. Whang-Peng, J. and Sieber, S.M.: Chromosomal damage by radiation and anti-tumour agents. In Stoll, B.A. (Ed.): Risk Factors in Multiple Cancer. Chichester, England, John Wiley and Sons, (In Press).
19. Whang-Peng, J., Lee, E.C., Kao-Shan, C.S., Seibert, K., Lippman, M.: Cytogenetic studies of human breast cancer lines: MCF-7 and derived variant sublines. J. Nat. Cancer Inst. (In Press).
20. Bracey, A.W., McGinniss, M.H., Levine, R.M., Whang-Peng, J.: Rh mosaicism and aberrant MNSs antigen expression in patient with chronic chronic myelogenous leukemia. Am. J. Clin. Path. 79: 397-401, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06700-10 M

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Program in Breast Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: Marc E. Lippman Senior Investigator M NCI

COOPERATING UNITS (if any)

Biometric Research Branch, NCI; Radiation Oncology Branch,
NCI; Surgery Branch, NCI

LAB/BRANCH

Medicine Branch and Division of Cancer Control and Rehabilitation

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3 1/2

PROFESSIONAL:

2 1/2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Medical Breast Cancer Section is responsible for the development of a clinical and laboratory program directed at breast cancer. Clinical trials in metastatic disease comparing chemotherapeutic, hormonal and chemohormonal regimens are underway. Biochemical and hormonal receptor studies are undertaken and coordinated by the Medical Breast Cancer Section. Clinical studies consist of a major chemotherapy trial aimed at synchronizing human breast cancer cells with hormonal agents for more successful cell cycle phase specific chemotherapy; a hormonal therapy trial aimed at prospectively evaluating the usefulness of steroid receptors for estrogens, androgens and progestins in human breast cancer; an advanced disease hormonal therapy trial comparing tamoxifen plus fluoxymesterone to tamoxifen plus danazol, and two Phase I and II trials, dihydroazacytidine (DHA) and CBDCA, respectively. A trial for stage IV no evidence of disease patients has been initiated. In addition there is an endocrine and chemotherapy program for male breast cancer. A cooperative trial between the Surgery, Radiation and Medicine Branches is underway comparing excisional biopsy plus definitive radiotherapy to simple mastectomy in clinical Stage I and II breast cancer. All patients have axillary dissections; A-C chemotherapy is given to all N+ patients; 118 patients are on study.

Finally, a prospective psychological study aimed at discovering whether or not patients' emotional responses to their disease influences outcome is underway.

Other:	Caroline Bagley	Nurse	M	NCI
	Margaret Wesley	Biostatistician	BR	NCI
	Allen Lichter	Senior Investigator	RO	NCI
	Sandra Levy	Senior Investigator	DCCR	NCI
	David Danforth	Guest Worker	M	NCI

Project Description:

The Medical Breast Cancer Service was established in July 1972, and the clinical program was initiated in January 1973. It was responsible to the Office of the Associate Director, COP, until its shift to the Medicine Branch in August 1974.

I. Clinical Trials

A. Recurrent disease trials.

1. MB-160, a randomized trial of chemotherapy + hormonal therapy aimed at inducing cell synchrony was initiated in August of 1976. This trial has approximately 101 patients on study, and preliminary analysis suggests ER+ patients may benefit from synchronization. Response duration and survival currently favor the synchronization arm; response rates are identical. This trial has just closed for general accrual but is ongoing for Stage III and IV disease.
2. A new pilot study combining intensive systemic therapy combined with local consolidation has just been initiated. The overall aim of this study is to attempt to improve response rate duration in patients with metastatic breast cancer by 1) evaluation of a new and intensive approach to chemotherapy; 2) testing the notion that patients achieving less than a complete response can be rendered free of disease by combined local and systemic therapy; 3) assessing the usefulness of a new tumor cell culturing technique to predict drug sensitivity; and 4) evaluation prospectively of the optimal timing of hormone stimulation of breast cancer cells prior to drug administration.
3. A new randomized primary endocrine trial comparing tamoxifen plus fluoxymesterone to tamoxifen plus danazol has recently been initiated to replace a randomized trial in which tamoxifen plus halotestin has been shown to be superior to tamoxifen alone.
4. A psychological study of how patient attitudes influence survival is ongoing.
5. A protocol for sequential endocrine approaches to male breast cancer with concurrent receptor analyses are ongoing. A trial of adjuvant therapy of Stage II MBC's also underway.
6. A randomized trial of radical radiation therapy versus simple mastectomy is underway with 118 patients on study.

II. Ancillary Studies

- A. Steroid Binding Proteins (SBP) SBP are being prospectively evaluated in all breast cancer samples. This includes analyses for androgen, estrogen, glucocorticoid and progesterin receptors. In addition, analyses are being performed on melanoma, ovary, colon, male breast and hematologic malignancies.

Studies of retinoic acid and retinol binding proteins in breast cancer are also being carried out. Several current publications resulting from these data are listed below.

III. Extramural Activities

- A. National Surgical Adjuvant Breast Project

Dr. Lippman is on the Endocrine Committee of the National Surgical Adjuvant Breast Project.

- B. Outside Teaching Responsibilities

Dr. Lippman is Associate Clinical Professor of Medicine and Pharmacology at the USUHS Medical School.

Publications:

1. Cowan, K. and Lippman, M.E.: Breast Cancer: What Steroid Receptor Analysis Can- and Can't- Tell You. Your Patient and Cancer 9: 56-62, 1982.
2. Cowan, K. and Lippman, M.E.: Steroid hormone receptors in cancer. In Rothfeld, B. (Ed.): In Vitro Nuclear Medicine. Lippincott, 1983, pp. 421-423, 1983.
3. Lippman, M.E.: Clinical implications of glucocorticoid receptors in human leukemia. Am. J. Physiol. 243: E103-E108, 1982.
4. Kerr, I.G., Lippman, M.E., Jenkins, J. and Myers, C.E.: The pharmacology of 13-cis-retinoic acid in man. Cancer Res. 42: 2069-2073, 1982.
5. Cassidy, J., Lippman, M., Lacroix, A. and Peck, G.: Phase II trial of 13-cis-retinoic acid in metastatic breast cancer. Eur. J. Cancer and Clin. Oncol. 18: 925-928, 1982.
6. Fisher, B., Redmond, C., Brown, A., Wickerham, D.L., Wolmark, N., Allegra, J., Escher, G., Lippman, M., Savlov, E., Wittliff, J., Fisher, E.R., and other contributing NSABP Investigators: The influence of tumor estrogen and progesterone receptor levels on the response to tamoxifen and chemotherapy in primary breast cancer. J. Clin. Oncol. 1: 227-241, 1983.

7. Tormey, D.C., Lippman, M.E., Edwards, B.K., Cassidy, J.: Evaluation of tamoxifen doses with and without fluoxymesterone in advanced breast cancer. Annals Intern. Med. 98: 139-143, 1983.
8. Seibert, K. and Lippman, M.E.: Hormone receptors. In Baum, M. (Ed.): Breast Cancer Clinics in Oncology, Vol. 1, No. 3. London, W.B. Saunders, 1982, pp. 735-793.
9. Silver, B.A., Barlock, A.L., Lippman, M.E., Anderson, T. and Fisher, R.I.: Phase II trial of chlorozotocin in malignant melanoma, breast cancer, and other solid tumors. Cancer Treat. Rep. 66: 1229-1230, 1982.
10. Lichter A.S., Limman, M.E., Gorrell, C.R., D'Angelo T, Edwards, B., and DeMoss, E.V.: Adjuvant Chemotherapy in Patients Treated Primarily with Irradiation for Localized Breast Cancer. Proceedings of the Symposium: Alternatives to Mastectomy, 1982. Lippincott (In Press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06702-08 M

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Hormone Dependence of Human Malignancy

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: Marc E. Lippman Senior Investigator M NCI

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NCI

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

10

PROFESSIONAL:

10

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer both in tissue culture model systems we have established and in clinical settings.

- A. We are studying intracellular pharmacokinetics of estrogen and anti-estrogen metabolism and efflux from human breast cancer cells using perfusion systems. These studies have led to new insights into hormone receptor interactions with the genome. Specifically, we have discovered that intracellular estrogen receptors are changed over time to a less easily extractable form associated with the onset of steroid induced effects. This "processed" receptor appears tightly bound to DNA, is extractable by nuclease digestion and may be the proximate receptor form involved in gene regulation.
- B. We are studying the detailed regulation of DNA synthesis in human breast cancer cells and as such have developed ways for accurately quantifying total DNA synthesis together with the scavenger and denovo pathways of pyrimidine biosynthesis.
- C. We have developed a soft agar cloning technique which has permitted the development of clones of antiestrogen resistant variant (putative mutant) cell lines derived from hormone dependent wild typed cells. These variant cells are currently being analyzed biochemically and via somatic cell hybridization.

Other:	Christine Berg	Medical Staff Fellow	M	NCI
	Anish Mohindru	Visiting Fellow	M	NCI
	Attan Kasid	Visiting Associate	M	NCI
	Richard Levine	Medical Staff Fellow	M	NCI
	Diane Bronzert	Technician	M	NCI
	Karen Huff	Technician	M	NCI
	Susan Aitken	Technician	M	NCI
	Leonard Zwelling	Staff Fellow	LBP	NCI

- D. We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous diseases, melanoma, colon carcinoma, ovarian cancer and male breast cancer.
- E. Assays for specific gene products - Thymidylate synthetase, aspartate tran carbamylase dihydrofolate reductase have been developed and we are studying the effects of steroid hormones on the activities and synthesis of these proteins.
- F. We are investigating glucocorticoid receptors in various subpopulations of normal and leukemic lymphoid cells. In addition we are studying glucocorticoid receptors in Burkitt's Lymphoma, hairy cell leukemia, ANLL and the lymphomas.
- G. We have developed assays for all of the enzymes in the denovo pyrimidine biosynthetic pathway and their hormonal regulation is under investigation.
- H. We are studying alterations in intercalating drug induced DNA damage by hormonal agents. Estrogen receptor complexes can cause a 50% increase in DNA breaks induced by iminodaunomycin and M-AMSA.
- I. We are developing probes to study estrogen related regulation of human breast cancer at the level of gene transcription.

Publications

- Scholl, S., Huff, K.K., and Lippman, M.E.: Antiestrogen Effects of LY 117018 in MCF-7 Cells. Endocrinology. (In Press).
- Cowan, K.H., Goldsmith, M.E., Levine, R.M., Aitken, S.C., Douglass, E., Clendeninn, N., Nienhuis, A.W. and Lippman, M.E.: Dihydrofolate reductase gene amplification and possible rearrangement in estrogen-responsive methotrexate-resistant human breast cancer cells. J. Biol. Chem. 257: 15079-15086, 1982.
- Seibert, K., Shafie, S.M., Triche, T.J., Whang-Peng, J.J., O'Brien, S.J., Toney, J.H., Huff, K.K. and Lippman, M.E.: Clonal variation of MCF-7 breast cancer cells in vitro and in athymic nude mice. Cancer Res. (In Press).

4. Bronzert, D., Hochber, R.B., and Lippman, M.E.: Specific cytotoxicity of 16-(125I) Iodoestraiol for estrogen receptor-containing breast cancer cells. Endocrinology 110: 2177-2179, 1982.
5. Jakesz, R., Kasid, A., Greene, G., and Lippman, M.E.: Characteristics of different cytoplasmic and nuclear estrogen receptors appearing with 6 hours of continuous hormonal exposure. J. Biol. Chem. (In Press).
6. Jakesz, R., Kasid, A., and Lippman, M.E.: Continuous estrogen in the rat does not induce loss of uterine estrogen receptor. J. Biol. Chem. (In Press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 CM 06708-04 M
PERIOD COVERED <p style="text-align: center;">October 1, 1982 to September 30, 1983</p>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <p style="text-align: center;">Genetic Regulation of the Immune Response</p>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) PI: Louis A. Matis Senior Staff Fellow M NCI Dan L. Longo Senior Investigator M NCI		
COOPERATING UNITS (if any) <p style="text-align: center;">Immunology Branch, NCI Laboratory of Immunology, NIAID</p>		
LAB/BRANCH <p style="text-align: center;">Medicine Branch</p>		
SECTION <p style="text-align: center;">Experimental Immunology Section</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NCI, NIH, Bethesda, Maryland 20205</p>		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <ol style="list-style-type: none"> 1. Examination of the cellular basis of the autologous mixed lymphocyte reaction and its ontogeny. 2. Examination of the mechanism by which the thymus acts to determine the self-restriction and antigen receptor repertoire of developing lymphocytes. 3. Analysis of the fine specificity of T cell antigen recognition and the influence of major histocompatibility complex (MHC) gene products expressed in the thymus and on antigen presenting cells on antigen recognition in T cell clones and T cell hybridomas. 4. Factors affecting antigen driven lymphokine release in antigen specific T cell clones. 5. Studies on the nature of T cell activation as a function of antigen concentration and MHC gene product concentration. 		

Other:	Toby T. Hecht	Cancer Expert	M	NCI
	Ada Kruisbeek	Cancer Expert	M	NCI
	Mary L. Davis	Clinical Associate	M	NCI
	Barry L. Gause	Clinical Associate	M	NCI
	Ronald Steis	Clinical Associate	M	NCI
	Tai-Chi Shan	Visiting Fellow	M	NCI
	Margaret Weston	Biologist	M	NCI
	Danny Dean	Biologist	M	NCI
	H. Mitsuya	Visiting Fellow	MB	NCI
	S. Broder	Senior Investigator	MB	NCI

Selected Highlights of Work Completed this Year:

- I. Studies on the autologous mixed lymphocyte reaction (AMLR): The AMLR is a proliferative T cell response by unprimed cells exposed to cells bearing autologous Ia molecules. Its physiological importance is unclear, however, the AMLR is absent in a number of autoimmune and immunodeficient states, including certain patients with cancer (e.g. Hodgkin's disease). Experiments with radiation-induced bone marrow chimeras indicate that the AMLR specificity is acquired by the developing T cell in its adoptive host. Anti-Ia monoclonal antibodies indicate that Ia antigens are the molecules being recognized. Finally, nude mice given thymus transplants reveal that the thymus imparts the recognition specificity.
- II. Thymic cell critical to thymic determination of T cell self and antigen recognition: The thymus determines the expression of antigen receptors by developing T cells. Since certain thymic cells (epithelium) derive from the 3rd pharyngeal pouch and others (dendritic or antigen presenting cells) derive from the bone marrow, it is possible to create an animal whose thymus gland is chimeric bearing epithelial cells of one genotype and dendritic cells of another. In general, T cells emerging from such a chimeric thymus gland appear to express the self-restriction and antigen-specific receptor repertoire of the bone marrow derived dendritic cell rather than the epithelial cell.
- III. Effect of the priming and developmental environment on the fine specificity of T cell antigen recognition: We have analyzed the fine specificity of antigen and MHC recognition by pigeon cytochrome c-specific T cell clones from two responder strains. The response of T cells from both strains is mapped to the same region of cytochrome c and antibodies directed at shared MHC determinants block the response in both strains. Since clones from both strains recognize similar antigen and MHC determinants, they were analyzed for the ability to recognize antigen on the other responder haplotype antigen presenting cell (APC). About 90% of T cell clones from each strain could recognize antigen only on self APC, but 10% of the clones responded to antigen on either responder MHCV but with altered fine specificity. Radiation induced bone marrow chimeras made by giving T cell-depleted bone marrow of one genotype to irradiated animals of the other reveal that; (1) the frequency of cross-recognizing

T cell clones is not increased by priming in the presence of both haplotypes; (2) suppression of antigen recognition in association with non-host MHC products is not the mechanism by which chimeric T cell function becomes restricted to host MHC determined responses; and (3) the thymus is responsible for the fine specificity of antigen recognition, i.e. the selection of a particular MHC antigen as self carries with it a family of antigen receptors that are expressed regardless of the MHC genes of the developing T cell precursor.

- IV. Complexities in lymphokine release from antigen specific T cell clones: Antigen-specific lyt 1+ T cell clones make high titers of gamma interferon upon exposure to the antigen for which they are specific on the appropriate antigen presenting cell. This interferon production begins about 4 hours after exposure to antigen and peaks at 48 hours after stimulation. While the kinetics of the response differ from the kinetics of antigen-specific proliferation, the amount of interferon produced parallels proliferation and the affinity of the receptor for the antigen controls both the amount of proliferation and the amount of interferon production. These results demonstrate that a single antigen-specific helper T cell is capable of providing interferon as well as helper factors to the cells with which it interacts. Furthermore, there are certain circumstances under which a stimulus to T cell clone activation resulted in the release of interleukin 2 but not interferon, while another stimulus resulted in release of both factors. This observation suggests that T cell activation need not be an all or none phenomenon and that factors other than affinity between a T cell and its antigen may act on this process.
- V. Effects of antigen and Ia concentration on T cell activation: We have examined the fine specificity of antigen recognition in Ia molecule-restricted long term T cell lines and clones specific for the protein pigeon cytochrome c. As reported above, the pattern of activation of such T cell clones by a number of species variant cytochrome c peptides varied with the MHC haplotype of the APC. These results unequivocally demonstrated a role for Ia molecules in determining the specificity of T cell antigen-induced activation, and suggest strongly that an antigen-Ia molecule interaction must occur in the APC membrane during T cell activation. Additional studies have demonstrated that the magnitude of proliferation of T cell clones is a function of the product of the antigen and Ia molecule concentrations, such that antigen concentration response curves of T cell clones shift to lower antigen concentrations in the presence of either greater numbers of APC or APC bearing more of the relevant Ia molecule (for antigen recognition) per cell. The magnitude of activation of T cell clones was further shown to be a biphasic function of the product of the antigen and Ia molecule concentrations, such that T cell proliferation was sub-maximal at high antigen concentration or in the presence of larger numbers of APC. We suggest that high $\sqrt{\text{antigen} \times \text{Ia molecule}}$ induced inhibition of T cell proliferation may represent a model for the induction of T cell tolerance.

Publications:

1. Longo, D.L., Paul, W.E.: Immune response genes and Ia antigens: the relationships between them and their role in lymphocyte interactions. In Parham, P. and Strominger, J.L. (Eds.): Transplantation Antigens. London, Chapman & Hall, Ltd., 1982, pp. 161-185.
2. Tse, H.Y., Mond, J.J., Longo, D.L.: B lymphocyte immune response gene phenotype is genetically determined. J. Exp. Med. 155: 1239-1244, 1982.
3. Schwartz, R.H., Glimcher, L.H., Hedrick, S.M., Singer, A., Longo, D.L.: Restriction to thymic major histocompatibility complex (MHC) and immune response (Ir) phenotype of T cells from allogeneic chimeras. Behring Institute Research Communications 70: 155-162, 1982.
4. Glimcher, L.H., Schwartz, R.H., Longo, D.L., Singer, A.: The restriction specificity of the syngeneic mixed lymphocyte reaction, a primary anti-I region T cell proliferative response, is imparted by the thymus. J. Immunol. 129: 987-991, 1982.
5. Hedrick, S.M., Matis, L.A., Hecht, T.T., Samelson, L.E., Longo, D.L., Heber-Katz, E. and Schwartz, R.H.: The fine specificity of antigen and Ia determinant recognition by T cell hybridoma clones specific for pigeon cytochrome c. Cell 30: 141-152, 1982.
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06709-03 M

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Drug Resistance

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert F. Ozols, Senior Investigator, Medicine Branch, NCI

COOPERATING UNITS (if any)

Laboratory of Medicinal Chemistry and Pharmacology
Clinical Pharmacology Branch

LAB/BRANCH

Medicine Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5

PROFESSIONAL:

4

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying the biology of ovarian cancer and the mechanisms of antineoplastic drug resistance in human tumors. This has required the development of human ovarian cancer cell lines in tissue culture and in nude mice xenografts. The dose response curves to antineoplastic drugs are generated using a clonogenic assay. Cell lines from previously untreated patients which are sensitive in vitro have been incubated with progressively increasing concentrations of melphalan, adriamycin and cisplatin to produce drug resistant variants. With these sets of cell lines [endogenously resistant, endogenously sensitive, and their resistant variants] we are examining the mechanisms of drug resistance at a cellular level and biochemical manipulations which can restore sensitivity in the resistant cell lines.

We have characterized 3 new ovarian cancer cell lines including a line which has steroid hormone receptors. We have also developed drug resistant variants which are 6-10 times more resistant than the primary cultures. We have demonstrated that melphalan resistance is linked to glutathione levels. Furthermore, using techniques to alter the levels of glutathione or to change the permeability of cell membranes, we have been able to restore drug sensitivity in melphalan and adriamycin resistant cell lines, respectively.

PI:	Robert F. Ozols	Sr. Investigator	M	NCI
Other:	Robert C. Young	Chief	M	NCI
	Karen Grotzinger	Med Technologist	M	NCI
	Wilma McCoy	Med Technologist	M	NCI
	A. M. Rogan	Visiting Fellow	M	NCI
	J. A. Green	Visiting Fellow	M	NCI
	Thomas C. Hamilton	Staff Fellow	M	NCI

Ongoing Studies:

1. Ovarian Cancer Cell Lines. We have established and characterized 3 long term cell lines of human ovarian cancer. One of the lines is noteworthy for the presence of steroid receptors and the hormonal requirements for growth are currently being investigated. The cell lines produce subcutaneous tumors in nude mice and studies are in progress to develop an intraperitoneal nude mouse model of ovarian cancer.
2. Drug Resistant Variant Cell Lines. We have developed variant cell lines from two cell lines established from previously untreated patients which were sensitive in vitro to antineoplastic drugs. Using step-wise incubation with either melphalan, adriamycin, cisplatin or thiotepa we have developed variant cell lines which are 6-10 fold more resistant to these drugs than the parent cell lines. These cell lines are being characterized as to cytogenetic features and patterns of cross resistance.
3. In Vitro Dose Response Curves. We have examined in vitro dose response relationships to various anti-cancer drugs using both established cell lines and clonogenic assays of fresh specimens of ovarian or testicular cancer. The result of the these studies have provided in part a rationale for clinical studies of high dose cisplatin, CBDCA, and intraperitoneal chemotherapy.
4. Mechanisms Of Drug Resistance. We are examining the mechanisms of drug resistance to melphalan and adriamycin in human tumors. We are evaluating the role of transport, metabolism, as well as of intracellular thiols in the expression of drug resistance.
5. Alternations Of Drug Resistance. We are examining the role of potential modifiers of drug resistance in human ovarian cancer cell lines. While we could not establish any beneficial effect of amphotericin B, we have demonstrated that verapamil will restore sensitivity to adriamycin in adriamycin-resistant variant cell lines and that manipulations of glutathione will alter sensitivity to melphalan. Studies are being performed using multiple concentrations and schedules of incubation to determine if drug resistance modification is clinically feasible.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06710-01 M

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Retroviruses and onc Genes in Human Malignancy and Immunodeficiency

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: Edward Gelmann Senior Investigator M NCI

COOPERATING UNITS (if any)

Laboratory of Tumor Cell Biology, NCI, Clinical Hematology
 Branch, MLBI, Laboratory of Immunology, NIAID

LAB/BRANCH

Medicine Branch

SECTION

Medicine Breast Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4

PROFESSIONAL:

4

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating the involvement of human retroviruses in human malignancy and the acquired immune deficiency syndrome and the involvement of human onc genes in human malignancies.

- A. We have cloned viral genes of two strains (I and II) of the human T-cell leukemia virus (HTLV) and are studying the detailed genetic structure of each.
- B. The HTLV-II has thus far been associated with only one case of chronic T-cell leukemia. A molecular survey of other leukemia case is underway to try to determine the specific disease spectrum associated with this virus.
- C. The HTLV-I genome has been identified in two patients' lymphocytes out of 135 specimens examined from individuals with the acquired immune deficiency syndrome. Molecular clones of one of these isolates have been made and a detailed characterization is underway to identify differences between this strain and the HTLV-I isolates from leukemic individuals.
- D. The HTLV gene hybridizes to endogenous sequences in human DNA. In addition, there are antigenic relationships between various cell surface proteins and HTLV structural proteins. We are cloning and characterizing human endogenous sequences homologous to HTLV to study their expression and significance.

Other:	Marc Lippmann	Senior Investigator	M	NCI
	Robert Gallo	Chief	LTCB	NCI
	Jacqueline Wang-Peng	Senior Investigator	M	NCI
	Neal Young	Senior Investigator	CHB	MLBI
	W. Clifford Lane	Senior Investigator	I	NIAID

- E. Using various techniques to enrich for populations of T lymphocytes from AIDS patients, we are trying to identify new individuals who may have active retrovirus infection.
- F. We are exploring the possibility that a human parvovirus (one of which can cause anemia in children) may be infecting AIDS patients.
- G. Human cytomegalovirus (HCMV) is highly associated with AIDS and Kaposi sarcoma. We have been studying nucleic acid homology between the myc onc gene and a genomic fragment of HCMV that is able to transform cells in culture after DNA-mediated gene transfer.
- H. The transforming potential of cancer associated dominant genes (oncogenes or tumor genes) has been explored by DNA-mediated gene transfer. We have been particularly interested in chemotherapy and radiation induced malignancies as a source for mutated sequences as which may have transforming potential. One gene has been isolated from an alkylator-induced acute myelogenous leukemia.
- I. Human onc genes are involved in some chromosomal translocations characteristic of specific malignancies such as Burkitt lymphoma. We have cloned both the translocation point and the reciprocal translocation site from the chromosomes 8 and 14 of a patient with Burkitt lymphoma and are studying the genetic structural changes induced by the translocation. By in situ chromosomal hybridization we are mapping onc gene translocations in other cases of Burkitt lymphoma.

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October 1, 1982 to September 31, 1983

ANNUAL REPORT OF THE NCI-NAVY MEDICAL ONCOLOGY BRANCH
OF THE DIVISION OF CANCER TREATMENT, NATIONAL CANCER INSTITUTE

1. Program Introduction

1.1 Overall Objectives

The research projects presented in this report represent the investigative efforts of the NCI-Navy Medical Oncology Branch of the Division of Cancer Treatment (DCT), National Cancer Institute. The research projects involve both clinical and laboratory investigation. The clinical projects are for the most part collaborative efforts with other NCI Clinical Oncology Program (COP) branches. The overall objective of the NCI-Navy Branch is thus the performance of clinical and laboratory research related to the treatment, diagnosis, staging, natural history, cellular and molecular biology of human tumors. Because of its location in the Naval Hospital, Bethesda (Naval Medical Command, National Capital Region) it has a unique opportunity for conducting clinical investigation on a variety of different tumor types. However, with this opportunity goes the responsibility of providing the best possible medical care for patients with cancer seen at the Naval Hospital and integrating the goals of the COP/NCI with those consistent with smooth function of the Naval Hospital. In addition, with the new directive of the Director, DCT, and Director, NCI, to integrate the clinical treatment studies of the adult medical oncology branches, the clinical research protocols require coordination with the other adult oncology treatment branches of the COP/DCT.

1.2 Primary Areas of Current Research

The primary areas of research at present include: 1) studies of the biology and treatment of lung cancer; 2) studies of the biology and treatment of adult T cell lymphomas; 3) studies of the biology, differentiation, genetics and treatment of B cell lymphomas; 4) treatment trials of all stages of breast cancer; 5) treatment trials of testicular cancer; 6) phase I, II trials of new cancer drugs and biologic response modifiers; 7) development, characterization, preclinical, and subsequently clinical trials of monoclonal antibodies, particularly those against lung cancer and T cell lymphomas; 8) molecular genetic and cytogenetic studies of cellular oncogenes and other genes related to tumor differentiation, growth, and drug/radiation resistance; 9) miscellaneous clinical trials, clinicopathologic correlations, staging studies, and studies of treatment toxicity. The clinical treatment studies are all in collaboration with one of several clinical branches of the COP.

1.3 History of the NCI-Navy Medical Oncology Branch

The NCI-Navy Medical Oncology Branch was derived from and took the place of the NCI-VA Medical Oncology Branch. The NCI-VA Medical Oncology Branch was started at the Washington VA Medical Center in 1971. The current senior staff of the NCI-Navy Branch came together in July 1975 at the NCI-VA Branch and worked at the Washington VA Medical Center until July 1981. The last site visit of the NCI-VA Branch was in July 1978.

With the construction of a new hospital building at the National Naval Medical Center, Bethesda, MD, negotiations began between the Director, DCT/NCI, and later the Director, NCI, with the Commanding Officer Bethesda Naval Medical Center to establish an interagency agreement between the NCI and the Naval Medical Center that would allow transfer of the NCI-VA Branch to the Bethesda Naval Medical Center. A general memorandum of understanding was signed among the Surgeon General, USN, Surgeon General, USPHS, Director, NIH, and Director, NCI, to develop such a relationship in 1979.

The Chief of the NCI-VA Branch began working on an interagency agreement with the military coordinator for the Bethesda Naval Hematology Oncology Program. This was approved in principle by the CO Naval Medical Center and the Surgeon General, USN, as well as by the Director, DCT, and Director, NCI. This led to: 1) the commitment of NCI funds for renovation of "temporary" (Tower Building 1) and "permanent" (Building 8) space for the offices, laboratories, and outpatient facility at the Naval Medical Center; 2) establishment of an oncology-hematology inpatient ward (6 West) in the new Naval Hospital Building; 3) transfer of NCI-VA personnel for clinical work to the Naval Medical Center in July 1981; 4) with completion of the temporary renovation of clinic and laboratory space in Building 1, transfer of NCI-VA personnel to the Naval Medical for laboratory research in April 1982. With the final transfer of all NCI-VA personnel, the NCI-VA Medical Oncology Branch became the NCI-Navy Medical Oncology Branch.

The relocation of the NCI-VA in Washington, D.C. to the NCI-Navy location in Bethesda, MD, across the street from the NIH permitted two features not previously possible. The first was an integration of clinical treatment protocols between the NCI programs at the Clinical Center, NIH, with the NCI-Navy program and the potential availability of Naval Hospital patients. The second was the recruitment of an excellent molecular genetics group. The first was made possible by the geography and the patient population seen at the Naval Medical Center. The second was also made possible by the geography and facilitated by the movement of many key personnel from the Laboratory of Molecular Genetics (LMG), National Institute of Child Health, to the Harvard Department of Genetics. Several key personnel from the old LMG were thus recruited to stay or return to the NCI-Navy Branch. Besides the physical relocation of the NCI-Navy Branch, the following problems have developed and have been resolved or are in the process of resolution. 1) There has been a series of changes in commanding officers (Admiral Horgan, Admiral Crews, Captain Admiral Crews, Captain Quinn and, in July, Captain Johnson) of the Bethesda Naval Hospital and changes in the Surgeon General and Bureau of Medicine (BUMED) USN. These have led to a series of reviews of the NCI-Navy Interagency Agreement after the NCI thought the agreement was approved. The longest of these required approval by the Policy Review Board of the BUMED which lasted over one year with final sign off in October 1982 after multiple revisions of the agreement. 2) Relocation of patients on NCI-VA protocols to the NCI-Navy Branch at the Naval Hospital. 3) Development of proper working relations with the Department of Medicine and Medical House Staff, Nursing, Pharmacy, Laboratory, Pathology Services, as well as working relationships with various internal medicine subspecialties, Surgery, and Radiotherapy Services. 5) Submission and approval of already approved NCI protocols

through the Institutional Review Board (IRB), Committee for the Protection of Human Subjects (CPHS), and Health Training and Education Command BUMED (HSETSC) as well as learning about the appropriate modes of dealing with the Navy review process. 6) Development of procedures for proper running of laboratories, offices, and clinics in accord with Naval Medical Center Policy. 7) Recruitment of clinical care support staff including nurses, secretaries, clinical lab technician, and pharmacist. 8) Development of final plans for renovation of Building 8 permanent space.

1.4 NCI-Goals for the NCI-Navy Medical Oncology Branch Program

A. Using methods consonant with optimal patient care to conduct clinical investigation and clinical treatment trials of patients with cancer using protocols developed by or in conjunction with the NCI-Navy Medical Oncology Branch.

B. To conduct laboratory investigation of tumor cell biology and matters pertaining to new methods for the diagnosis, treatment, and prevention of cancer, and on matters of fundamental biologic importance.

C. By virtue of points A and B above, to establish a clinical treatment program for human cancer at the Bethesda Naval Hospital of the first rank and through this program to provide a service to the Naval Hospital and to train Fellows and housestaff in the care of cancer patients.

1.5 Naval Hospital Goals for the Program

A. To provide the highest quality of health care for cancer patients eligible for treatment at the center in a manner consistent with the overall mission of the Bethesda Naval Medical Center.

B. Through use of Clinical Research Protocols to provide intelligent, consistent, and monitored cancer treatment.

1.6 Summary of Sections composing the NCI-Navy Medical Oncology Branch

In 1981, the Branch was organized into the Office of the Chief and four sections.

A. Office of the Chief: Dr. John D. Minna, Chief, NCI-Navy Medical Oncology Branch.

This office functions to coordinate administrative and overall scientific action of the Branch and to serve as the nodal point for interaction with Naval Hospital authorities including the Oncology-Hematology Branch, Department of Medicine, and Navy Administration as well as the COP administrative structure, other clinical branches within the COP, and other laboratory research efforts at NIH.

B. Clinical Investigations Section: Dr. Daniel C. Ihde, Head

This section coordinates all intramural COP and NCI-Navy protocols for scientific and ethical review; plans, develops, and coordinates the data management system for clinical investigations; coordinates collaborative interactions with other branches in the COP and other organizational components in the DCT, NCI; has responsibility for organizing and monitoring of clinical excellence of staff physicians, clinical fellows, outpatient nurses and clinical support staff.

C. Cell Kinetics Section: Dr. Paul A. Bunn, Jr., Head

This section conducts research on the cell kinetics of normal and malignant cells; plans and directs laboratory, preclinical, and clinical studies of biologic response modifiers such as monoclonal antibodies and interferon; plans and directs studies on the factors regulating and required for the growth and differentiation of malignant human lymphomas; as a service to the Branch supervises and administers the operation of a multiparameter cell sorter, along with coordinating the Branch's use of the instrument in clinical and laboratory investigations.

D. Human Tumor Cell Biology Section: Dr. Adi F. Gazdar, Head

This section uses the tools of cell biology and pathology to study the growth and differentiation of human solid tumors with a special emphasis on human lung cancer. Human tumor cell models including cultured cell lines, tumor cell cloning assays, nude mouse heterotransplants are established and then used to study: the expression and regulation of differentiated functions; the selective expression of certain fetal determinants; the embryologic origin of human tumors; the nature of tumor stem cells; the drug and radiation sensitivity and resistance patterns of human tumors including determination of these for use in clinical trials; and the occurrence and mechanisms of clonal heterogeneity of human tumors. Expertise in anatomic pathology for clinical trials and in immunohistochemistry is also provided. As a service to the Branch, this Head also acts as Deputy Chief of the Branch for administering laboratory affairs and supervises the maintenance and use of the Branch's rodent colony and nude mouse facility.

E. Genetics, Molecular Biology, and Immunology Section: Dr. John D. Minna, Head

This section is responsible for investigating the genetics of human tumors through the use of somatic cell, molecular genetics, and cytogenetic techniques and for the development of new monoclonal antibody reagents for studies and treatment of human cancer. There is a particular emphasis on lung cancer and B cell lymphomas. These laboratory studies involve close interactions with the other Sections and focus on: the identification of genes coding for or suppressing malignancy and those involved in differentiation, including cellular oncogenes; hormones and growth factors regulating the growth of tumor cells; isolation and characterization of genes coding for drug and radiation resistance.

As a service for the rest of the Branch the monoclonal antibody production facility and molecular genetics technical resources are located in this section for use by the entire Branch.

F. Brief Explanation of Overall Branch Emphasis

The major emphasis of the NCI-Navy Branch is to integrate clinical and laboratory research to study the biology and etiology of human tumors and develop new methods of prevention, diagnosis, staging, and treatment of human malignancy. The location of a human tumor cell biology program and the technology of molecular genetics and monoclonal antibodies together with the clinical program provide a unique opportunity to achieve at least some of these goals.

Each Senior Investigator of this Branch has been encouraged to pursue his primary research interests, be it in the clinic or laboratory. All participate in the development of clinical research protocols and each has accepted various clinical service, clinical investigation, or scientific teaching and administrative responsibilities. For example, where each clinical protocol has principal investigators, all the senior medical oncologists during the four months a year when they are "on service" in either the outpatient clinic, consult service, or 6 West inpatient service, supervise the conduct of the clinical protocols as well as the clinical care of the patients with cancer at the Naval Hospital. The board certified pathologists not only review the pathologic materials on all patients entering NCI trials but also provide service functions to the Naval Hospital in anatomic pathology. The Senior laboratory staff not only perform their own research but teach a variety of molecular genetic techniques to fellows in training in the lab as well as to other staff and integrate these new methodologies into the overall research program of the Branch.

The types of clinical and laboratory research projects studied by this Branch are summarized in the following section. While the research interests of the various senior investigators are unique, they are interdependent on many levels. For example, all of the clinical protocols (which often are chaired by other senior investigators) are coordinated out of the Clinical Investigations Section and in fact some of the major clinical-laboratory interactions (such as the drug sensitivity testing) are actually chaired by the chief of this section. The Human Tumor Cell Biology Section provides resources and cell lines for use of the branch as a whole and, in turn, relies on monoclonal antibodies and molecular genetic techniques for studying these materials and the Clinical Investigation Section for designing trials to obtain tumor tissue. The Cell Kinetic Section provides cell sorter assays and new sources of tumor cells to work with in vitro, and also relies on monoclonal antibodies and molecular genetics to study these materials. The Genetics, Molecular Biology, and Immunology Section provides new reagents and assays and relies on the other sections to provide interesting biologically relevant cell lines to work on.

1.7 Administrative Structure and Clinical Integrations

The NCI-Navy Medical Oncology Branch is an intramural part of the Clinical Oncology Program (COP), Division of Cancer Treatment (DCT), National Cancer Institute (Table 1). Together the NCI-Navy and the Naval Hospital Hematology-Oncology Branches make up the Oncology Hematology "Program" for the Bethesda Naval Hospital (NHBETH). This Oncology-Hematology Program is part of the Department of Medicine of the Naval Hospital. The civilian coordinator for the program (Dr. Minna) is chief of the NCI-Navy Branch while the military coordinator for the program (Dr. Veach) is head of the Naval Hospital's Hematology Oncology Branch. However, for all day to day clinical operations the two branches function as one. For administrative affairs Dr. Minna has responsibility for reporting back through NCI channels while Dr. Veach has responsibility for reporting through Naval Hospital Channels as shown in Table 2. As a practical matter, the Administrator (Ms. M.A. Anerino) detailed by the Office of the Director, DCT, to work full time at the NCI-Navy branch handles many of the administrative affairs with various Naval Hospital offices (e.g., for civilian personnel, construction, public works, administration). For medical affairs both report to the Chief of Medicine.

While the administrative structure outside the program is by necessity complex, the day to day clinical workings of the program are straightforward. There is a senior medical staff composed of four NCI medical oncologists (Drs. Minna, Ihde, Bunn, and Carney), two NCI pathologists (Drs. Matthews and Gazdar) one of whom (Dr. Gazdar) is also Deputy Chief for Laboratory Administration, one Navy oncologist (Dr. Veach) and two Navy hematologists (Dr. Phares and Dr. Dainer, also boarded in oncology). (Dr. Phares also serves as the head of Hematology and the administrative director of the outpatient clinic.)

There are weekly senior staff meetings where matters of general importance are discussed and decisions made as a group. There is one outpatient clinic and one inpatient service. A yearly attending schedule is made consisting at any one time of a senior inpatient (6 West) attending physician, and an outpatient clinic-general hospital inpatient consult service attending. This is rotated among the senior staff. Currently there are five NCI medical oncology fellows (all first year) and two Navy Oncology and two Navy Hematology fellows (2 first year, 2 second year). All clinical conferences (e.g., tumor board, pathology, combined modality planning) and lectures are handled as for one department. Each of the approximately 1,100 patients are divided among the fellows and senior staff. Thus, each patient has a primary physician responsible for his or her care, with supervision of the fellows by the senior staff attending physicians. The major decisions concerning initiation of treatment, entrance onto protocols, major changes in a patient's course (e.g., tumor progression or regression), major therapy toxicities, or unusual clinical features are discussed at one of the major clinical conferences by the group as a whole. Thus, the clinical care program is integrated in terms of oncology and hematology, and in terms of civilian and military senior staff and oncology fellow physicians.

Hematology Service and Functions

The Hematology Section is an integral part of the Hematology/Oncology Division of the Department of Internal Medicine, Naval Hospital, Bethesda, sharing clinical spaces, utilizing common facilities and resources and having a staff which shares responsibilities in both Hematology and Oncology.

The mission of the Hematology Service is to provide in and out patient consultative services for patients with a wide variety of hematologic disorders, provide ongoing primary care for patients with hematologic disorders, provide fellowship training in hematology and to provide hematology training for Internal Medicine residents and students. The service is also dedicated to the support of and participation in ongoing research and clinical trials, in close liaison, cooperation and coordination with the oncology service of the division and the NCI-Navy Medical Oncology Branch of the NCI.

Currently, the staff assigned to the Hematology Branch consists of two individuals who alternate as attendings for the Hematology Clinic and inpatient Hematology consultative services, serving both functions simultaneously. The staff at times, when not attending on the hematology service, serve in rotation with the oncology staff members as attending on the oncology outpatient, consultative and inpatient services.

The Navy sponsors fellowship training programs at the Naval Hospital, Bethesda, offering training positions in Hematology and Oncology, either individually for two years or as a combination for three years. In recent years the majority of military sponsored fellows have chosen the three years combined program. Currently, there are four Navy sponsored trainees, two at the first and second level in the combined program. Two fellows currently serve on the hematology service with one each at the first and second year level. The second year fellow in the previous academic year trained on the oncology service and the first year fellow will in the next academic year serve on the oncology service.

The Hematology Outpatient Clinic is part of the combined Hematology/Oncology Clinic, sharing spaces, personnel, resources and facilities with the Oncology Services.

The Hematology Service maintains no inpatient beds and serves the inpatient population primarily in a consultative setting. A number of inpatients, predominantly those with hematologic malignancies, require extensive support and specialized care as is available on the oncology inpatient ward. To provide for the optimal care for these patients and insure appropriate exposure and training for fellows, this type of patient frequently is admitted to the oncology ward with the hematology fellow following the patient in close coordination with the ward staff, including attendance at ward rounds. The fellow is responsible to the ward attending as well as to the hematology staff.

Research protocols and projects are equally available to the Hematology and Oncology Services and no distinction between services is made in the

support, application or utilization of available studies and projects with the Hematology Service, actively supporting ongoing research.

Both the Hematology and Oncology sections have established teaching and working conferences. Individuals from both disciplines are encouraged to and do regularly attend these conferences without distinction to their being hematology or oncology.

In summary, the Hematology Service works in close liaison with the Oncology Service of the division sharing staff and other personnel, facilities, resources and participation in research protocols and projects.

1.8 Oncology - Hematology Fellowship Program

The NCI-Navy Oncology Clinical Associate Medical Oncology training program is that of the COP fellowship program. Currently, the NCI fellows spend six months at the Naval Hospital and then rotate and spend six months at the Clinical Center, NIH, during their first year. During this time the patients cared for by the first set of fellows are rotated to the oncoming fellows. The Navy Oncology Fellows do the same thing if they rotate to the Clinical Center. During the second and third years the NCI fellows work in specific laboratory or clinical research within the DCT, attend clinical conferences and follow selected patients.

The Navy fellowship program is a three year oncology-hematology fellowship devoted to patient care. The rotation of the Navy fellows to the NCI clinical center is an individual decision made by them and the Military Coordinator for the program.

1.9 Pathology

Two of the senior staff physicians, Drs. Matthews and Gazdar, of the NCI-Navy Branch are Board Certified in Pathology. Dr. Matthews, a reference

pathologist for the VA Surgical Adjuvant Trials, Lung Tumor Study Group Trials, NCI Long Term Lung Cancer Survivor Registry, and WHO, serves as the reference pathologist for NCI-Navy patients entering onto NCI trials. Her office is located in the Division of Anatomic Pathology of the Naval Hospital Laboratory Service where she fulfills a role as a senior staff pathologist. Dr. Gazdar, while having major laboratory research and administrative responsibilities in the NCI-Navy Branch, also does reference pathology work for the NCI trials and some clinical work for the Naval Medical Center. These two pathologists provide the Oncology-Hematology Program with a strong link to Anatomic Pathology. This is important not only to coordinate clinical pathologic review between the Naval Hospital and the Clinical Center for patients on NCI trials, but also to provide a source of tumor materials for the cell biologic studies in the laboratory. In fact, Dr. Worsham, head of the Naval Hospital's Anatomic Pathology, and Dr. Gazdar are Co-Principal Investigators on the tumor procurement protocol.

Another strong link is the location of the bone marrow procedure room, staining facilities, and hematopathology review room in the Oncology-Hematology

Programs Outpatient Clinic Area. Dr. H. Schumacher, the Chief of Hematopathology and his staff, and Dr. Garvin, the Assistant Chief of the Laboratory Service, who also has a great interest in hematopathology, thus perform their pathologic reviews on site in the outpatient clinic. In addition, Dr. Garvin and Dr. Matthews together have coordinated review of all patients entering onto NCI lymphoma protocols with Dr. Jaffe in the Pathology Branch at the Clinical Center, NIH.

1.10 Radiotherapy

The major decisions about starting Oncology-Hematology patients on radiotherapy or radiotherapy chemotherapy are made at the Friday morning Combined Modality Treatment Planning Conference. This conference is attended by the combined senior staffs of the Oncology Hematology Program, the senior staff Radiotherapists of the Naval Hospital (Dr. Whittington and Herst), Dr. E. Glatstein, Dr. Hancock, Dr. Findlay, and Dr. Lichter of the Radiation Oncology Branch (ROB) and all of the NCI-Navy, and Navy Hematology-Oncology program fellows as well as the ROB fellows. This conference thus serves as a major focus of cancer treatment planning. Currently, it is the best clinical cancer conference the NCI is involved in.

The radiotherapy of patients treated at the Naval Hospital may be performed either at the Naval Hospital or at the Clinical Center, NIH, by the Radiation Oncology Branch, NCI, depending on individual patient, and protocol (e.g., Small Cell Lung Cancer) requirements. Currently, the majority of patients are treated at the Naval Hospital by Dr. R. Whittington, Dr. Herst or one of the ROB fellows in training assigned to the Naval Hospital. Dr. E. Glatstein, Chief of the NCI ROB, is also a professor of Radiotherapy at the Uniformed Services University of the Health Sciences (USUHS) and Chairman of the Radiotherapy Training Program, not only for the NCI but also for USUHS. Patients where special treatment planning or equipment require the use of NCI facilities are treated at the Clinical Center, ROB, facility.

At present there are only two radiotherapists left in the Navy, namely, Drs. Whittington and Herst, and both of these will be leaving the Navy within the next 18 months. Because of this, the Radiotherapy/Radiology Departments of the Naval Hospital and the Commanding Officer of the Naval Hospital have approached Dr. Glatstein to provide senior staff and supervise radiotherapy for the Naval Hospital. At present this plan, which would become part of the NCI's Interagency Agreement with the Naval Hospital, is being developed for consideration and necessary approval by the Director, DCT, Director, NCI and the Naval Hospital Commanding Officer. As an interim measure, Dr. Alan Lichter, Chief of the ROB's Radiation Therapy Section, will provide backup coverage to Dr. Whittington's Department at the Naval Hospital. To facilitate this, the NCI-Navy Branch will provide laboratory and office space for Dr. Lichter. Because of Dr. Lichter's interest in delivery of radiotherapy via monoclonal antibodies and the NCI-Navy Branch's interest in monoclonal antibodies, there are a variety of laboratory collaborations and then clinical studies that will naturally develop. If this type of Radiotherapy interaction between the ROB and the Navy can be arranged, it would be of great benefit to the Naval Hospital, NCI, ROB, and the NCI-Navy Branch.

1.11 Surgery

Currently, nearly all the surgery on cancer patients at the Naval Hospital is performed by the Naval Hospital Surgeons for head and neck cancer, gynecologic oncology, and chest thoracic surgery. To date, the participation of the Oncology-Hematology Program staff and fellows in these has been very limited. This should, however, change in two specific areas: Gynecologic Oncology at the Navy Hospital is extremely strong with Dr. W. Hoskins, the head of this division. Dr. Hoskins has his own GYN oncology fellowship training program and, combined with the Walter Reed Army Medical Center, a strong clinical research connection with the Gynecologic Oncology Group (GOG). Thus, during the first two years the NCI-Navy group has not been involved in GYN Oncology. However, at Dr. Hoskins' invitation the Oncology Program is starting to see difficult and interesting cases in consultation with the GYN Oncology and to provide backup chemotherapy support as needed. In addition, because of Dr. Lichter's interest in GYN radiotherapy, the radiotherapy of these malignancies should be benefited if he works at the Naval Hospital.

There have been several changes of leadership of Thoracic Surgery at the Naval Hospital so it has been difficult to develop a strong working relationship with the Navy thoracic surgeons. Until this settles down at the Naval Hospital, the NCI-Navy Branch has taken three approaches: 1) the development of the tissue procurement protocol with the head of Anatomic Pathology; 2) the development of close ties with the Pulmonary Service (Dr. T. Walsh, Chief); and 3) development of ties with Dr. J. Roth, Head of Thoracic Surgery for the Surgery Branch, Clinical Center, NIH. Thus, currently the plan is to have any investigative thoracic surgical procedures performed by Dr. Roth at the Clinical Center and to try to dovetail in with the new thoracic surgeons at the Naval Hospital, for all standard procedures.

1.12 Other Naval Hospital Resources

The Naval Hospital has a large number of resources which make it attractive to the NCI-Navy Program. The relations that have developed with the Division of Medicine are very good, including close communication and contact with the Chief of Medicine, participation in "Morning Report", and the interaction with the internal medical housestaff on the 6 West Oncology-Hematology Inpatient Service. Currently there are three interns and one first year resident on the 6W floor full time. Two medical oncology fellows are assigned at a time to make rounds and work with the medical housestaff, in addition to having one of the full time Oncology Hematology program senior staff serve as the senior attending physician. Other strengths within the Division of Medicine include the Pulmonary Branch, the Infectious Disease Branch, the Renal Branch, and the Cardiology Service.

The Naval Hospital has a joint Medical - Surgical Intensive Care Unit (ICU) (Dr. T. Rainey, Chief), as well as post operative recovery rooms, overnight stay post op rooms, and a Coronary Care Unit. The Oncology Hematology Program patients requiring care in one of these units (most commonly the ICU) have

received excellent care provided by the full time attending staff and specially training nurse and supporting staff.

Other Divisions within the hospital besides those already mentioned which have provided superb help to the Oncology Hematology Program patients include: Dermatology, Rehabilitative Medicine, Dental Service, Social Service, and the Dietary Service.

1.13 Nursing Service

The 6 West inpatient nursing service is composed of civilian personnel on NCI billets and Navy and civilian personnel on Naval Hospital billets. The daily supervision of the inpatient nurses is under a head nurse assigned to the ward (currently LCDR R. Frayley), and nursing supervisor (currently CDR K. Ryan). The inpatient nurses thus work under and within the Bethesda Naval Hospital Nursing System. The Oncology-Hematology Program Staff have worked with the inpatient nurses to train them for work on an oncology-hematology ward. This includes chemotherapy and supportive care for patients with cancer at many disease stages and after receiving complex chemotherapy and combined modality regimens. The current plan of the Division of Medicine and endorsed by the Commanding Officer is to increase the bed capacity from 20 to 30 beds as originally planned in the first approved version of the Inter-agency Agreement. This expansion requires the staffing with additional nursing billets. Currently there are 16 staff nursing billets (8 Navy and 8 NCI) assigned to 6 West although all the NCI billets are not yet filled because of problems of nursing recruitment in the National Capital area. The NCI-Navy Branch is requesting five additional NCI nursing billets for the 10 bed expansion.

The outpatient nursing in the Oncology-Hematology Clinic consists of three full-time and one part-time NCI-Navy nurses, and one Navy civilian nurse. These nurses function in the care and treatment (chemotherapy delivery) of the patients as well as in data monitoring and collection. They are under the supervision of the Oncology-Hematology Program.

1.14 Pharmacy

With the exception of GYN oncology, nearly all the chemotherapy administered at the Naval Hospital is under the supervision of the Oncology-Hematology Program. The NCI-Navy Branch provides a full time outpatient pharmacist for the preparation, monitoring, and inventory of chemotherapy drugs administered by the Oncology Hematology Outpatient Clinic. This represents a great advantage to the delivery of quality care and the conduct of clinical treatment trials. The pharmacy services prepares the drugs administered to the in-patients. In addition, the NCI provides all the chemotherapy drugs to be used at the Naval Medical Center.

1.15 Patient Population and Census

The patients treated by the Oncology Hematology Program include active duty military, retired military, and various dependents, as well as small

numbers of other government employees entitled to care at the Naval Hospital (e.g., FBI, USPHA, diplomats). In addition, as part of the Interagency Agreement, a special category (Secretary of the Navy Designees, or SECNAV) exists for treatment of civilian patients on NCI protocols. Currently, the NCI-Navy Branch restricts this latter category to patients with small cell lung cancer and adult T cell lymphomas, both primary research interests of the NCI-Navy Branch representing about 10% of all patients treated.

The patient population requiring cancer treatment at the Bethesda Naval Medical Center and the potential ability to recruit these patients into NCI trials is the major reason for the Interagency Agreement. Summaries of currently available patient statistics are given in Tables 3 & 4. The Bethesda Naval Hospital is the second largest cancer referral center in the military hospital system (after Walter Reed Army Medical Center), and the oncology hematology patient load of over 1,200 active patients, most of whom require regular follow-up, is large. The wide spectrum of tumors both common and rare, as well as the number of patients eligible to enter ongoing or prospective NCI protocols, are important assets. While the number of patients entered onto NCI protocols is still small this is explained by two facts. First, for Navy approved NCI protocols pending the final review of the BUMED of the Interagency Agreement, the Health Training and Education Command of the Navy ruled that no patients could be entered onto NCI protocols. Second, there has been an extremely long delay in getting some protocols approved by the Naval Hospital. A series of meetings were held with the Commanding Officer and the Chairpersons of the various approval committees. While part of this delay represented the NCI-Navy Branch learning the Navy system, and several significant changes in the format required by the Navy (e.g., in the informed consents), the most significant reason for the delay represented staffing problems in the local IRB of the Naval Hospital. The Commanding Officer determined that the Hospital would supply an administrative Naval Officer (and has done so) if the NCI would provide a secretarial billet to work on this IRB. This billet has been requested by the NCI-Navy Branch from the DCT. The IRB and the Commanding Officer have determined that with the appropriate support a two month turn around time for protocols with no major scientific criticism could be obtained.

1.16 Statistics and Data Management of the Clinical Trials

The statistical input in terms of design, monitoring and evaluations of the Oncology Hematology Program trials is carried out by the Biometric Research Branch of the COP. Currently, Dr. Robert Makuch has done this job in an excellent fashion. He also serves as the interface for statistical interactions with other COP Branches involved in collaborative clinical trials. Major changes are taking place in the whole COP computerized data management system. As part of the new data management system being developed by Dr. Makuch for the NCI-Navy Branch, will be a computerized patient census, tracking, and follow-up system for the patients under its care. This should aid in the best use of the patient assets as well as the data collection for protocol study.

1.17 Collaborative Clinical Trial Interactions with other NCI Branches

Since Dr. E. Glatstein's arrival in 1977 to head the ROB, nearly all trials conducted by the NCI-VA and now NCI-Navy Branch have been developed and performed in collaboration with the ROB of the NCI. One Phase I trial was conducted with the Clinical Pharmacology and Medicine Branches. A major decision has been recently made by the Director, DCT, and Director, NCI, in the conduct of clinical trials in adult medical oncology; namely, that these trials should be in collaboration rather than in competition. This was particularly brought into focus with the creation of the NCI-Navy Branch because of the large number of patients eligible for entry onto NCI protocols developed originally by other NCI branches (e.g., lymphomas, Hodgkin's disease, testicular cancer, and breast cancer). To aid in this, a large number of existing protocols of the Medicine Branch, after appropriate revision by the NCI-Navy Branch, were submitted for approval at the Naval Hospital. (See Table 3 for a list of clinical treatment protocols and their origin and Table 6 for the NHBETH Protocol Approval flow chart.) A decision was made, strongly reinforced by the desires of the Navy Oncologists-Hematologists, that Navy patients on NCI protocols be treated at the Naval Hospital rather than being sent to the Clinical Center, except where unique resources were available. Every attempt has been made by the Oncology Hematology Program staff to identify and enter patients onto these NCI protocols.

The NCI-Navy Branch fully supports the plan for an integrated clinical research treatment program of the COP to maximize patient and other resources in the conduct of NCI clinical trials. However, it is clear that close cooperation in all areas of clinical trials including: design, patient recruitment, staging, pathology review, onstudy forms, delivery of treatment, follow-up, staging conferences, data collection and monitoring, and statistical analysis, preparation of data for presentation and publication, and fair representation of authorship will have to be developed and carried out. This is currently being instituted with combined medical oncology staff meetings of the NCI-Navy, Medicine, and Radiation Oncology Branches, as well as some joint staging conferences. However, much needs to be done and this represents a major problem area for the NCI-Navy Branch and the COP treatment studies as a whole.

1.18 Molecular Genetics Group

Because of the dramatic advances in the technology and results of recombinant DNA techniques in analyzing eukaryotic cells including tumor cells, it was felt highly desirable, if not imperative, to establish this expertise in the NCI-Navy Branch. In fact, the clinical, cell biologic, and molecular genetic approaches are complementary: the former provide interesting systems of biologic relevance to study while the latter provides clear answers not available by other methods.

Several approaches were possible including collaborating with outside units (which will be done), having one or two trained personnel, or establishing a small molecular genetics group. The latter approach was selected for the following reasons: the molecular genetic approach needs a critical mass; the

technology is rapidly advancing and several persons are required to develop expertise in many areas to provide all this technology; if people are working on different but related projects in this area their results can synergistically cross feed one another. Finally, one would want people of the highest quality and such a group would be required to attract the best people. During the past year a unique event occurred at the NIH; namely, one of the major recombinant DNA labs, that of Dr. P. Leder, moved from its prior location (Laboratory of Molecular Genetics (LMG) in the Child Health Institute) to become a part of the Department of Genetics at Harvard. There were several outstanding young investigators and technical personnel in this group who had worked very well together for several years who were interested in either staying at or returning in the near future to NIH. After a series of discussions with these people the following was worked out: four investigators, most of them young, were interested in coming. This included Dr. Michael Kuehl (M.D.) (formerly Professor of Microbiology at the University of Virginia, on Sabbatical in the LMG), Dr. Ilan Kirsch (M.D., Ph.D.) (Research Associate, who was interested in completing training in Pediatric Oncology at the NCI), Dr. Gregory Hollis (Ph.D.) and Dr. James Battey (M.D., Ph.D.) who went with Dr. Leder to Boston to work for 9 months and 18 months, respectively. In addition, Ms. Marion Nau (GS-11) was a highly skilled professional level technician who had played a significant part in the LMG in Bethesda and wished to stay at the NIH. All of these individuals came with different backgrounds to the LMG lab and all demonstrated excellence in molecular genetics during their tenure in Dr. Leder's lab. With the exception of Dr. Kuehl who came from a tenured position, and established reputation as an independent investigator, the other professionals were as yet untested as to their ability to perform independent research. The plan worked out was to have them come as a group. The young investigators would each get lab space, budgetary support, a post doctoral fellow to work with them, and encouragement to establish their own independent research projects in molecular genetics. Many of these appeared to be in the area of tumor cell biology, and many appear to be related to the ongoing human tumor cell biology program at the NCI-Navy Branch. The three young investigators would be considered to be in "tenure track" positions and their performance at the NIH could thus be systematically evaluated for later conversion. Rather than providing technical staff, it was considered by the group that it was important to have a continuing source of young post doctoral fellows to keep up with the rapidly developing field. By the same token this approach would provide the most flexibility to the NCI in evaluating the program for continued support or curtailment. All of the group come with strong recommendations from Dr. Leder and their current interests nicely interrelate: Dr. Kuehl with the biology and differentiation of B cells; Dr. Kirsch molecular cytogenetics; Dr. Hollis molecular genetics of processed genes and oncogenes; Dr. Battey molecular genetics of oncogenes and immunoglobulin genes. All but Dr. Battey are currently on a NCI-Navy Branch billet; however, the group will only finally come together in July, 1983, with Dr. Battey planning to arrive in October-November 1983. In preparation for this, Dr. Minna, Ms. Nau and Dr. Kuehl have been setting up the laboratory and beginning preliminary experiments related to lung cancer (Dr. Minna) and B lymphocytes (Dr. Kuehl). The first one to two years of the group will be involved in completing current projects, establishing technology in a variety of areas, teaching the molecular geneticists about human tumor

cell biologic problems, the biologists about molecular genetics, and performing pilot experiments. Thus, the immediate "future plans" are not as detailed as some of the other research projects with more prior work. In addition, with completion of the "permanent" laboratory space in Building 8 of the Naval Hospital the group has already assisted in the redesign of this space, and will work towards the equipping and establishment of the space in working conditions when it is ready in November, 1984.

1.19 Space Renovation

The layouts of the "temporary" (Building 1) and "permanent" (Building 8) space at the Naval Hospital for the offices, labs, and outpatient clinic of the Oncology Hematology Program are provided in Table 7/a-f. The renovation of these spaces has proceeded as follows and had to be integrated into the overall plan for the retrofit of the Naval Hospital.

1. The inpatient unit was moved into the new Naval Hospital Building 10, ward 6 West as of July, 1981. This required no renovation or changes.

2. The Outpatient Clinic was moved first (July 1981) to temporary structure later demolished while the fourth and fifth floors of the old hospital (Building 1) were partially renovated including cleaning, painting, carpets, ceilings, and some wall changes, new electrical outlets, and some new lab benches installed. Basically, the outpatient clinic moved into the area previously occupied by the Radiology Service and the Delivery Rooms, while the laboratory portion moved into the old laboratory service facilities. When this was completed (in April 1982) the Clinic and the NCI-VA Laboratory (which had been working at a separate geographic location in its prior space at the Washington VA Medical Center in the District of Columbia, from July 1981 to April 1982), moved into the Naval Hospital, Building 1 space. The renovation of the "permanent" space in Building 8 then began which involved considerable internal demolition and wall changes, power, utilities and ventilation changes. All of this was handled by a separate interagency construction agreement funded by the NCI and contract construction supervised by the Navy as part of the larger Bethesda Naval Hospital retrofit. The design and layout of the buildings were carried out by the firm of Dalton, Dalton, and Newport under contract to the Navy for the retrofit. This was monitored from July 1982 by the NCI's Construction Management Branch as well as the NCI Contract Management Branch. With the planning beginning in 1979, and final occupancy in 1984, changes in the layout and equipping of the labs occurred of necessity, particularly with the development of the molecular genetics group. This latter group designed the biochemical/molecular genetic research portion of the lab. Currently, the first phase of the permanent renovation is completed. A contract is being let for the second phase which will include running utilities, and installing laboratory bench space and equipment for final beneficial occupancy anticipated for November 1984. This should provide the NCI-Navy Branch, and the Oncology Hematology Program as a whole, with an excellent, integrated, office, outpatient clinic, and research laboratory facility.

1.20 Resources

A. Budget. The budget allocated by the Clinical Oncology Program to the NCI-Navy Medical Oncology Branch for both intramural and extramural expenses for the past six years are shown in Table 8a. This table also shows the breakdown of intramural funds versus the unique Interagency funds that have been utilized for Branch operations. A summary of intramural, extramural (Interagency) and Special Ambulatory Care Program (SACP) costs for the NCI-NMOB are shown in Table 8b. A summary of extramural support contracts for the entire Clinical Oncology Program is illustrated in Table 8c. Table 9 shows the specific costs associated to the FY 1983 Interagency Agreement with the Naval Hospital, Bethesda. In order to display the costs per investigator and per project, Tables 10a, 10b, and 10c were developed. These tables also provide a breakdown of the direct cost operating budget for the Branch. A comparison of the NCI-Navy Medical Oncology Branch budget to the other Clinical Oncology Program Branch budgets is presented in Table 11. The following is an explanation of the categorical and direct cost calculations for the Budget tables.

1. TRAVEL

a. Category Description: Travel reflects requirements for employees (i.e. Clinical Associates, Civil Service and Medical Staff Fellows) joining and leaving the Branch; and travel for scientific meetings, training purposes, and program travel (e.g., laboratory visits, lectures, and consultations).

b. Direct Cost Calculation: Travel direct costs are calculated by taking the total obligated in Object Code 21 less 25 percent which is considered as associated indirect costs.

2. SUPPLIES AND SERVICES

a. Category Description: Direct costs for supplies and services includes all of object code 26 (supplies) and sub-object code 23.44 only. Direct costs, therefore, include chemicals, drugs, reagents, enzymes, other laboratory supplies, clinical supplies (e.g. tissue culture flasks, tubes, pipets, scintillation vials, etc.), subscriptions for professional journals, and the charges for the NIH Scientific Equipment Rental Program. Indirect costs for supplies and services include rental charges for equipment, copying machines, word processors, and related office equipment, GPO reprinting of annual reports, reprints of journal articles, and the printing of various clinical and office forms. Indirect costs also include professional service contracts, IPA salaries, laboratory tests provided by non-government sources in support of the basic research laboratories (e.g., the Equipment Rental Program. Indirect costs for supplies and services preparation of tissue culture reagents), equipment maintenance contracts, photographic services, word processors, and renovations.

b. Direct Cost Calculation: To calculate the supplies and services direct costs take the total spent in Object code 26 only less 4 percent, and then add the total obligation in Sub-Object code 23.44.

3. EQUIPMENT

a. Category Description: Direct cost for equipment include the purchase of scientific equipment only. Indirect costs include the purchase of office equipment, books, and computer hardware.

b. Direct Cost Calculation: To calculate equipment direct costs take the totals spent in sub-object codes 31.2H, 31.51, 31.9H, 31.9T and 31.9X only.

4. SALARY AND WAGES

a. Category Description: This covers all of object classes 1100 and 1200 and include the salary and wages of all employees regardless of type of appointment (civil service, commissioned corps, visiting program, temporary part time, etc.).

b. Direct Cost Calculation: The total of OC 11 and 12 less 25% for associated fringe benefits that are normally considered as "indirect costs" in the private sector.

5. TOTAL DIRECT COSTS

Total the direct costs calculated for travel, supplies and services and equipment salaries as described above.

6. TOTAL INDIRECT COSTS

This total is derived by taking the total obligated for all object codes minus the direct cost total obtained above. No single indirect cost rate is provided since it varies between branches and from year to year. The various percentages mentioned in the calculations above were derived from analysis of comparing the Federal accounting system to that of the private sector and non-profit institutions.

B. Personnel. The NCI-Navy Medical Oncology Branch currently has 54 full-time equivalent slots, allocated by the Division of Cancer Treatment. Table 12a provides a breakdown of these positions by section within the Branch. The personnel assigned to the NCI-Navy are also shown by position category in Table 12b. The individuals assigned by the Navy to the NCI-Navy Hematology/ Oncology Program are shown in Table 12c. Organizational charts for the NCI and Navy are in Table 13a & 13b, respectively. A comparative staffing profile of the entire Clinical Oncology Program is presented in Table 14.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 CM 03024-14 NMOB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical Trials and Other Clinical Investigations		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Dr. Daniel C. Ihde, Chief, Clinical Investigations Section, NCI-NMOB		
COOPERATING UNITS (if any) See attached sheet		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Clinical Investigations Section		
INSTITUTE AND LOCATION NCI-Navy Medical Oncology Branch National Naval Hospital, Bethesda, Maryland		
TOTAL MANYEARS: 55	PROFESSIONAL: 15	OTHER: 40
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The NCI-Navy Medical Oncology Branch studies new methods of evaluating and treating patients with malignant disease and provides general medical oncology consultations for the National Naval Medical Center. Clinical investigations are carried out in patients with small cell lung cancer and other types of lung cancer (epidermoid, large cell, and adenocarcinoma), mycosis fungoides and the Sezary syndrome, lymphomas, breast and testicular cancer, and multiple myeloma and other plasma cell dyscrasias. New Phase I and Phase II agents, both chemotherapeutic and immunotherapeutic, are studied. Other interests involve general medical oncology and miscellaneous cancers. Within each disease category, investigations are centered in one or more of the following areas: 1) therapeutic trials and complications of treatment; 2) staging procedures, prognostic factors, and natural history; 3) clinical cell biologic correlations; 4) review articles. Some 30 oncology consultations per month are seen in the NNMC and outpatient care (200 visits/week) provided for patients requiring chemotherapy who are not eligible for any protocol studies.		

Senior Staff Oncologists, PI:

Z01 CM 03024-14 NMOB

John D. Minna, M.D.	Chief	NCI-NAVY MOB NCI/NHBETH (USPHS)
Paul A. Bunn, M.D.		NCI-NAVY MOB NCI/NHBETH (USPHS)
Daniel C. Ihde, M.D.	(Deputy Chief, Clinical)	NCI-NAVY MOB NCI/NHBETH (USPHS)
Desmond N. Carney, M.D.		NCI-NAVY MOB NCI/NHBETH

Affiliated Senior Staff Oncologists (Hematology/Oncology Branch, NHBETH)

CDR Stephen R. Veach, M.D.
 CDR John C. Phares, M.D.
 CDR Paul U. Dainer, M.D.

Senior Staff Other, PI:

Adi F. Gazdar, M.D.	Deputy Chief (Lab) Pathologist	NCI-Navy MOB NCI/NHBETH
Mary J. Matthews, M.D.	Pathologist	NCI-Navy MOB NCI/NHBETH

Clinical Associates and Medical Staff Fellows:Third Year

Cameron Little, M.D.		NCI-Navy MOB NCI/NHBETH
Jeffrey Ochs, M.D.		NCI-Navy MOB NCI/NHBETH
James Mulshine, M.D.		NCI-Navy MOB NCI/NHBETH (USPHS)
Martin Brower, M.D.		NCI-Navy MOB NCI/NHBETH (USPHS)

Second Year

Gerald Batist, M.D.		NCI-Navy MOB NCI
Austin Doyle, M.D.		NCI-Navy MOB NCI/NHBETH
Richard Knop, M.D.		NCI-Navy MOB NCI
George Morstyn, M.D.		NCI-Navy MOB NCI
Charles Winkler, M.D.		NCI-Navy MOB NCI/NHBETH
LCDR Jeffrey Crane, M.D.		Heme/Onc Branch/NHBETH
CDR George Savides, M.D.		Heme/Onc Branch/NHBETH

First Year

Marcia Browne, M.D.		NCI-Navy MOB NCI/NHBETH
Bruce Johnson, M.D.		NCI-Navy MOB NCI/NHBETH
Andrew Kraft, M.D.		NCI-Navy MOB NCI/NHBETH
Neal Rosen, M.D.		NCI-Navy MOB NCI/NHBETH
Edward Sausville, M.D.		NCI-Navy MOB NCI/NHBETH
Carmen Allegra, M.D.		NCI-Navy MOB NCI/NHBETH
Steven Averbuch, M.D.		NCI-Navy MOB NCI/NHBETH
Nancy Davidson, M.D.		NCI-Navy MOB NCI/NHBETH
Claude Harmon, M.D.		NCI-Navy MOB NCI/NHBETH
Richard Sorace, M.D.		NCI-Navy MOB NCI/NHBETH
Mark Browning, M.D.		Heme/Onc Branch/NHBETH
Vincent Shen, M.D.		Heme/Onc Branch/NHBETH

Other Full-Time NCI-Navy MOB:

Joyce Eddy, RN	Clinical Research Nurse	NCI-Navy MOB NCI/NHBETH
Mercedes Gilliom, RN	Clinical Research Nurse	NCI-Navy MOB NCI/NHBETH
Susan Schwartz, RN	Clinical Research Nurse	NCI-Navy MOB NCI/NHBETH
Maria Poblet, RN	Clinical Nurse	NCI-Navy MOB NCI/NHBETH
Delphine Knop, R.Ph.	Clinic Pharmacist	NCI-Navy MOB NCI/NHBETH

Cooperating UnitsNHBETH

LCDR Richard Whittington, M.D.	Chief, Radiation Oncology Service
CAPT Kevin O'Connell, M.D.	Chief, Urology Service
CDR Fred Worsham, M.D.	Pathology Service
CAPT David Garvin, M.D.	Pathology Service
CAPT Thomas Walsh, M.D.	Pulmonary Branch

Cooperating UnitsNational Institutes of Health

S. Broder, M.D.	Assoc. Director for COP	DCT/NCI
R. Fisher, M.D.	Senior Investigator	MB/DCT/NCI
E. Glatstein, M.D.	Chief	ROB/DCT/NCI
E. Jaffe, M.D.	Senior Pathologist	LP/NCI
T. Kinsella, M.D.	Senior Investigator	ROB/DCT/NCI
A. Lichter, M.D.	Senior Investigator	ROB/DCT/NCI
M. Lippman, M.D.	Senior Investigator	MB/DCT/NCI
D. Longo, M.D.	Senior Investigator	MB/DCT/NCI
R. Makuch, M.D.	Statistician	BRB/DCT/NCI
K. McIntire, M.D.	Senior Scientist	LID/DCBD/NCI
R. Oldham, M.D.	Assoc. Director for BRMP	DCT/NCI
T. Waldman, M.D.	Chief	MET/DCBD/NCI
J. Whang-Peng, M.D.	Senior Staff (Cytogenetics)	MB/DCT/NCI
R. Young, M.D.	Chief	MB/DCT/NCI
M. Zweig, M.D.	Assistant Chief, Clinical Laboratories	CC/NIH

Cooperating Units, Other:

F. Hirsch, M.D.	Finsen Institute, Copenhagen, Denmark
L. Napoli, M.D.	Radiologist, Providence Hospital
I. Royston, M.D.	University California, San Diego
R. Yesner, M.D.	Yale University, New Haven, Connecticut
J. Aisner, M.D.	University of Maryland Cancer Center, Baltimore, Maryland

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Title: CLINICAL STUDIES IN SMALL CELL CARCINOMA OF THE LUNG

Personnel

A. Permanent Senior Staff: D.C. Ihde, P.A. Bunn, Jr., D.N. Carney, J.D. Minna, S.R. Veach, A.F. Gazdar, M.J. Matthews

B. Collaborating Senior Staff: E.J. Glatstein (ROB), A.S. Lichter (ROB), K. Cowan (MB), R.W. Makuch (BRB), R. Whittington (Radiotherapy, NHBETH), T. Walsh (Pulmonary Sect., NHBETH), N.R. Dunnick (Diagnost. Radiol. NIH), J. Aisner (UMCC), J. Becker (Psychiatry Dept., NHBETH), H.H. Hansen (Finsen Inst.), M.H. Cohen (Oncology Sect., WVAMC), T. Shields (VASOG), P.A. Marangos (Clin. Psych. NIMH), A.B. Deisseroth (POB), G.P. Schechter (Hematology Sect., WVAMC), B.J.L. Sauerbrunn (Nuclear Med. Sect., WVAMC), M.H. Zweig (Clin. Chem., NIH)

Collaborating Branches

Radiation Oncology, Pediatric Oncology, Biometric Research and Medicine Branches (COP, NCI); Radiotherapy, Pulmonary, and Psychiatry Divisions (NHBETH); Hematology, Oncology, and Nuclear Medicine Divisions (Washington VAMC); Diagnostic Radiology and Clinical Chemistry (Clinical Center, NIH); Finsen Institute (Copenhagen, Denmark); Veterans Administration Surgical Oncology Group; University of Maryland Cancer Center; Clinical Psychobiology Branch (NIMH)

I. Staging and prognostic studies and clinicopathologic correlations

Several benefits can be derived from careful determination of the extent of tumor dissemination prior to therapy for neoplastic disease. The choice of treatment may be affected, with localized therapies generally being reserved for patients with localized disease. An estimate of prognosis can be made. From observations on sites of tumor relapse, particularly from complete remission, deductions concerning the relative efficacy of the treatment for localized and systemic disease can be made. Finally, knowledge of patient characteristics allows comparison of different forms of therapy employed in clinical trials. In small cell lung cancer, it is not yet clear whether stage-specific therapy, most importantly chest irradiation in patients with limited disease, leads to major survival benefits. Since with current therapeutic philosophies all patients will require combination chemotherapy, the staging process becomes less important in terms of therapy selection. However, careful staging studies, analyses of prognostic factors, and clinicopathologic correlations have greatly increased our understanding of the clinical behavior of this usually fatal neoplasm.

1. Prognostic factors

We conducted a multivariate statistical analysis of prognostic factors in 106 consecutive patients with small cell lung cancer (SCLC) treated with aggressive induction chemotherapy with cyclophosphamide, methotrexate, and CCNU (1). Minimum follow-up was three years, in essence making this a "completed" analysis. Disease stage as traditionally defined (limited versus extensive) did not significantly influence survival. Survival was associated with the number of organ systems involved with metastatic disease, however,

with substantially poorer survival in patients with three or more sites involved. Other factors with major survival impact were performance status and liver metastases. Patients with more sites of distant metastases were significantly more likely to have poor performance status and liver metastases. Involvement of bone and distant soft tissue sites had little adverse effect. In carefully staged patients given aggressive combination chemotherapy, we concluded that certain sites or a small number of sites of extensive disease can be treated almost as successfully as limited stage SCLC. It should be noted that fewer than 15% of these patients received chest irradiation.

Performance status is the dominant prognostic factor in most reported trials of therapy in SCLC, yet it can be quite subjective and is sometimes difficult if not impossible to determine in retrospective chart review. We found that the prognostic information contributed by performance status in 56 of our SCLC patients could be accounted for by knowledge of the serum albumin and hemoglobin levels prior to therapy (2). A more objective prognostic index based on simple laboratory values, if confirmed, would be very useful in analysis of SCLC data.

Defective immune status as assessed by skin testing for delayed hypersensitivity has been found to correlate with prognosis in SCLC patients treated surgically, but it is unclear whether this factor is independent of disease stage. Little information exists with regard to patients treated with chemotherapy. We studied the association of skin test reactivity with other prognostic factors and survival in 154 consecutive SCLC patients given aggressive combination chemotherapy (3). One hundred twenty-one reacted to at least one of five test antigens, and 33 were anergic. Non-anergic patients lived significantly longer than anergic ones, and there was a significant trend for reactive patients to have good performance status and more limited tumor burden. Skin test reactivity was not of independent prognostic value except in good performance status, limited stage patients.

2. Staging of intrathoracic tumor

We have previously shown that fiberoptic bronchoscopy can detect residual tumor in up to one-third of chemotherapy-treated patients in apparent complete response on chest X-ray. Even with bronchoscopy, however, particularly in patients receiving chest irradiation, determination of complete response can often be somewhat arbitrary. We are currently prospectively investigating whether chest CT scans obtained at diagnosis and at the time of response are of value in determining whether a patient is in complete remission. We have also attempted to grow tumor colonies from bronchoscopic washings in a soft agar assay of clonogenicity. Although significantly more colonies were present in cytologically positive washings than in cytologically negative ones, the number of colonies was too small to allow drug sensitivity testing (4).

3. Bone metastases

Radionuclide bone scans are included in the staging evaluation in many SCLC treatment protocols, but detailed information on their value has been scanty. We scanned 119 systematically staged patients before and during aggressive combination chemotherapy (5). Scans were positive in 41%; positivity correlated with the presence of bone marrow metastases, positive skeletal

radiographs, and elevated serum alkaline phosphatase. Non-osseous sites of distant metastases were significantly more likely to be detected as the number of focal abnormalities on bone scan increased. Patients with limited disease, extensive disease confined to bone, and extensive disease confined to non-bony sites had insignificantly different survival, while patients with bony and non-bony extensive sites of disease fared substantially worse. Of 36 patients with initially positive bone scans and tumor regression documented by other means, scan findings improved at the time of response in 67%. Abnormal bone scans in SCLC that are not explained by non-malignant osseous disease provide reasonably accurate staging and prognostic information in SCLC, and are probably due to metastatic tumor in the great majority of cases.

4. Intraabdominal metastases

Retroperitoneal structures such as pancreas, adrenals, and lymph nodes are involved with SCLC at autopsy in approximately 50% of patients, but evaluation of these areas during initial staging has been infrequent. We performed abdominal computed tomography (ACT) on 77 previously untreated SCLC patients. Mass lesions thought to be due to tumor were seen in 34%, with abnormalities confined to the liver in 15 patients, confined to the retroperitoneum in eight, and present in both areas in three. Most positive studies were in patients already known to have extensive disease. Only three of 29 patients otherwise staged as having limited disease had evidence of abdominal metastases on ACT. In 71 patients with pathologic confirmation of liver status, ACT had similar sensitivity and specificity to radionuclide liver scan in assessing the liver. During combination chemotherapy, ACT lesions improved or disappeared in 11/12 patients whose tumor response was documented by other methods. In only 3/46 patients, however, did ACT provide therapeutically relevant information not discernible from other staging tests (6). We conclude that ACT can demonstrate metastatic dissemination of SCLC not detectable by other standard staging tests, but has little value in the routine management of SCLC.

5. Central nervous system metastases

Background and Completed Studies: Early studies from the NCI-VA Medical Oncology Branch demonstrated that central nervous system (CNS) metastases were more frequent in SCLC than in non-small cell lung cancers. Subsequent studies from our group established that about 10% of SCLC patients present with CNS metastases, that the frequency of CNS metastases increased dramatically with lengthening survival to 80% for 2-year survivors, that 65% of autopsied patients had CNS metastases, that leptomeningeal (7) and spinal metastases were common (though not as frequent as intracranial metastases), that radionuclide brain scans and lumbar punctures were not sufficiently sensitive to detect asymptomatic CNS disease, and that chemotherapy did not reduce the rate of CNS relapse. While primary CNS relapses occurred, they were not common. Therapeutic cranial irradiation was quite effective in controlling CNS relapses, particularly if detected early. While therapy with cranial irradiation and intrathecal (or intraventricular) methotrexate seemed most efficacious for leptomeningeal tumor, it provided effective long-term palliation in a minority of patients (7).

Other groups reported a reduction in CNS relapse rate when prophylactic cranial irradiation was employed. A large review by Bunn and Ihde showed a reduction in CNS relapse rate from an average of 22% to an average of 8%. Similarly, 5 of 7 randomized trials showed a significant reduction in CNS relapses. A compilation of these randomized studies shows a similar reduction from 22% to 6%. None of these trials demonstrated an effect on overall or median survival. Long term survival was not analyzed nor were the results analyzed with respect to the effects of systemic therapy. Our recently reported review (8) of our own experience with PCI also demonstrated a significant reduction in CNS relapse rate from PCI. The effect on relapse and survival was most striking for patients in complete response from systemic therapy. The 2-year CNS relapse rate was reduced from 52% to 25% in this group ($p = .06$) and 2-year survival increased from 16% to 38% ($p = .18$). There were fewer CNS relapses in partial responders given PCI, but there was a continuing high relapse rate for patients surviving more than 6 months. An international consensus panel concluded that PCI need not be given to partial responders, but should be considered in complete responders pending results of randomized trials.

Future Plans: One small randomized trial recently reported that PCI reduced CNS relapses in complete responders, but did not influence survival. Several recent trials have suggested some late toxicities from PCI which are particularly worrisome given similar studies in acute leukemia. In our own experience in a small number of long-term survivors, minor but not major neurologic deficits and brain CT scan abnormalities were found in approximately half the patients whether or not they received PCI (9). Thus, in collaboration with the Finsen Institute, we are initiating a randomized study of the value and toxicity of PCI for complete responders.

Our recent experience has also shown that computer assisted tomography (CT scans) of the cranium are more sensitive than radionuclide scans for detecting symptomatic intracranial metastases. However, CT scans were insufficiently sensitive for routine use in screening asymptomatic patients. Preliminary studies of cerebrospinal fluid from patients with intracranial and leptomeningeal metastases have demonstrated elevated levels of several marker substances including bombesin, neuron specific enolase, and calcitonin. These studies will be continued and expanded to routine CSF analysis.

A recent review has also shown that cranial irradiation in doses up to 4000 rad generally provides symptomatic relief for our patients with brain metastases. However, there is a high relapse rate reaching 100% at 10 months for patients with long survival. Since this group consists almost exclusively of patients with solitary CNS metastases at diagnosis or at relapse from remission, we are exploring the routine use of higher doses of radiotherapy (5100 rad/3 weeks) for these patients.

6. Liver metastases

The liver is involved at autopsy in approximately 75% of cases of SCLC, and in several groups' experience a poor prognosis is conveyed by hepatic metastases at diagnosis. The work of others has played down the value of radionuclide liver scan and liver function tests (LFT's) as methods of assessing liver involvement. We compared the results of multiple pretreatment staging

procedures including liver scans and LFT's with the results of staging liver biopsy in 118 patients with SCLC. Percutaneous liver biopsy was positive in 15% of patients, and multiple biopsies at peritoneoscopy were positive for tumor in 12%, most of whom had had negative percutaneous biopsies. Overall, liver biopsies were positive in 24% of patients, in 6% as the sole site of distant metastatic disease. When compared with biopsy data, the sensitivity and specificity (82% and 92%) of radionuclide liver scan were markedly superior to LFT's and physical examination of the liver. A combination of liver scan and LFT's allowed segregation of two groups of patients with extremely low (less than 5%) and high (over 90%) likelihood of pathologic liver involvement. Without liver biopsy, the liver was incorrectly staged in 13% of patients by reliance on scan alone, but only 3% of patients were incorrectly scored as limited or extensive stage disease. If liver status must be known for therapeutic or other reasons in SCLC, liver scan and LFT's are quite accurate in assessing involvement when both are positive or both are negative, but biopsy is required in other patients (approximately 60% of the total) with discordant scan and LFT results. Peritoneoscopic biopsies are the most sensitive method of establishing the presence of metastases.

7. Clinicopathologic correlations

The World Health Organization classification divides SCLC into lymphocyte-like (oat cell), intermediate, and combined (SCLC plus other cell types of lung cancer) subtypes, but conflicting data exists as to the clinical and biologic relevance of this subclassification. Dr. Mary Matthews of our Branch participated with two other experienced lung cancer pathologists in a blinded review of microscopic slides from 93 patients thought to have SCLC by one of the pathologists. Agreement among the three panelists that the diagnosis was SCLC rather than non-small cell carcinoma was obtained in 91% of cases. Agreement as to subtype, however, could be obtained in only 54% by the current WHO classification and 38% by an older WHO subgrouping (10). This suggests that SCLC subtyping does not appear reproducible, and is consistent with our previous experience that subtype (of lymphocyte-like versus intermediate) does not correlate with clinical characteristics or prognosis in SCLC patients given combination chemotherapy. Furthermore, we have found that multiple SCLC subtypes are present at the time of diagnosis in 29% of patients and have never observed the lymphocyte-like or oat cell subtype in any of our SCLC cell lines or nude mouse tumors. Currently, proposed subtypes of "pure" SCLC probably do not reflect clinically or biologically meaningful differences and may be related to artifacts of specimen preparation.

In contrast to the lack of clinically relevant differences between lymphocyte-like and intermediate subtypes of SCLC, we have found the combined subtype of small cell-large cell carcinoma to be present in 6-12% of SCLC patients at diagnosis and to have negative prognostic implications (11). Among 122 previously untreated SCLC patients given equivalent aggressive chemotherapy programs, 19 (12%) had a significant component of large cell carcinoma in their diagnostic biopsy specimens, and were designated mixed small cell/large cell (SC/LC) carcinoma. These patients were prospectively identified over a 5-1/2 year period by a single pathologist (Dr. Matthews). SC/LC patients were comparable to the 103 "pure" small cell patients in terms of performance status and extent of disease, but complete (16%) and overall (58%) response rates and median survival (6 months) were significantly inferior to results

obtained in the "pure" SCLC patients. One SC/LC patient attained long-term disease-free survival, however. We conclude that SC/LC patients should be treated aggressively, but with expectations of inferior results compared to "pure" SCLC patients, and should therefore be segregated when results are reported. It is conceivable that some reports of responsiveness of large cell carcinoma of the lung to chemotherapy include SC/LC patients. At the Finsen Institute, another pathologist reviewing different patient material has also found that SC/LC patients are less responsive to current treatment regimens for small cell lung cancer.

There are occasional reports in the literature of tumors that appear pathologically identical to SCLC apparently arising in extrapulmonary sites. There is little information concerning whether these tumors possess the same responsiveness to chemotherapy as do their pulmonary counterparts. Over a 6-year period, eight (4%) of 203 consecutive prospectively staged and treated patients with small cell carcinoma had no evidence of pulmonary or mediastinal tumor on chest X-ray, tomography or CT scan of the thorax (in 5 cases), and fiberoptic bronchoscopy (12). In four cases, possible primary tumors were present in the skin and soft tissues, esophagus, and salivary gland. In the other four, only involved lymph nodes or widespread metastatic disease was present. One complete and two partial responses to chemotherapy occurred in six evaluable patients. Two other patients treated with locoregional therapy and adjuvant chemotherapy are currently disease-free at 15 and 28 months. At postmortem examinations in five patients, a possible primary pulmonary tumor was found in only one. We conclude that extrapulmonary small cell carcinoma includes at least two clinicopathologic entities and can be responsive to chemotherapy. Initial systemic treatment should be encouraged.

Dr. Matthews has provided pathologic review for studies of the Veterans Administration Surgical Oncology Group (VASOG). In a recent report (13) of 132 surgically resected SCLC patients with all pathologic material reviewed by Dr. Matthews, pathologic TNM stage was shown to have a major impact on survival. Actuarial 5-year survival estimates were 60% for T1N0M0 patients and 30% for remaining Stage I and Stage II patients. Approximately half the patients received modest doses of postoperative adjuvant chemotherapy, but this did not appear to influence survival with one possible exception. This study firmly establishes that certain patients with SCLC can probably be cured by surgical resection alone (although this represents only a tiny fraction of all SCLC patients), and encourages revision of current thoracic surgical dogma that SCLC should not be subjected to surgical exploration. In another recently reported VASOG study, post-resection adjuvant chemotherapy with CCNU and hydroxyurea was found to be without benefit in terms of survival or delayed disease recurrence (14). There was a suggestion of benefit ($p = 0.16$) in patients with SCLC, but this was statistically insignificant, possibly due to small patient numbers. It is of interest that a marginally active drug (CCNU) and a drug for which no information on anti-tumor activity exists in SCLC (hydroxyurea) could possibly have demonstrated therapeutic effects in an adjuvant situation. These results encourage the use of more appropriate chemotherapy post-surgery in resected SCLC. The results of both of these studies have added validity with central pathologic review, in view of the difficulties some pathologists still have in distinguishing SCLC from other cell types of lung cancer.

8. Biomarkers in SCLC

With the exception of gestational and germ cell carcinomas, the value of serum biomarkers in actual disease management has been marginal in most cancers. We have recently examined the relationship of serum levels to disease stage and disease status in serial serum samples before and during aggressive therapy of patients with SCLC. The markers selected for assay were neuron-specific enolase (NSE) and creatine kinase-BB (CK-BB). They were chosen because both are secreted by virtually all SCLC cell lines in long-term culture in significantly higher amounts than can be detected with cell lines of non-small cell lung cancer. Serum NSE was elevated in 69% of 94 patients, including 39% of those patients with limited and 87% of with extensive stage disease (15). No independent prognostic information with regard to survival was contributed by NSE levels, however. During therapy, NSE levels seemed to parallel disease status in most patients, but knowledge of the marker level rarely provided therapeutically relevant information that could not be deduced from our routine staging studies.

Similar results were found in a comparable study of serum levels of CK-BB in 106 patients. Only 2% of patients with limited disease had elevated CK-BB levels in pre-treatment serum, while the marker was elevated in 40% of extensive stage patients. Marker levels appeared to be closely related to tumor burden, since 22% of patients with one, 40% with two, 75% with three, and 100% with four or more sites of extensive disease had abnormal CK-BB values. In serial samples, most responding and progressing patients had falling and rising CK-BB values, but once again the marker provided little clinically useful information beyond that which was apparent from our routine staging studies.

9. Long-term survivors with SCLC

Three years ago, we identified 20 of 167 patients (12%) with SCLC entered on therapeutic trials at the National Cancer Institute who had survived at least 2-1/2 years free of disease from the initiation of treatment. Since that time, two patients have died of second malignancies (stomach cancer in one patient and acute erythroleukemia and epidermoid lung cancer in a second), and five have pathologically documented relapses of SCLC. Continued relapse from complete remission for at least five years was predicted by our group in the past, based on observations of relatively long tumor doubling times on chest X-rays (median 77 days). We are currently in the process of recalling 5-year survivors for assessment of pulmonary status, neurologic examination, psychometric testing, and karyotypic examination of bone marrow.

II. Therapeutic Trial in Limited Stage Small Cell Lung Cancer

Background: Clinical studies during the 1960's established that few patients had stage I or II disease, that 5 year survival following surgery was < 1%, and that these poor results were due to early tumor dissemination. Radiation therapy was shown to provide longer median survival and a rare 5 year survivor when compared to surgery for potentially resectable cases in a randomized trial conducted by the British Medical Research Council. Early chemotherapy trials demonstrated significantly higher response rates in small cell than in non-small cell lung cancers. An early trial conducted by the VA cooperative group demonstrated the single agent cyclophosphamide more than

doubled the survival of patients with extensive stage when compared to placebo. Subsequent studies in the 1970's confirmed the superiority of combination chemotherapy over single agent or sequential single agent therapy.

Combined modality trials in limited stage SCLC in the early 1970's compared radiation therapy to radiation plus chemotherapy. Though most of these trials employed chemotherapy which would be considered inferior by today's standards, nearly all showed a positive effect for the chemotherapy. Non-randomized combined modality trials in the mid 1970's employing more intensive chemotherapy and higher doses of radiation gave results which were superior to the earlier combined trials with less intensive treatments. These trials reported median survivals exceeding one year (12-16 mos) and a significant minority of 2 year disease-free survivors. Yet, these trials also demonstrated increased toxicity from combined modality treatment, particularly to the esophagus, lungs, skin, heart and CNS.

Non-randomized trials employing intensive chemotherapy alone for patients with limited stage SCLC gave results which were similar to those in combined modality trials, particularly with respect to objective response rate, complete response rate and median survival. However, compilations of data from the literature by Salazar and Creech showed a lower local failure rate associated with a combined modality approach and the review of Bunn and Ihde showed a higher percentage of 2 year disease-free survivors in the combined modality trials. Thus, a major question was whether radiation added to intensive combination chemotherapy improved results over chemotherapy alone, especially since the combined modality approach appeared more toxic. An international consensus panel recently concluded that this was one of the major issues in limited stage SCLC.

Two early randomized trials showed no benefit for the combined approach when sequential chemotherapy with intermittent "sandwich" radiotherapy was employed. A non-randomized trial from the NCI suggested that the simultaneous use of chemotherapy and radiotherapy beginning on day 1 was superior to a sequential approach. Moreover, three weeks of simultaneous treatment, while associated with slightly lower local control rates than more protracted radiotherapy schedules, yielded acceptable toxicity with the highest 2 year survival rates. Data from this study as well as those of Cox's group suggested 4000 rad/15 fractions/3 weeks may be an optimal radiation dose when combined with chemotherapy.

The NCI-Navy Medical Oncology Branch had shown that a high dose 3-drug combination of cyclophosphamide + methotrexate + CCNU (CMC) was superior to lower doses of the same drugs. Unfortunately, cyclophosphamide doses in excess of 1500 mg/m² or more frequent drug administration seemed to add toxicity without improving results. Thus, our high dose CMC regimen which provided up to 10% 2-year disease-free survival in limited stage SCLC was chosen as the chemotherapy standard.

Objectives: To determine whether the simultaneous use of optimal chemotherapy plus optimal radiotherapy is superior to the use of chemotherapy alone in patients with limited stage SCLC. To compare the toxicity of the two regimens.

Progress to Date: The current randomized study compares CMC chemotherapy alone to CMC with simultaneous thoracic radiotherapy. The trial was instituted in 1979 and, to date, 67 patients have entered the trial. An interim analysis completed in November 1982 showed a potential benefit for the combined modality approach. The complete response rate was higher (81% versus 47%, $p = .013$); the median survival was longer (17 mo versus 12 mo); and the 2-year survival and disease-free survival rates were higher (39% versus 18%, and 32% versus 15%, respectively). Interim analyses also showed increased toxicity for the combined approach with respect to myelosuppression, esophagitis, and treatment-related deaths (5 versus 1). Recent studies reporting results of long-term complications, particularly employing combined modality therapy, have shown significant pulmonary compromise and 7 cases of acute non-lymphocytic leukemia (ANLL). It is too early to determine whether there are differences in these parameters in our treatment groups.

Future Plans: Interim statistical analyses have shown that there will be a significant survival difference between our groups if the present trends continue and additional 30-40 patients are entered. Thus, we plan to continue the trial for an additional 1-2 years to accrue these patients.

III. Therapeutic Trials in Extensive Stage Small Cell Lung Cancer (NCI-Navy Medical Oncology Branch)

1. Background and recently completed studies

Despite major advances in the chemotherapy of SCLC over the past decade, more patients with this form of lung cancer will eventually die of their tumor. The prognosis is especially grim for patients with disseminated or extensive disease, defined as tumor beyond the confines of one hemithorax, the mediastinum, and the supraclavicular nodes. With presently employed treatment, a large literature review completed in 1980 by Bunn and Ihde revealed overall objective response rates of 65-85%, complete response rates of 20-30%, expected median survival of 7-11 months, and 2-year disease-free survival of only 1-3% in extensive stage patients.

The dose-response curve of response to chemotherapy in SCLC is fairly steep, with improved response rates and survival when the doses of chemotherapy are raised from those producing minimal to modest, to those producing moderately severe toxicity. In an early prospective randomized trial from the NCI-VA Medical Oncology Branch, doubling the doses of cyclophosphamide, methotrexate, and CCNU (CMC) for the first six weeks of therapy resulted in doubled median survival (5 versus 10 months). Furthermore, complete responses were seen only with high dose treatment, and one patient on the high dose arm is alive free of disease after almost 10 years. Another recently reported randomized trial in much larger numbers of patients showed that doubling the cyclophosphamide dose to 1500 mg/m² in a modified CMC regimen led to improved survival in limited stage disease. We have increased the cyclophosphamide dose to even higher levels in the CMC regimen and have observed only increased toxicity without any apparent increased anti-tumor effect. We therefore initiated a trial in extensive stage SCLC using increased doses of drugs in a different active program which we termed CAPO.

Forty-nine consecutive previously untreated patients with extensive stage SCLC received cyclophosphamide 1000 mg/m², doxorubicin 50 mg/m², etoposide (VP-16) 125 mg/m², and vincristine 1.4 mg/m² as induction chemotherapy (16). Thirty-four patients were given high intensity therapy, receiving these drugs on both days 1 and 8 of two or three 21-day induction cycles. Because of excessive toxicity on the intensive programs, 15 other patients were treated with moderate intensity, receiving CAPO only on day 1 of two 21-day cycles. There were 94% complete or partial remissions (including 24% complete) among high intensity patients, while there were 73% complete or partial (including 20% complete) responses in the moderate intensity group. There was no marked tendency for higher response rates in the high intensity group ($p = 0.22$), and survival experience was very similar in the two groups, with median survival of approximately 12 months in each. The high intensity regimens were much more toxic, with 18% induction deaths compared to 7% in the moderate intensity patients ($p = 0.59$). Only two patients, both in the high intensity group, were alive and free of disease at 24 months, and one relapsed at 32 months. Increasing the intensity of induction chemotherapy with the drugs in CAPO did not significantly improve response or survival in our extensive stage SCLC patients.

Our results with the CAPO program and other results from Johns Hopkins and M. D. Anderson led us to conclude that increasing drug doses during initial chemotherapy with cyclophosphamide/doxorubicin based combinations beyond a certain point led only to increased toxicity without improved antineoplastic effects. In other words, the dose-response curve to induction chemotherapy in SCLC is steep, but appears to plateau at cyclophosphamide doses approximating 1000-1500 mg/m² every 3 weeks. Because of results in animal tumors and human acute myelogenous leukemia which suggested that very intensive therapy might be most efficacious in situations in which complete remission of clinically detectable tumor had already been induced, we initiated a trial of late intensive combined modality therapy (LICMRX) in extensive stage SCLC.

Twenty-nine consecutive patients of performance status 3 or better (ECOG-Zubrod scale) received 6 weeks of CMC induction chemotherapy, followed by 6 weeks of treatment with vincristine + doxorubicin + procarbazine. Autologous bone marrow was then collected in consenting good performance status patients in complete or partial response with negative bilateral bone marrow examinations. LICMRX consisted of 2000 rad/5 fractions/5 days to all sites of initial tumor except bone marrow, followed by cyclophosphamide 60 mg/kg IV qd x 2 + VP-16 200 mg/m² IV qd x 3. Autologous marrow was infused two days after completion of chemotherapy. Elective cranial irradiation and no maintenance chemotherapy were given thereafter. Due to lack of tumor regression, early disease progression, and poor performance status, only 10 patients (34%) were eligible for LICMRX. Two refused, so only eight received it. Three patients with complete response prior to LICMRX had recurrence of SCLC 4, 8, and 15 months after LICMRX was begun. Of five partial responders, one achieved complete response but recurred at 3 months, two remained in partial response and progressed at 2 and 4 months, and two died without recovery from LICMRX. One of the latter two had no evidence of tumor at autopsy.

The median time from autologous marrow infusion until recovery of granulocyte count to $500/\text{mm}^3$ was 18 days in the six survivors of LICMRX (range 12-22). There were three documented bacteremias, including one which proved fatal. The remaining toxic death was due to cardiac arrhythmia. Severe esophagitis, presumably due to the combination of chemotherapy and thoracic irradiation, occurred in seven patients and lasted a median of nine days (range 5-18). Among all 29 patients, complete response occurred in six (21%, five prior to or in patients not receiving LICMRX), partial response in 16 (55%), and no response in six (21%). There was one (3%) early death before response could be assessed. Complete response duration was 6-18 months from initiation of therapy in four patients who received LICMRX, and seven and 18 months in the two who did not. Median survival of all patients was 8 months, and there were no 2-year survivors. In 67 consecutive extensive stage SCLC patients we previously treated with high-dose CMC induction chemotherapy without LICMRX, 30% attained complete response and there were six (9%) 2-year survivors. Since 95% confidence limits for 2-year survival are 0-9.8% in the 29 patients entering this study, it is highly unlikely that the current treatment program will yield a substantial improvement in long-term survival in extensive stage SCLC.

Several hypotheses may be advanced to explain the poor therapeutic results of this treatment program. The initial 12 weeks of therapy very likely produced insufficient tumor cell kill for any putative increased sensitivity of lower tumor burden to late intensive treatment to become apparent. The LICMRX itself was relatively ineffective, as only two of five partial responders attained complete response, and one response was of evanescent duration. Finally, the patients may have been "contaminated" by viable tumor cells in the autologous bone marrow infusion, despite examination of aliquots from the collected marrow by multiple cytologic slides, measurement of DNA content by flow cytometry, and clonogenic assay on soft agarose. It must also be admitted that the autologous marrow may have been unnecessary for the majority of all of the patients to tolerate this program, since hematologic recovery occurred as early as 12 days from marrow infusion.

Since only three patients with complete response prior to LICMRX were treated, it is still possible that a modest degree of survival extension could be demonstrated in this favorable group if additional patients were subjected to LICMRX. Given the 25% toxic death rate, we were unwilling to continue the study since the duration of unmaintained remissions was unimpressive. Limited stage patients, with lesser tumor burden and a higher complete response rate to chemotherapy or chemoradiotherapy, may be better candidates for such an approach than are SCLC patients with extensive disease. However, we must conclude that LICMRX as given in this protocol can be administered to only a minority of extensive stage SCLC patients, and there is no indication thus far that impressive survival benefit for the entire patient population will result.

2. Planned therapeutic study in extensive stage small cell lung cancer

Most programs which have investigated the efficacy of very intensive induction chemotherapy in SCLC have utilized high doses of only two drugs--cyclophosphamide and, to a lesser extent, doxorubicin. Higher than standard doses of other agents have not been studied in any detail. Etoposide (VP-16)

and cisplatin are two agents which are highly synergistic in the treatment of murine leukemia. There is little experience with this combination in SCLC, but VP-16 is known to be active in pre-treated and very active in previously untreated patients. Studies principally conducted in patients failing other chemotherapy suggest only modest activity at best for cisplatin. Preliminary information on the VP-16/cisplatin combination in SCLC appears to indicate that this combination has much greater activity than would be anticipated from the single agent data. In two studies in which this combination was given for the first six or 12 weeks of therapy, overall response rates of 88-95% and complete remission rates of 40-55% were reported. Follow-up is too short to determine whether major benefits in terms of survival have occurred. In patients failing other chemotherapy, others have found that VP-16/cisplatin appears to yield higher response rates (40% versus 20%) than VP-16 alone.

Additional data suggests that the study of higher doses of VP-16/cisplatin might be of value in SCLC. Cisplatin in doses of 120 mg/m² produces responses in testicular cancer patients progressing on lower doses of the drug. Cisplatin 200 mg/m² at the Medicine Branch (NCI) has produced responses in ovarian cancer patients failing cisplatin-containing regimens. Finally, a combination of cisplatin 200 mg/m² + VP-16 500 mg/m², along with vinblastine and bleomycin, appears to have major activity (complete response rate 86%) in poor-risk germ cell cancer in a collaborative study of the Medicine Branch and our group. Administration of cisplatin in this dose has been associated with little or no nephrotoxicity when the drug is prepared in hypertonic saline and given in a setting of hydration with 250 ml/hr normal saline over a 5-day period. We therefore plan to compare the activity of high dose versus standard dose VP-16/cisplatin as induction chemotherapy for extensive stage SCLC. Patients at higher risk of toxicity during induction chemotherapy will receive standard doses of the two-drug combination. After the first six weeks of treatment, the most critical time of therapy in SCLC, all patients will receive standard doses of the drugs for six more weeks.

This study will also attempt to collect prospective information concerning the ability to obtain in vitro drug sensitivity information from SCLC cell lines established from the patient prior to therapy, and the efficacy of a chemotherapy regimen constructed on the basis of in vitro drug sensitivity testing in producing new complete remissions in patients in partial or no response after 12 weeks of VP-16/cisplatin. We believe that considerable experience in our laboratory in the establishment of malignant tumor cell lines from patients with SCLC makes this study feasible.

In our experience, only 23% of tumor biopsies cloned directly into soft agar will yield sufficient SCLC colonies to allow testing of in vitro drug sensitivity of even one drug. This data is similar to that obtained by many other groups studying many different tumor types. Therefore our group has concentrated on attempting to establish cell lines from patient biopsies. With the aid of a serum-free defined medium termed HITES (hydrocortisone, insulin, transferrin, estradiol, and selenium) which suppresses the growth of stromal cells but not small cells, cell lines for drug testing of a large number of drugs can be established from 70-80% of positive biopsies. Furthermore, retrospective analysis indicates that in vitro drug sensitivity of cell lines and of tumor biopsies subjected to direct agarose cloning yield equivalent

correlations with in vivo tumor response in the patient from whom the in vitro data was derived. In both instances, in vitro sensitivity is associated with in vivo response 75-90% of the time, and in vitro resistance is associated with in vivo resistance 100% of the time.

In this protocol, the details of which are given in the clinical protocol book which is appended to the Site Visit package, patients will undergo biopsies of bone marrow, effusions, and peripheral nodal and soft tissue masses. If these are negative for tumor, mediastinal exploration--either by mediastinoscopy or parasternal incision--will be performed in order to obtain tumor tissue for cell culture in consenting patients in good medical condition. We estimate approximately half of newly diagnosed extensive stage patients could undergo this procedure which will be performed in collaboration with the Surgery Branch (NCI). If cell lines are successfully established from a tumor biopsy, seven drugs known to be active in SCLC will be tested in vitro against the tumor cells. From the results of this in vitro testing, either one of 13 known active three-drug regimens comprised of three of the seven drugs or VP-16/cisplatin will be determined to be the "best regimen" for the patient. After appropriate stratifications for major prognostic factors, patients will be randomized to high-dose versus standard-dose VP-16/cisplatin. After 12 weeks of chemotherapy, all patients, both those randomized initially and those assigned to standard-dose drugs because of high risk of toxicity, will be fully restaged.

Further therapy after week 12 will depend upon the results of restaging. Patients in complete remission will continue on standard dose VP-16/cisplatin for another 12 weeks. This will allow a determination not only of the complete response rate, but also of the duration of complete remission associated with high dose and standard dose drugs. Whether the complete responders are the patients who also had the greatest degree of in vitro sensitivity to VP-16 and cisplatin can also be noted in patients for whom this data is available. Treatment for partial responders and patients with no response will depend upon whether a "best regimen" based on in vitro drug sensitivity data is available. If it is, the patient will receive this "best regimen," and any new complete responses (which we have never observed in any patient given any chemotherapeutic regimen after 12 weeks of treatment) will be noted. Partial and non-responders without in vitro drug sensitivity data will receive vincristine + doxorubicin + cyclophosphamide, a standard program for SCLC, and the frequency of new complete remissions determined. This will allow an estimate of how frequently new complete responses might occur after 12 weeks of VP-16/cisplatin, a two-drug regimen, when treatment is changed to a known active chemotherapy program rather than to an in vitro-determined regimen which is "individualized" to the patient.

Additional tumor biopsies for attempted cell culture are also provided in the protocol after 12 weeks of therapy and at the time of progressive disease. We believe this study will provide clear-cut information concerning 1) the efficacy of high dose versus standard dose VP-16/cisplatin as induction chemotherapy for SCLC; 2) an estimate of how frequently in vitro drug sensitivity information can be feasibly obtained in SCLC if good-risk consenting patients are taken to mediastinal biopsy; 3) whether a "best regimen" constructed on the basis of in vitro data is any better than simply changing therapy to a standard program in terms of new complete responses for patients

in partial or no response after 12 weeks of chemotherapy; and 4) the frequency with which changes in in vitro drug sensitivity of SCLC can be detected after 12 weeks of therapy and at the time of tumor progression compared to pre-treatment observations.

IV. Other therapeutic programs and complications of treatment in small cell lung cancer

1. Phase II studies in patients failing combination chemotherapy

Four Phase II studies involving both investigational and non-investigational drugs in SCLC patients who failed initial combination chemotherapy have recently been completed. Only one of eighteen patients had a brief partial response to cisplatin 100 mg/m^2 IV every 3 weeks (17). Hematologic toxicity, particularly thrombocytopenia, was impressive in this heavily pre-treated population. This negative result, which was duplicated by two other groups, is of interest in view of the apparently excellent activity of VP-16/cisplatin in newly diagnosed patients. High dose methotrexate, given every 2 weeks in a dose of 1500 mg/m^2 over 30 hours followed by leucovorin rescue, produced no objective responses and five cases of severe thrombocytopenia in 17 patients (18). All patients but three had previously received the CMC regimen which contains low doses of methotrexate. To our knowledge, this was the first study investigating the value of high doses of methotrexate in SCLC, despite the fact that several groups have added high doses of the drug to combination regimens. A more recent study from Vanderbilt University suggests that high dose as opposed to standard dose methotrexate is of no value when added to other drugs as part of initial therapy in extensive stage SCLC.

Two other Phase II studies have been conducted jointly with the University of Maryland Cancer Center. We contributed approximately 15% of the patients. Neither vindesine (19) in a dose of 3 mg/m^2 weekly, nor aziridinybenzoquinone (20) in a dose of 20 mg/m^2 on days 1 and 8 every 4 weeks produced any objective responses in 18 and 16 patients, respectively. The former finding is of interest in view of two other studies of vindesine which reported activity in SCLC patients failing other chemotherapy. This suggests that our Phase II study patients may have extremely aggressive previous treatment and be resistant to therapy prior to entering these trials. This is a major problem which has been discussed by many investigators active in the field, and new strategies for seeking new active agents in SCLC, principally administering Phase II agents to extensive stage patients for one month prior to switching to a standard regimen, are under consideration.

Our current phase II study has just been initiated. In order to gain experience with the VP-16/cisplatin regimen to be studied in our new extensive stage protocol, we are employing this program in patients failing other chemotherapy. Thus far, we have seen no responses in three patients.

2. Chemotherapy in late relapses of SCLC

Five patients who developed pathologically-proven relapse of SCLC after two years or more of continuous complete remission received "reinduction"

combination chemotherapy including some or all of the drugs with which they had initially been treated (21). Four patients responded for a median of 10 months (range 2-18). These results are much better than would be anticipated with any chemotherapy regimen given to patients who developed relapsing or progressive tumor while receiving chemotherapy, and suggest that greater intensity or longer duration of initial treatment might have cured some of the patients. To our knowledge, this is the first comprehensive report of re-treatment of "late" relapse, a problem which may become increasingly apparent with longer follow-up of SCLC patients. In our own experience, 6/31 patients (19%) with two-year disease-free survival eventually developed a documented relapse of their SCLC. It is unlikely that the majority of these cases represent new small cell cancers, since only half of our patients had evident tumor in the lung.

3. Thymosin Fraction V as an adjuvant to induction chemotherapy of SCLC

Complete follow-up is now available on a trial reported several years ago in which SCLC patients undergoing induction chemotherapy with CMC were randomized to receive one of two doses of thymosin Fraction V twice weekly for six weeks or no treatment. Sixty-seven patients were randomized and did not differ significantly with regard to the important prognostic factors of performance status and disease stage. Thymosin was without observable toxicity except for local effects at the subcutaneous injection site. Multivariate analysis indicates that patients receiving thymosin 60 mg/m² had significant survival prolongation relative to patients receiving no thymosin treatment, while patients receiving 20 mg/m² had very similar survival to the no treatment group. Four of the five two-year disease-free survivors among the 67 patients received thymosin in the higher dose. The validity of this observation remains uncertain because of the small numbers of patients randomized in this trial and the varying treatments given in addition to CMC induction, and confirmation is required.

4. Chest irradiation for progressive intrathoracic SCLC developing on combination chemotherapy

Thirty-two patients with SCLC received chest radiotherapy to progressive intrathoracic tumor after failing combination chemotherapy. Most of the 25 evaluable patients received split course treatment (4000 rad/10 fractions/4 weeks). Sixteen of 25 (62%) had an objective response. Median time to local progression within the tumor port was 16 weeks. All 10 autopsied patients had tumor within the treated hemithorax. Two patients, one of whom was given concurrent combination chemotherapy with agents to which she had not previously been exposed, achieved permanent local control and lived more than 18 months. One remains free of disease at 31+ months. Short-term palliation of chest disease and rare long-term survival is possible with this regimen. SCLC is less responsive to irradiation as second-line than as initial therapy, but doses of 4000 rad or more can offer symptomatic relief of intrathoracic symptoms at a much higher rate than is usually observed with Phase II chemotherapeutic regimens.

5. Complications of treatment and hematologic effects of chemotherapy

Among eight SCLC patients followed for a minimum of 2-1/2 years without relapse, one developed erythroleukemia with extensive chromosomal abnormalities 34 months after starting chemotherapy and seven months after a prodrome of macrocytic anemia and thrombocytopenia developed (23). A second patient had persistent pancytopenia with macrocytic anemia two years after discontinuing chemotherapy. The remaining six patients had normal peripheral blood counts and smears.

Fifteen anorectal infections occurred in 6.4% of 188 intensively treated patients with SCLC. Granulocytopenia was present at onset in 13 episodes. Ten infections resolved with recovery from granulocytopenia, but five patients required incision. Septicemia accompanied four episodes, and one patient died. Early recognition of this potentially fatal complication will become increasingly important as more patients with solid tumors receive increasingly intensive chemotherapy (24).

Eleven consecutive patients with SCLC received cyclophosphamide, doxorubicin, etoposide (VP-16), and vincristine (CAPO) on days 1 and 8 of three 21-day cycles of induction chemotherapy (16). Four had severe peripheral neuropathy, leaving them virtually bed-ridden, and the remaining seven had mild or moderate neuropathy. Of the next 14 patients treated similarly except for administering the vincristine only on day 1 of each 21-day cycle, mild-moderate neuropathy was noted in eight and no neuropathy in six. In view of independent reports of peripheral neuropathy associated with administration of VP-16, it is possible that VP-16 led to enhanced neurotoxicity of vincristine in this therapeutic program (25).

Circulating numbers of granulocyte/macrophage hematopoietic stem cells (CFUc) were measured in the peripheral blood of 20 extensive stage SCLC patients receiving CAPO chemotherapy. As leukocytes declined following chemotherapy, CFUc also declined (26). However, as leukocytes recovered from their nadir, circulating CFUc numbers per ml of blood and per mononuclear cell substantially expanded in 19 and 17, respectively, of the patients studied. The median CFUc expansion, compared to pre-treatment values, was 7.9-fold per mononuclear cell and 6.7-fold per ml of blood. The magnitude of this amplification suggests that appropriately timed peripheral blood buffy coat collections could be used to provide hematopoietic stem cells in sufficient numbers to allow marrow recovery after subsequent marrow-ablative therapy. CFUc were also measured in limited stage SCLC patients receiving CMC chemotherapy with or without chest irradiation. CFUc amplification was inconsistent and not of great magnitude. Moreover, CFUc were depressed to a significantly greater degree in patients receiving concurrent irradiation.

V. Review articles on clinical aspects of small cell lung cancer

During the past 2-1/2 years, 23 review articles concerning the staging and prognosis, pathology, and treatment of SCLC have been published by members of our Branch (27-49).

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Title: CLINICAL TRIALS IN NON-SMALL CELL LUNG CANCER

I. Personnel

- A. Permanent Senior Staff: Drs. J. Minna, D. Ihde, P. Bunn, Jr., D. Carney, A. Gazdar, M. Matthews
- B. Clinical Associates/Medical Staff Fellows: Drs. J. Mulshine, M. Brower, A. Doyle
- C. Other Professional Staff: None
- D. Technical Staff: E. Russell, H. Sims, A. Simmons, V. Bertness

II. Collaborators/Collaborating Branches:

- NCI: J. Roth (Thoracic Surgery), S. Hancock, A. Lichter, E. Glatstein (ROB):
- Naval Hospital Bethesda: T. Walsh (Pulmonary Service), R. Penniston (Thoracic Surgery), F. Worsham, M. Bibro (Anatomic Pathology); R. Whittington (Radiation Branch); S. Veach (Oncology)

III. Introduction

A. Objectives

To develop new ways to treat non-small cell lung cancer including use of combined modality therapies and in vitro assays of drug, radiation, and biologic response modifier sensitivity testing of tumor cell lines to select therapy.

B. Rationale and Background:

Non-small cell lung cancer (NSCLC) including adenocarcinoma, epidermoid carcinoma, and large cell carcinoma represents 75% of all pulmonary malignancies with nearly 100,000 new cases diagnosed in the USA every year. Current therapy for the majority of patients with this disease is dismal with over 80% of patients dying of their disease within one year of diagnosis (1). While it is possible that advances in control of the primary tumor may occur, the big problem is the presence of microscopic metastatic disease in the large majority of patients even when post surgical staging indicates control of the local problem. Thus, in addition to preventive measures, methods to treat this microscopic disease as well as to deal with overt metastatic disease are urgently needed.

Can effective systemic therapy be provided by chemotherapy? There is a long history of both single and combination chemotherapy trials of non-small cell lung cancer in patients with overtly metastatic disease. In brief, most drugs have given objective tumor response rates of less than 5%. Currently, the most active single agents include: vindesine, mitomycin C, cisplatin, adriamycin, and VP-16-213 with response rates ranging from 14-27% (1). Recent results of combination chemotherapy with the most "active" regimens include objective tumor response rates of 13-63% with an overall average of around 30-40% (1,2). However, "complete" clinical remissions only occur in less than 2% of patients. In addition, when larger number of patients are treated at

several institutions, or the regimens are tested in a cooperative group, the "best" combinations have response rates of 20% or less (3). Currently, a favorite "active" regimen is a combination of cis-platin and either vindesine or velban with response rate of over 40% (2). Regimens like these can cause enough tumor shrinkage to give symptomatic relief in some patients. However, they also have considerable toxicity associated with their usage. In addition, while patients whose tumors respond to therapy have better survivals than those that are unresponsive, survival benefit for the group of NSCLC patients as a whole has never been convincingly demonstrated to be improved by chemotherapy (3). With these results, it is not surprising that large numbers of randomized trials of chemotherapy given as an adjuvant to surgery in NSCLC have failed to show any benefit (1). All of these findings indicate the need to find new drugs with activity against NSCLC, or to better utilize the currently available agents.

While the NCI-VA Branch undertook several combination chemotherapy trials in NSCLC we have obviously also been impressed by the resistance of this disease to chemotherapy. We have completed a Phase II study of the combination of adriamycin and mitomycin-C (4). In 45 patients the overall response rate was 25% (4). We began the trial giving mitomycin-C on a q 3 weekly basis because of early reports of great activity using this schedule (4). However, we encountered unexpected pulmonary toxicity with this regimen and were forced to revert to a standard q 6 weekly schedule.

Another approach is to use in vitro tumor cell lines of NSCLC to test the efficacy of new drugs, drug combinations, drug schedules, or biologic response modifiers and then test active regimens in Phase I-II trials. A related approach is to test tumor cells and tumor cell lines from individual patients for their drug sensitivity/resistance and use this information to select therapy for individual patients. Both of these approaches, while attractive in concept, have not been shown to convincingly work in any human tumor and need to be studied in a prospective fashion. The first approach deals with the cell lines as a "panel" of patients. The second approach assumes that NSCLC patients differ in their sensitivity to different chemotherapy regimens and that the best responses currently seen occur because by chance the patients got regimens to which their tumors were sensitive.

There are a variety of potential problems in using in vitro assays of drug sensitivity and resistance and these are covered in detail in the section on the NCI-Navy Branch's new trial in extensive stage SCLC and the protocol itself (5,6). These include ability to grow tumor cells, prepare single cell suspensions, know appropriate drug concentrations and schedules to use in vitro, development of in vitro assays of tumor cell kill (e.g., clonogenic assays), use of fresh tumor samples vs. short and long term cell lines. However, preliminary results with the currently available NSCLC lines suggest they are resistant to single agent chemotherapy including agents with some activity in the clinic. These include lines from patients without prior chemotherapy (See Dr. Carney's report).

To conduct a prospective in vitro drug and radiation sensitivity testing trial will require several stages. These include learning how to best obtain tumor tissue from individual patients consistent with their clinical care; establishment of working relations with thoracic surgeons, pulmonary physicians, anatomic pathologists, and radiotherapists, so that tumor tissue can be obtained, and palliative or appropriate curative therapy can be given; learning how to grow NSCLC reproducibly from individual patients, and learning how to best perform drug sensitivity assays.

While our group has had the most experience in growing SCLC we have begun to work on NSCLC (see sections by Drs. Gazdar and Carney). Currently, approximately 60-70% of all NSCLC tumor specimens will yield colonies in a soft agarose cloning assays but only 20% of all NSCLC tumor containing specimens will yield enough colonies to test even a few drugs (NCI-Navy Unpublished). Continuously replicating tumor cell lines provide large amounts of material for clonogenic testing. Approximately 30-40% of all NSCLC tumor specimens can be established into a cell line or nude mouse heterotransplant (which can then be turned into a cell line or used for cloning assays). However, many of these attempts represent suboptimal amounts of tumor coming mostly from small biopsies. In any event, as described by Drs. Gazdar and Carney there are new developments in the growth of NSCLC as well as SCLC including serum free, growth factor supplemented medium. With availability of better specimens and experience, this rate should improve just as it did for SCLC.

C. Specific Aims and Outline of Methodology:

We plan a three pronged attack on this problem:

1. To test all of our NSCLC lines for sensitivity or resistance to currently available drugs and various combinations including new drugs coming into clinical trials.
2. To obtain NSCLC primary lung tumors at the time of surgical resection and grow these in vitro. Currently we have a tissue procurement protocol by Drs. Gazdar and Worsham (Head of Naval Hospital Anatomic Pathology) to allow this (NCI-Navy 82-12). It will provide us material to work with, as approximately 40-50 pulmonary carcinomas are resected at the Naval Hospital each year.
3. Using our cell lines, and the fresh surgical material, we will systematically examine the influence of growth factors, peptide hormones, and conditioned medium extracts to improve our ability to clone and grow NSCLC as described in the section by Dr. Carney.
4. To establish a clinical protocol to obtain tumor tissue from NSCLC at the time of diagnostic or therapeutic surgical procedures, or when the patient's clinical condition permits a biopsy to be done with reasonable safety. Then, while the patient is receiving standard therapy (surgery, radiotherapy, chemotherapy), to grow the cells in short term culture, and when enough tumor cells are available,

to test them for sensitivity and resistance to a variety of available drugs and combinations (as in the extensive stage SCLC protocol NCI-Navy 83-13). If drug sensitivity is seen in vitro, to try to integrate this into the patient's clinical treatment program.

D. Methodology

Patients with (or suspected of having) NSCLC will have standard clinical staging as appropriate for their individual situation. Tumor tissue will then be obtained at the time of standard diagnostic or surgical procedures, or if this is not available, we will perform the minimal surgical procedure necessary to obtain tumor tissue. The tumor tissue will be grown in vitro in an attempt to establish a cell line, and then will be tested for drug and radiation sensitivity or resistance. Currently, surgical procedures deemed "reasonable" for getting tumor for growing NSCLC, include biopsy of peripheral lymph nodes, skin nodules, pleural effusions, and pleural based masses, liver biopsies under peritoneoscopy, bone marrow biopsy, and in selected cases, parasternal mediastinal node biopsies. As the trial progresses we can consider more aggressive procedures such as thoracotomy. Other than standard biopsies, thoracic surgical approaches to obtain tumor tissue not called for under standard therapeutic conditions will be performed by Dr. J. Roth (Thoracic Surgery, NCI). In all cases we will physiologically evaluate the patient to determine if the biopsy can be done with limited risk to the patient. Our methodology and results of cell culture are described in the sections by Drs. Gazdar and Carney. We will use serum supplemented and serum free medium, implantation in nude mice, and cloning in soft agarose with transplantation of the colonies into nude mice. Drug sensitivity testing will be done on early and late passage cell lines using soft agarose cloning techniques unless and until other tumor cell sensitivity assays are devised.

Currently, the clinical trial is in the protocol planning stage. However, a likely scenario is that we will use "Standard therapy" for stage I and II patients (surgery), while radiation therapy with curative intent will be administered to stage III M0 patients. The remaining stage III patients with distant metastases will receive palliative radiotherapy and chemotherapy as needed. If in vitro sensitivity data can be developed for the individual patient this will be administered in the following situations:

1. For post surgical stage I and II disease at the time of disease recurrence, or possibly in an adjuvant setting in those patients at high risk for recurrence.
2. For stage III M0 disease after primary therapy is completed.
3. For patients with distant metastatic disease at the time palliative therapy is completed.

Once it is clear that a reasonable number of specimens can be grown and that sensitivity data can be obtained, patients can be randomized to

receive only standard therapy (including some standard chemotherapy) vs. therapy selected by the in vitro assays.

Initially our objectives are to see if we can obtain and grow tumor tissue and perform the assays in a reasonable period of time. Currently, the earliest the assay data would be available is about 6-12 weeks. We will also be able to assess, in individual patients with evaluable tumor masses, response to the chemotherapy. We would use the approach described in the extensive stage SCLC trial of selecting from a list of combination chemotherapy regimens for which published experience already exists in terms of dose, schedule, and toxicity.

IV. Progress Report

We have obtained approval for the tumor tissue procurement protocol. This involved considerable time with various clinical services, and administrative committees.

We have developed new data on growth factors for NSCLC (see Dr. Carney's report) and experience cloning NSCLC lines, some fresh specimens, and have some drug and radiation sensitivity data (5-8). Dr. Gazdar is beginning to make a major effort in developing techniques to grow NSCLC. Tests of a new Phase I agent tiazofurin on our cell lines revealed that some NSCLC are sensitive to this agent while others are not (9). The sensitive lines are resistant to other standard chemotherapy agents used in the treatment of NSCLC. We plan to introduce this into a Phase I-II trial (NCI-Navy 83-7, 83-C-31) (see section by Dr. Ihde).

We have begun protocol discussions with the many services necessary to run this trial including pulmonary and thoracic surgical services at the NCI.

V. Future Plans:

Develop and obtain protocol approval and continue related laboratory studies.

VI. Publications, References and Abstracts:

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Title: CLINICAL TRIALS IN THE CUTANEOUS T-CELL LYMPHOMAS

I. Personnel

- A. Permanent Senior Staff: P.A. Bunn, D.C. Ihde, J.D. Minna, D.N. Carney, M. J. Matthews, A. F. Gazdar
- B. Clinical Associates: C.F. Winkler
- C. Other Professional Staff: J. Eddy, R.N.
- D. Technical Staff: None

- II. Collaborating Units: E. Glatstein (Radiation Oncology Branch, NCI); S. Broder (AD, COP); L. Matis (Medicine Branch, NCI); R. Makuch (Biometry Branch, NCI); D. Hendricks (Dermatology Branch, NHBETH); A. B. Fischmann (Dermatology Section, Washington, VA Medical Center); G.P. Schechter (Hematology Section, Washington VA Medical Center).

A. Background

The treatment of cutaneous T-cell lymphomas (CTCL) prior to the 1950's was restricted to local orthovoltage irradiation of local tumors and ulcers and other nonspecific topical treatments including antibiotics and later corticosteroids. During the 1950's several new treatment options were developed and later used to treat patients with CTCL including: electron beam irradiation, topical and systemic chemotherapy. Later during the 1960's and 1970's treatment with ultraviolet A light preceded by oral doses of methoxsalen (PUVA) was described primarily in patients with psoriasis, but soon was extended to studies of patients with CTCL.

The primary lesions in CTCL are located in the skin and the early systemic nature of these lymphomas was not recognized until the 1970's; hence, early studies employed topical chemotherapy or electron beam irradiation.

1. Topical Chemotherapy

Haserick et al in 1959 first reported the ability of topically applied mechlorethamine (nitrogen mustard, HN₂) to produce skin clearing. Since this report, a number of investigators have confirmed the initial observations. In the largest series, Vonderheid et al reported an overall clinical complete remission rate of 64%, varying from 94% for T1 lesions to 59% for T4 lesions. The median response duration was 18 mos; by 36 mo. only 13% of patients were disease free. Relapses continued after this time suggesting that topical HN₂ is not a curative modality. The major advantages of topical HN₂ are its wide availability, relative low cost, and absence of systemic toxicities. The major disadvantages are the need for continuous daily whole body applications, the lack of systemic effects and the development of hypersensitivity reactions

in up to 40% of patients. Some of these can be overcome by topical desensitization or use of HN_2 in aquaphor rather than water. A large number of other agents have been evaluated but none, with the possible exception of BCNU, have proven sufficiently effective and non-toxic for routine use.

2. Electron Beam Irradiation [EBRT]

Following the lead of Trump et al a number of investigators evaluated electron beam irradiation in the 1950's. The early trials demonstrated that total body application of the beam was necessary to prevent the emergence of lesions in non-irradiated areas. The Stanford group also showed a clear dose response relationship with higher CR rates in patients receiving 3000 rad or more. In the Stanford experience, complete remissions were achieved in 84% of all patients, the rate being directly related to skin stage. The median duration of response was 16 months with 20% of patients remaining disease-free at 3 years. In their experience, relapse after 3 years was rare, suggesting that some patients (only those with early stage plaque lesions without adenopathy, blood or visceral involvement) were cured. While the main advantage of EBRT is the potential cure of some patients without excess systemic toxicity, there are a number of drawbacks. Adverse cutaneous reactions including alopecia, atrophy, dryness, edema, radiodermatitis, and second skin cancers are common. The technique is expensive, available in limited number of centers and requires considerable technical expertise to avoid toxicity. The majority of patients relapse within 2 years of treatment. In addition, other groups have not been able to reproduce the high "cure" rate reported from Stanford, though they have used different techniques.

3. Photochemotherapy

Ultraviolet (UVA) light is a form of irradiation known to produce transient responses in some CTCL patients. Methoxsalen (8-methoxy-psoralen) is a phototoxic furocoumarin compound which binds to DNA and when activated by UVA light damages the DNA. Thus, the effective irradiation is exclusively to the epidermis and upper dermis. The experience with psoralen and UVA, (PUVA) in CTCL is more recent so the long term effects are unknown. The complete response rate (overall 62%), median duration of response, and survival, of patients treated with PUVA appears to be similar to those achieved with topical HN_2 . Maintenance therapy is required, and continuing relapses over time seem to occur suggesting PUVA is not curative. PUVA may be non-cross resistant with other modalities as patients who have failed topical HN_2 or EBRT may respond. The major disadvantages are the inability to treat deep lesions, the cost and inconvenience of maintenance treatment and the potential for second skin cancers.

4. Systemic Chemotherapy

A wide variety of drugs have been evaluated in CTCL, though the total number of patients is small except for the alkylating agents and methotrexate. In general, the objective response rates to a variety of agents (mechlorethamine, cyclophosphamide, methotrexate, adriamycin, VP 16

and vinca alkaloids exceeds 60% with complete responses in 10-20% of patients. The response duration is relatively short with a median of 4-20 months. Drug combinations have been evaluated in small numbers of patients. There is a suggestion that the objective and complete response rates (about 85% and 25% respectively) and median duration of response (5-20 months) may be slightly superior to single agent therapy. This has not been established in a randomized trial. The experience with both single agents and drug combinations has been confined to patients with advanced stages; nonetheless, there are no reports of cure using chemotherapy alone.

5. Combined Modality Therapy

The reasons for the inability of these treatment modalities to cure patients are unknown. The frequent finding of small numbers of extracutaneous tumor cells in patients with early stages of disease suggests that unsuspected systemic lymphoma accounts, in part, for the inability of the topically directed therapies to cure early stages. The natural history of the cutaneous T cell lymphomas is very similar to that of their B-cell counterpart, the "favorable histology" non-Hodgkin's lymphomas. Systemic chemotherapy also has thus far been unable to cure patients with these B-cell histologies despite the high rates of objective response to chemotherapy.

These concepts have led to trials of multi-modality therapies. The Stanford group has reported preliminary observations from a study comparing EBRT plus topical HN₂ to topical HN₂ alone with an early advantage for the combined modality. It is too early to determine whether long-term disease free survival was improved. The University of Chicago reported preliminary results from a non-randomized trial employing EBRT followed by MOPP or COPP chemotherapy for patients with advanced stage. In comparison with a historical series of EBRT alone or local therapy alone, the combined modality improved disease-free and overall survival. The actuarial disease free survival and overall survival were 67% and 88% respectively at 3 years. We conducted a pilot combined modality trial whose results were encouraging but were inferior to these results from Chicago (vide infra)

B. Objectives

1. To determine whether combined modality therapy with whole body EBRT plus systemic chemotherapy can be administered safely and whether it is superior to a conservative approach employing sequential therapies with topical HN₂, PUVA, EBRT and systemic chemotherapy.

2. To gain an understanding of the natural history of these disorders through serial staging and restaging studies.
3. To perform biologic studies of the malignant cells obtained from patients.

C. Progress to Date

1. Pilot Combined Modality Study.

From 1976 to 1979 we conducted a pilot study of whole body EBRT plus systemic chemotherapy to determine whether this approach was safe and promising. The schema for the study is attached. Patients with plaques confined to skin (Stage I) received EBRT plus single agent intravenous mechlorethamine. Patients with more extensive lesions (Stages II-IV) received EBRT plus alternating 3 drug regimens (vinblastine + adriamycin + bleomycin/cyclophosphamide + methotrexate + prednisone). There were 38 patients entered on the trial; long term follow-up is continuing. The complete response rate (pathologically documented) was 77% for the 13 Stage I patients and 28% for the 25 Stage II-IV patients. At 5 years the actuarial survival was 92% for Stage I and 38% for Stage II-IV. Disease free survivals were 56% and 8% respectively. These results were quite similar to those obtained at Stanford University using EBRT alone (see attached Figure) with the possible exception of relapse free survival in Stage I patients. However, there were very small numbers in the pilot trial. There was moderate toxicity from the combined modality approach, particularly to cutaneous sites. These included moist desquamation (radiodermatitis) in 21%, ulcerations in 18%, atrophy in 13%, alopecia in more than half, peripheral edema in 13% and gynecomastia in 8%. Second skin cancers have occurred in 3 patients. Severe myelosuppression was rarely a problem and platelet counts of less than 50000/u1 and WBC less than 1000/u1 were observed in only one instance each. There were no deaths related to therapy. The frequency of moist desquamation and ulcers has diminished to a negligible fraction as we have become more familiar with EBRT (no cases since Dr. E. Glatstein became Chief of the ROB).

This pilot study established that full doses of chemotherapy and EBRT could be administered together with relative safety. The combined modality therapy produced high objective response rates, particularly in Stage I disease where the 3-year disease free survival was excellent. However, the results must be tempered by the small numbers and the fact that median survival for patients treated with EBRT or topical HN₂ approaches 10 years. While useful palliative responses were seen in 96% of more advanced stages, only 8% were relapse free at 3 years. The overall survival of CTCL patients (see attached Figure) and the continuing decline in relapse free survival over time are analogous to data from the "favorable histology" B cell lymphomas.

Recent studies in these lymphomas suggests that a no initial therapy approach (or irradiation to local areas) may be equivalent to initial chemotherapy or combined modality therapy. Randomized trials addressing this issue are in progress at our institution (vide infra) as well as other institutions.

2. Present Randomization

We feel the major therapeutic issues in CTCL, at present, are:
1) Is a potentially curative combined modality approach superior

to a conservative palliative approach? 2) Can patients be cured? 3) Do patients undergo histologic conversion when treated or untreated? (similar to that seen in the "favorable prognosis" B cell lymphomas) 4) Are there subsets of patients who benefit more from one approach? To answer these questions we instituted a randomized clinical trial in 1979. A protocol schema is attached. After initial staging patients are randomized to receive palliative topical HN₂ versus combined EBRT plus combination chemotherapy with cyclophosphamide + adriamycin + VP 16 + vincristine (CAPO). This regimen was chosen for the following reasons: the University of Chicago data suggested higher CR rates could be obtained with more intensive chemotherapy, we had observed complete responses in patients given this regimen following relapse on our initial regimens, all the agents have established activity in CTCL and we wished not to use procarbazine or prednisone.

Results will be presented at the time of the site visit.

D. Future Plans

We plan to continue the randomized clinical trial until at least 50 patients have been entered on each arm of the trial. There are no plans for major protocol modifications until the trial is complete.

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Title: STUDIES OF THE NATURAL HISTORY, STAGING AND CLINICOPATHOLOGIC
CORRELATIONS IN THE CUTANEOUS T-CELL LYMPHOMAS

I. Personnel:

- A. Permanent Senior Staff: Drs. P.A. Bunn, M.J. Matthews, A.F. Gazdar
- B. Clinical Associates: Dr. C.F. Winkler
- C. Other Professional Staff: J. Eddy, R.N.
- D. Technical Staff: P. Jewett, R. Hubert (Med. Tech.)

II. Collaborating Units:

- G.P. Schechter (Hematology, WVAMC); A.B. Fischmann (Dermatology, WVAMC);
- J. Whang-Peng (Cytogenetics, MB, NCI); R. Makuch (Biostatistics, NCI);
- S.M. Hsu (Pathology, NCI); S. Rosen (Oncology, Northwestern University).

III. Introduction

A. Objectives

1. To gain an understanding of the natural history of the cutaneous T cell lymphomas, and determine the prognostic significance of a number of parameters.
2. To evaluate the utility of a series of pre- and post-therapy staging procedures.
3. To establish criteria for the morphologic diagnosis of skin involvement, lymph node involvement, and peripheral blood involvement.
4. To evaluate biologic properties of the malignant cell with respect to DNA content, cytogenetics, monoclonal antibody binding and to assess the prognostic significance of these factors.
5. To perform epidemiologic studies on background exposures to chemicals, drugs, physical agents and viruses (HTLV).

B. Background

The fact that the malignant cell in mycosis fungoides and the Sezary syndrome is a malignant lymphocyte was not appreciated until 1970. The observation that the malignant lymphocytes were T cells was reported in 1973 and the ability of Sezary cells to function as "helper" cells was reported in 1976. Preliminary cytogenetic studies without Giemsa banding performed during this period suggested that aneuploidy was a frequent finding although specific abnormalities were not reported.

Autopsy series and several large clinical series suggested that extra-cutaneous lymphoma was more frequent than initially reported and rarely if ever represented the development of a new lymphoma. It was appreciated that patients with palpable lymph nodes had a worse prognosis, that circulating malignant cells were often observed, and that spread to visceral organs conveyed an especially poor prognosis. The lack of

routine pretherapy staging evaluation led to several small laparotomy studies where unsuspected extracutaneous lymphoma was often found in the liver and spleen.

Methods for the early detection and diagnosis of cutaneous lymphoma were unsatisfactory and there was considerable controversy over histologic interpretation of skin and lymph node biopsies. Documentation of circulating cells and the significance of this finding were also controversial. The etiology was uncertain and there were few epidemiologic observations.

IV. Progress Report

We have performed a routine series of pretherapy staging evaluations on all of our CTCL patients. These studies have included routine blood, x-ray, and scanning studies. In addition, we have evaluated cytogenetics, electron microscopy, DNA content analysis and more recently monoclonal antibody binding patterns. We have shown that nearly all patients have evidence for extracutaneous lymphoma. Even when infiltration of a node or organ is not conspicuous by light microscopy, small numbers of malignant cells may be found by cytogenetics or DNA content analysis. We have shown that peripheral blood involvement, advancing lymph node involvement, and visceral organ involvement convey a poor prognosis.

Drs. Matthews and Gazdar have developed a new classification schema for pathologic interpretation of lymph node biopsies, and this classification has been shown to have prognostic significance.

The malignant cells in nearly all CTCL cases have a specific monoclonal antibody phenotype, characteristic of mature "helper/inducer" T cells (i.e., T1⁺, T4⁺, T8⁻, T9⁻, T10⁻, Ia⁻ and anti Tac⁻). The malignant cells in HTLV+ malignancies have the same phenotype though they are often Ia⁺ and always Tac⁺. In some CTCL cases, cutaneous malignant cells have a slightly different phenotype than those in the circulation and may be Ia⁺, T9⁺ or anti Tac⁺, suggesting they are "activated." Whether patients whose cells have this phenotype have a worse prognosis is under investigation.

In most CTCL cases the malignant cells have a diploid or near-diploid DNA content. When hyperdiploidy is present, the prognosis worsens, patients are more likely to have a large cell tumor, and the fraction of cells in DNA synthesis is higher. In some instances, a shift to higher ploidy levels has been associated with relapse and histologic conversion to larger cells. The DNA index is highly correlated with modal chromosome numbers. We have been unable to identify aneuploid clones by chromosome analysis in most CTCL patients with early stages. While most patients with advanced stage have such clones, the clone markers have varied from patient to patient. No single translocation or other abnormality has been found in all patients. Cytogenetic analysis often demonstrates chromosomal abnormalities in the absence of pathologic involvement of several sites, suggesting low numbers of malignant cells may be detected with this method.

We have shown that nearly any visceral organ can be involved, but that metastases to most organs are clinically silent until late in the course. Bacterial sepsis is extremely common, particularly in patients with advanced stages due to the skin breakdown and deficiencies in cellular immunity. While staph species are nearly always cultured, secondary infections with unsuspected gram negative organisms may be life threatening.

CTCL patients have an excess in exposures to drugs, chemicals, and physical agents. The mechanism for the carcinogenesis of these agents is unclear. Some recent laboratory data suggests that CTCL patients may have fragments of the HTLV genome even though they have no anti-HTLV antibodies and viral particles have not been isolated. The etiologic significance of this finding awaits further study.

V. Future Plans

We plan to continue to collect the data from the routine pretherapy staging evaluations. We have performed all or part of these investigations on about 120 patients to date. In the next 6 months we plan to perform a multivariate analysis of these results to determine which factors have independent prognostic importance.

We will continue to evaluate the usefulness of monoclonal antibodies in classification, diagnosis and in assigning prognosis. Cytogenic and DNA content studies will be continued to assess the prognostic information they provide in a multivariate analysis.

We are also assessing the value of a number of radiographic and imaging studies including lymphangiograms, gallium scans, liver spleen scans, bone scans, and abdominal CT scans. We will be imaging with radio-labeled monoclonal antibodies in the future.

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Title: CLINICAL TRIALS IN RETROVIRUS ASSOCIATED ADULT T CELL LYMPHOMAS

I. Personnel

- A. Permanent Senior Staff: Drs. P.A. Bunn, Jr., D.C. Ihde, D.N. Carney, M.J. Matthews, A.F. Gazdar.
- B. Clinical Associates: Dr. C.W. Winkler
- C. Other Professional Staff: J. Eddy, R.N.
- D. Technical Staff: R. Hubert (Med. Tech.)

II. Collaborating Branches

E. Glatstein (Radiation Oncology Branch, NCI); R. Young, (Medicine Branch, NCI); W. Blattner, D. Blayney (Environmental Epidemiology Branch, NCI); M. Robert-Guroff, R. Gallo (Laboratory of Tumor Cell Biology, NCI); S. Broder, T. Waldmann (Metabolism Branch, NCI and AD COP).

III. Introduction

A. Objectives

1. To study the natural history of HTLV associated adult T-cell lymphoma.
2. To increase the complete remission rate and long term survival of these patients by utilizing 2 "non-cross resistant" chemotherapy regimens (ProMACE and MOPP). To determine the toxicity of this regimen in these patients.
3. To determine the ability of high dose methotrexate and prophylactic cranial irradiation to prevent CNS relapse.
4. To determine the biologic and prognostic significance of lymphocyte surface markers.
5. To establish tumor cell lines and to perform other biologic studies (see section on Laboratory Studies on Biology of Malignant T cells).
6. To perform epidemiologic studies of these patients and their families.
7. To perform virologic and molecular genetic studies on these tumor cells.

B. Background

In 1977 while conducting clinical and laboratory studies on mycosis fungoides a 27 year old black male presenting with cutaneous skin nodules which appeared rapidly and within several months spread over his entire body was referred to the NCI. A skin biopsy containing Pautrier's micro-abscesses as well as an infiltrate of atypical lymphoid cells in the upper dermis was interpreted as mycosis fungoides. He was said to have

the rare d'emblee tumor form of this disease. Interestingly, he developed bone pain and was found to have multiple lytic bone lesions. A bone biopsy from one of the lytic lesions demonstrated a scalloped paratrabecular pattern of bone resorption by osteoclasts. Hypercalcemia was noted and attributed to a bone resorbing lymphokine (1). A permanent T-cell line, Hut 102, was established in the lab by Dr. Gazdar and coworkers and possessed all the characteristics of mature helper T-cells (2). The cells contained characteristic irregular nuclear contours. Drs. Gallo, Polesz, and coworkers first isolated the human T-cell lymphoma virus or HTLV from these cultured cells and the patient's serum was shown subsequently to contain antibody to this virus, (3,4).

In 1977 Uchiyama and coworkers described a syndrome termed adult T-cell lymphoma which was characterized by high white blood cell counts, skin lesions, lymphadenopathy, organomegaly and a rapidly fatal course. This disease was endemic in the Southwestern provinces of Japan. This fact and a seasonal variation in incidence suggested the possibility of an infectious etiology. Later, after hearing of our case, Catovsky, et al. described a similar syndrome in Blacks from the Caribbean region. These patients were also noted to have hypercalcemia. Both Japanese and Caribbean groups of patients were subsequently shown to have HTLV particles and antibodies. Since the presentation of this patient, we have followed 10 additional patients with retrovirus associated T-cell lymphoma who define a new subset of high grade T-cell lymphomas (6). We are continuing to investigate the clinical, epidemiologic, and virologic aspects of these lymphomas (5,6,7,8)

IV. Progress Report

A. Clinical Studies.

We have followed a series of 11 cases each of whom had serum anti-HTLV antibodies. In 7 instances virus particles have also been obtained from cells in culture. The 11 patients in our series were generally young, with a median age of 33 and a range of 24 to 62 years. There was an 8 to 3 predominance of black patients and a 7 to 4 predominance of males. Seven of the black patients were born in the Southeastern United States. Two patients were born in Jamaica and one each in Ecuador and Israel.

The onset of symptoms was acute, and primarily related to rapidly arising cutaneous lesions and/or hypercalcemia. Six patients initially presented with skin lesions and 2 others developed skin lesions prior to therapy. Five patients initially presented with symptoms related to hypercalcemia. The interval between the onset of symptoms and diagnosis was unusually short with a median of 2 months and a range of 1 to 12 months. The skin lesions varied considerably. Two patients presented with large and discrete tumor nodules and 3 patients presented with smaller nodules which were sometimes nearly confluent. Three patients presented with more nonspecific plaques and papules, or erythroderma. The initial biopsy diagnosis of lymphoma was established by skin biopsy in these 6 patients. In addition to the 5 cases presenting with hypercalcemia, 5 others developed hypercalcemia prior to therapy. The 11th patient without

initial hypercalcemia developed hypercalcemia at relapse. The serum calcium levels were generally quite high with a median value of 16mg/dl and levels as high as 24mg/dl. Serum phosphorus levels were usually within the normal range. Calcium levels were always difficult to control until treatment with combination chemotherapy was instituted.

There was striking evidence for osteoclast activation and increased bone turnover in all patients. The typical bone scan was abnormal with increased symmetric accumulation of radioactive tracer throughout the skeleton and most prominent in the joints and skull. Scans with these abnormalities suggestive of metabolic bone disease have been called "superscans". They are highly otherwise unusual in patients with malignant lymphoma.

Eight of the 11 patients had elevated white blood counts with a range from 6,700 to 97,000. A lymphocytosis was documented in 8 patients with lymphocyte percentages ranging from 15 to 93% with a median of 46%. Overall, circulating atypical malignant lymphocytes were documented in 9 of the patients. Anemia and thrombocytopenia were uncommon.

Lymph node enlargement was noted in nearly all the patients though the nodes were initially quite small in several patients. Rapid enlargement was documented in several patients during the pretherapy evaluation. Overall 9 of 11 patients had peripheral adenopathy which was generalized in 7 and local in 2. Retroperitoneal adenopathy was demonstrated in 4 of 7 patients who underwent abdominal CT scans or lymphangiograms. Hilar adenopathy was noted on chest roentgenogram in 4 patients but none of the 11 patients had a mediastinal mass. Six patients had hepatomegaly on physical examination and 3 had splenomegaly by physical examination or scans.

After completion of initial staging procedures, all 11 patients had stage IV disease with visceral organ involvement. This was documented in the skin in 8 patients, by lytic bone lesions in 4 patients, by positive bone marrow biopsy in 6 patients, by positive gastric biopsies and/or ascites in 3 patients, by bilateral interstitial pulmonary infiltrates with or without pleural involvement in 4 patients, by lymphomatous leptomeningitis with positive CSF cytology in 3 patients and by liver biopsy in 2 patients. When sites of disease at relapse were considered, involvement in these sites became even more frequent with additional patients developing skin, pulmonary, hepatic and central nervous system lymphoma.

Opportunistic infections were extremely common in these patients despite having normal serum immunoglobulins. Pneumocystis carinii pneumonia was pathologically documented in 2 patients and clinically present in a third prior to therapy. Fungal sepsis and/or esophagitis was documented in 3 patients and one patient had cryptococcal meningitis while granulocyte levels were normal. Bilateral pulmonary infiltrates of unproven etiology but suspected to be infectious developed in 3 patients and bacterial sepsis in 4 patients who were granulocytopenic following chemotherapy. Treatment with combination chemotherapy resulted in prompt clinical complete remission in 7 of 10 evaluable patients. Three patients died during their induction therapy

and were not considered as responders though 2 had no evidence of lymphoma at post mortem. One patient is currently undergoing initial therapy. Unfortunately, 5 of the 7 responding patients later relapsed after a median of 13 months with a range of 6 to 26 months. All failed in sites of initial disease though several also failed in new sites including the skin, liver, and central nervous system. Second and third line chemotherapies were generally unsuccessful in inducing complete responses. Monoclonal antibody therapy also failed to induce objective response in 2 patients.

We concluded that retrovirus associated adult T-cell lymphoma should be strongly suspected on clinical grounds when young black adults, particularly from the Southeastern United States present with the rapid onset of lymphomatous skin lesions or metabolic bone abnormalities with or without hypercalcemia. The diagnosis should be confirmed by pathologic, serologic and virologic studies. Prompt recognition of metabolic and infectious complications and prompt staging with emphasis on pulmonary and CNS lesions is important. Intensive combination chemotherapy produced a high rate of complete response and has led to development of our current prospective trial.

B. Epidemiologic Studies

A number of our patients have been blacks from the Southeastern United States. In collaboration with the Environmental Epidemiology Branch, the family members of the index cases and a small population sample from this area have been studied for evidence of HTLV infection (7). The results to date suggest that HTLV infection is endemic in certain parts of the Southeastern U.S. at rates similar to that seen in Caribbean blacks but at a lower rate than observed in Southwestern Japan. We have also studied the family members of all of our patients and preliminary results show that family members are more likely to possess HTLV-specific antibody than the normal population supporting the infectious nature of HTLV (8). The infectivity of HTLV was further suggested by studies of one family in which 4 of 5 members possessed either serum anti-HTLV antibodies or whose cells expressed HTLV proteins or released intact viral particles.

C. Biologic, virologic, molecular, immunologic and cytogenetic studies (see laboratory section).

V. Future Plans

Clinical Studies: We plan to enlarge our current series of cases and continue our prospective study of treatment with ProMACE/MOPP. We will continue the adjunctive biologic and epidemiologic studies. We will also perform pilot therapeutic studies in patients who fail ProMACE/MOPP. These will include the monoclonal anti-Tac and radiolabeled T101 studies, the relapsed acute leukemia protocol and possibly 2'DCF. We are coordinating studies with the Chinese to establish parallel studies of HTLV incidence, epidemiology, biology and therapy in China.

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INTERFERON (RECOMBINANT LEUKOCYTE A INTERFERON)

I. Personnel

- A. Principal Investigators: P.A. Bunn, D.C. Ihde, D.N. Carney, J. D. Minna
- B. Clinical Associate: C. Winkler
- C. Other Professional Staff: J. Eddy, R.N., J. Sakol, R.N.

II. Collaborating Branches

S. Sherwin, Biologic Response Modifier Program; D. Longo, Medicine Branch, NCI

III. Introduction

A. Objectives

1. To determine the activity of recombinant leukocyte A interferon (IFLrA) in cutaneous T-cell lymphomas failing conventional therapies.
2. To determine the toxicity of IFLrA.
3. To determine biologic correlates of the interferon for predicting antitumor effects.

B. Background and Rationale:

Interferons are a family of low molecular weight proteins classified into three main groups: alpha, gamma, and beta. Alpha interferon was previously classified as type I interferon, is acid stable and is the predominant interferon produced by virus stimulated leukocytes and lymphoblastoid cell lines. Scientists at the Roche Institute and Genentech were able to clone the gene for human leukocyte A interferon, insert it into E. coli and produce highly purified (> 96%) recombinant leukocyte A interferon.

Large Phase I trials were conducted at many centers, including the NCI, with various doses and schedules. At the NCI, 50 X10⁶ units IM 3 times weekly was determined to be the maximum tolerated dose in Phase I trials, and was selected as the starting dose for Phase II trials. Antitumor activity was noted in indolent lymphomas during the Phase I trials.

IV. Progress Report

Eight patients have entered the study to date. Objective partial remissions have been obtained in six patients with minor regressions in two patients. All patients had failed prior therapies and all patients had advanced stage II-IV disease. All types of cutaneous lesions, plaques, tumors and erythroderma (Sezary syndrome) were represented.

Responses were noted in peripheral blood and lymph nodes as well as skin lesions. It is too early to determine the median response duration, but 2 PR's and one MR have progressed in less than six months.

There has been considerable toxicity and all patients have required dose reductions; most to 10% of the starting dose. All patients have developed fever on initial injections to at least 102°F. All patients have generalized fatigue and malaise which has been sufficiently severe to require dose reductions in most patients. One patient developed lipoid nephrosis, nephrotic syndrome and acute renal failure which cleared with discontinuation and reappeared with rechallenge at a lower dose. Two patients have developed increases in hepatic enzymes.

V. Future Plans

We plan to treat up to 20 patients on this study to determine an approximate response rate and response duration. If significant responses are observed, we will consider using the agent in future front-line protocols. If moderate activity is observed and we are not ready to develop a new front line study, we may alter the dose and schedule. We plan to test the IFLrA in vitro on our human T cell lymphoma lines.

DEOXYCOFORMYCIN

I. Personnel

- A. Principal Investigators: P.A. Bunn, D.C. Ihde, D.N. Carney, J.D. Minna
- B. Clinical Associates/Medical Staff Fellows: C.F. Winkler

II. Collaborating Branches

- L. Matus, Medicine Branch; D. Poplack, Pediatric Oncology Branch, NCI;
- B. Mitchell, Department of Medicine, University of Michigan

III. Introduction

A. Objectives

1. To determine the therapeutic efficacy of 2'-deoxycoformycin in cutaneous T-cell lymphomas.
2. To determine the toxicity of 2'-deoxycoformycin administered in a fixed dosage schedule.
3. To determine whether biochemical changes in vitro can predict for response or toxicity.

B. Background

2'-deoxycoformycin (2'-dCF), a potent inhibitor of the enzyme adenosine deaminase (ADA), is a nucleoside analog produced by the organism *Streptomyces antibioticus*. 2'-dCF is a transition state analog of

the reaction in which adenosine is deaminated to inosine, a reaction catalyzed by ADA. 2'-dCF is a tight-binding inhibitor with a K_i of 2.5×10^{-12} M for human ADA.

ADA is an enzyme of known importance in lymphocyte metabolism. It is present in the body in highest amounts in lymphoid tissues and absence of the enzyme, as occurs with genetic deletion of ADA, results in defective lymphocyte maturation and function and the immunodeficient state known as Severe Combined Immunodeficiency Disease (SCID). Inhibition of ADA by 2'-dCF has been shown to result in immunosuppression in several experimental systems. Inhibition of *in vivo* delayed hypersensitivity skin test responses to nonspecific mitogens has been documented.

Interest in 2'-dCF as an antileukemic agent was stimulated by the report of a 23-fold elevation of ADA in the blast cells of patients with acute lymphocytic leukemia. More recently, it has been demonstrated that the E-rosette positive blast cells of ALL patients with "T cell disease" have considerably higher ADA levels than "null" lymphoblasts, prompting speculation that, should it be effective against leukemia, 2'-dCF may have some "specificity" for those poor prognostic patients with "T" lymphoblasts. When 2'-dCF is used as a single agent in murine test systems, it is without antileukemic activity except when supplemented with the physiological nucleoside 2'-deoxyadenosine. This observation supports the theory that the impaired lymphocyte maturation and function seen in ADA deficiency in man is due to 2'-dATP accumulation, and that in man accumulations of toxic levels of the latter nucleoside can occur if the degradation of endogenous 2'-deoxyadenosine, through deamination by ADA, is blocked. In the mouse, this accumulation after ADA inhibition and without 2'-deoxyadenosine supplementation is seen only in newborns.

Recently, a phase I study of single agent 2'-dCF conducted at the Royal Marsden Hospital yielded clinical evidence of antileukemic activity in humans. Partial remissions were noted in three of seven ALL patients treated in that trial. One patient had a 70% reduction in circulating lymphoblasts, a second individual demonstrated complete clearing of lymphoblasts from peripheral circulation and a 50% reduction of bone marrow blasts, and a third patient, who was circulating 82,000 blasts per mm^3 at the start of therapy, manifested complete clearing of both peripheral and bone marrow lymphoblasts. This latter individual was thrombocytopenic and died from a cerebrovascular bleed on the 7th day after starting 2'-dCF treatment. His bone marrow at post-mortem showed a hypocellular marrow with no leukemic blast cells present.

These reports stimulated interest in the treatment of other lymphoproliferative malignancies and resulted in the initiation of phase I-II trials. Kufe, et al., reported their preliminary results of a phase I-II trial of 2'-deoxycoformycin in the treatment of T-cell malignancies. Of the five patients they treated, one patient with mycosis fungoides had complete resolution of his skin disease lasting for more than 2 months without further treatment. Recently, Bisaccia and Grever et al., reported the results of 2'-deoxycoformycin treatment in four patients with advanced mycosis fungoides. They obtained two complete responses and two partial responses lasting from 2+ to 9+ months.

In view of the apparent *in vivo* responses to single agent 2'-deoxycoformycin of T-cell ALL and the cutaneous T-cell lymphoma, mycosis fungoides, and the *in vitro* response of 2'-deoxycoformycin in murine systems when 2'-deoxyadenosine pools are supplemented (a requirement which appears unnecessary in man because of species differences in the ability to form 2-deoxyadenosine tri-phosphate), the current phase I-II trial in patients with advanced refractory cutaneous T-cell lymphoma is being instituted.

IV. Progress Report

We have examined the biochemical properties of the malignant cells from patients with cutaneous T-cell lymphomas and HTLV retrovirus associated adult T-cell lymphomas with respect to purine metabolism. The malignant cells had enzyme levels of ADA, 5'N, and PNP which were similar to normal mature T cells in contrast to immature T-cell malignancies and thymocytes (e.g., T-ALL or lymphoblastic lymphoma (1)). (See attached figures.) These results support the concept that Sezary cells are well-differentiated with respect to the T-cell axis. These data would also suggest that the ADA inhibitor, 2'-deoxycoformycin might not cause the profound metabolic effects, including accumulation of dATP, in these cells compared with thymocytes or immature malignant cells. We have been conducting these experiments *in vitro* in collaboration with Drs. B. Mitchell and C. Koller at the University of Michigan. Preliminary results suggest that this is the case; accumulation of dATP is far less in Sezary cells or HTLV+ cells than in immature T cells after exposure to 2'-dCF(2). This, in theory, would suggest one would expect less activity for 2'-dCF in CTCL than in ALL or lymphoblastic lymphoma. Though there are few data at present, early clinical studies suggest the response rates may be equivalent.

We have treated only one patient with 2'-dCF to date. Unfortunately, the patient developed bacterial sepsis on the second day of treatment which rapidly progressed to pneumonia and acute respiratory failure. The patient subsequently expired and we were unable to determine the exact role of 2'-dCF in terms of response or toxicity.

V. Future Plans

We plan to continue this study in an additional 15-20 patients and to continue the *in vitro* correlative studies. If the drug is effective but too toxic, we will alter the schedule or look for active analogs based on the *in vitro* data. We also plan to develop a rapid *in vitro* method of performing the metabolic studies by NMR in collaboration with Dr. Richard Knop.

VI. References

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T101 MONOCLONAL ANTIBODY (see section on monoclonal antibodies)

Title: OTHER TRIALS IN LYMPHOPROLIFERATIVE DISEASES

- A. Permanent Senior Staff: P.A. Bunn, D.C. Ihde, D.N. Carney, J.D. Minna, S.R. Veach, M.J. Matthews, A.F. Gazdar, J.C. Phares, P. Dainer
- B. Collaborating Senior Staff: G.P. Schechter (Hematology Section, WVAMC), L. Pearce (Hematology Section, Washington Hospital Center), R.I. Fisher (MB), E. Glatstein (ROB), T. Kinsella (ROB), R. Whittington (Radiotherapy NHBETH), D. Longo (MB), D. Garvin (Pathology NHBETH), F. Worsham (Pathology NHBETH), E. Jaffe (Pathology NCI), R.C. Young (MB), R.W. Makuch (BRB), R. Kaplan (UMCC)
- C. Collaborating Branches: Medicine, Biometric Research, and Radiation Oncology Branches (COP, NCI), Radiotherapy and Pathology Services (NHBETH), Hematology Section (Washington VAMC), Hematology Service (Washington Hospital Center), University of Maryland Cancer Center
- I. Multiple myeloma and other plasma cell dyscrasias

A. Completed therapeutic study

Introduction of non-cross-resistant combinations of chemotherapeutic agents prior to the development of tumor progression on the initial therapeutic program is an attractive strategy for dealing with the problem of drug-resistant cells presumed to be present in most human cancers. We have completed a single-arm study of two treatment programs alternated every 15 weeks in multiple myeloma, with special attention to the effects of the two regimens on M-protein levels. Therapy was begun with melphalan and prednisone (MP), which was alternated for a total of 60 weeks with vincristine + cyclophosphamide + doxorubicin + prednisone (VCAP). BCNU was added to MP from Weeks 31-45, and all treatment was discontinued at Week 61 in stable patients. Overall, 60% of 35 patients responded to therapy, with a median survival of 26 months in all treated patients. These results are similar to many other studies of single regimens given until progression in patients with equivalent prognostic factors. Three patterns of M-protein decline were found in responding patients: some responded to MP only, some to VCAP only, and some to both programs. However, there were no significant survival differences in the three groups, although all did better than nonresponding patients. To date, 12/17 patients in whom therapy was stopped after 60 weeks have relapsed. After reinstitution of MP/VCAP therapy, 5/12 patients again responded (1/6 who relapsed after less than 12 months off treatment and 4/6 who relapsed after a longer time period). We conclude that these alternating drug combinations failed to lead to major improvement in the therapy of myeloma, possibly (as suggested by the M-protein data) because they were not cross-resistant in many patients or because the interval with which the combinations were altered was too long.

B. Planned Study

Until recently, prospective randomized studies of three- and four-drug combinations and of alternating "non-cross-resistant" drug combinations

have shown no advantage over standard MP in the therapy of multiple myeloma. A recent Southwest Oncology Group trial which employs vincristine as one of four drugs in two combinations does appear to have demonstrated that alternating combinations yields superior results to MP alone. This is consistent with historical SWOG data that vincristine-containing regimens have produced superior survival. However, it is unclear from the SWOG study whether addition of vincristine to initial therapy or the alternation of two drug combinations was the critical factor in attaining the observed results. In collaboration with the Washington VA Medical Center and the Washington Hospital Center, we are initiating a study in which treatment with a four-drug combination of vincristine, melphalan, cyclophosphamide, and prednisone (VMCP) for 60 weeks will be compared in a prospective randomized fashion with VMCP alternating monthly with vindesine, doxorubicin, BCNU, and prednisone. This should directly test the value of frequent alternation of two vinca-containing programs compared to use of a single such combination. This protocol has recently been approved by most of our IRB's and should be instituted shortly.

C. Staging Study

The frequency and prognostic implications of abnormal DNA content as assessed by flow cytometry were studied in 35 patients with multiple myeloma and plasma cell dyscrasias (1). Abnormal DNA content was found in 72% of multiple myeloma patients during the course of their illness, including 57% at diagnosis, 39% in remission, and 79% at relapse. None of three patients with macroglobulinemia had aneuploidy. Aneuploid myeloma patients were more likely to have advanced stage (Salmon-Durie system) and renal failure than were those with diploid tumor cells. Multivariate analysis indicated that a significant negative association of aneuploidy with survival still existed after accounting for disease stage.

II. Diffuse Aggressive Lymphomas

A. Therapeutic study in progress in advanced (Stages II-IV) disease

Advanced diffuse histiocytic and other diffuse aggressive lymphomas can be cured with combination chemotherapy alone in a proportion of patients. Many regimens produce 25-40% long-term disease-free survival. A recent single-arm study of the Medicine Branch (NCI) appeared to yield superior results, with an estimated 60% of patients alive free of disease at four years. The therapeutic program, consisting of cycles of prednisone, high dose methotrexate, doxorubicin, cyclophosphamide, and etoposide (VP-16) alternating with cycles of standard MOPP (mustargen, vincristine, procarbazine, and prednisone), was termed ProMACE-MOPP. We are participating in a succeeding randomized study originated in the Medicine Branch, in which patients are randomized between ProMACE drugs given on Day 1 and MOPP drugs given on Day 8 (with high dose methotrexate on Day 15) of each cycle, and ProMACE (Day 1) with CytaBOM (cytarabine, bleomycin, vincristine, and high dose methotrexate) on Day 8. The purpose of the study is the early introduction (within one week) of presumably non-cross-resistant combination chemotherapy and the evaluation of

whether the possibly less toxic CytaBOM regimen, which is based on COMLA and similar regimens from Yale University and the University of Chicago, might be as effective as MOPP in this disease setting. We have contributed six patients to this study, all in recent months. Overall, with 47 patients randomized, the two study plans have similar complete response rates of 75% and 81% in evaluable patients, with only one complete responder relapsing to date. The toxicities of the two regimens appear similar, with 4% treatment-related deaths.

B. Therapeutic study in progress in Stage I diffuse aggressive lymphomas

The results of treating laparotomy-staged Stage I diffuse aggressive lymphoma with radiotherapy alone are excellent, with over 70-90% disease-free survival. However, without laparotomy staging, survival is not as good when irradiation is used as the sole therapeutic modality. Recently, some groups have treated non-laparotomy staged patients with combination chemotherapy, with or without involved field irradiation. In a study originated in the Medicine and Radiation Oncology Branches (NCI), patients are given four cycles of reduced dose ProMACE-MOPP chemotherapy followed by involved field radiotherapy. We have contributed one of 14 patients to this study. Thus far, toxicity has been very modest and 8/9 patients completing therapy remain in complete remission.

III. Indolent or favorable histology lymphomas

A. Therapeutic study in progress for advanced (Stages III-IV) indolent lymphoma.

This study, originated by the Medicine and Radiation Oncology Branches (NCI), compares a "watch-and-wait" policy of symptomatic irradiation only with immediate aggressive ProMACE-MOPP chemotherapy followed by consolidative low-dose total lymphoid irradiation in previously untreated patients not requiring immediate treatment. We are planning to enter eligible patients who accept randomization into this study. Thus far, 18 patients have been randomized to immediate aggressive treatment (83% complete responses and 1 death), 14 were judged to require immediate aggressive treatment (69% CR's and 4 deaths), and 19 were randomized to "watch and wait" (7 have required aggressive treatment with 1 CR and 2 deaths).

IV. Hodgkin's Disease

A. Therapeutic study in progress in advanced Hodgkin's disease

Previously untreated patients with Stages IIIA-2, IIIB, and IV Hodgkin's disease are entered on this study originated by the Medicine Branch and the University of Maryland Cancer Center. The patients are randomized between a minimum of 6 courses of standard MOPP and alternating monthly courses of MOPP and SCAB (streptozotocin, CCNU, doxorubicin, and bleomycin) for a minimum of 6 months. SCAB has been shown to produce complete remissions in patients failing other chemotherapy, and this trial is attempting to evaluate the merits of alternating non-cross-resistant

chemotherapy in Hodgkin's disease. We have contributed four patients to this study, all but one in recent months. Overall, 28 patients have received MOPP, with 83% complete remissions in patients with six month follow-up, 3 relapses, and 3 deaths; and 37 have received MOPP/SCAB, with 84% CR's in evaluated patients, 5 relapses, and 2 deaths. The study is early, but disease-free survival results are excellent and MOPP certainly does not appear inferior to the alternating regimen.

B. Therapeutic study in progress in Hodgkin's disease with massive mediastinal involvement

We have contributed one patient to this study originated by the Medicine and Radiation Oncology Branches (NCI). Patients receive six cycles of monthly MOPP alternating with monthly ABVD (doxorubicin, bleomycin, vinblastine, and DTIC), followed by 3500-4000 rad to a mediastinal field, with the initial 1500 rad to the original extent of disease. This study attempts to improve on the minor survival benefit which has been associated with previous combined modality programs for massive mediastinal Hodgkin's disease that employed a single chemotherapeutic regimen and radiotherapy. Fourteen patients have been entered on this program (7 very recently). Of the initial 7, six are in complete remission and one has relapsed.

C. Therapeutic study in progress in early stage Hodgkin's disease

We are planning to enter consenting patients on this study originated by the Medicine and Radiation Oncology Branches, and have submitted the protocol to our IRB's. In this protocol, patients with pathologically-staged Stages I, II, and IIIA-1 disease are randomized between appropriate radiotherapy and MOPP chemotherapy. Thus far, 26 patients have received radiotherapy, with 24 complete responses and 9 relapses. Twenty-seven patients have received MOPP, with 17/19 CR's in patients with sufficient follow-up, 1 relapse, and 1 death.

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Title: MISCELLANEOUS CLINICAL TRIALS

- A. Permanent Senior Staff: D.C. Ihde, P.A. Bunn, D.N. Carney, J.D. Minna, S.R. Veach
- B. Collaborating Senior Staff: R.C. Young (MB), C.E. Myers (CPB), R.F. Ozols (MB) R.W. Makuch (BRB), K. O'Connell (Urology Service, NHBETH), M.H. Zweig (Clin. Chem. NIH), W. Belville (WRAMC), H. Bensimon (Urology Serv. WVAMC), N. Javadpour (SB)
- C. Collaborating Branches: Medicine Branch, Biometric Research Branch, Surgery Branch, and Clinical Pharmacology Branch (COP, NCI), Urology Service (NHBETH), Clinical Chemistry Service (NIH), Urology Services (WRAMC and Washington VAMC)
- I. Carcinoma of the prostate

A. Completed Therapeutic Study

A previous trial of combination chemotherapy with cyclophosphamide and doxorubicin in Stage D-2 prostatic cancer was conducted by the NCI-VA Medical Oncology Branch in men who had progressive tumor after one or more hormonal manipulations. This demonstrated to our satisfaction that 1) tumor regressions can occur at a rate of 25-30% in advanced prostatic cancer treated with cytotoxic chemotherapy, and 2) with careful serial multiple staging procedures, these regressions can be objectively documented. We therefore began to treat newly diagnosed Stage D-2 patients with combination chemotherapy, followed by hormonal therapy at the time of progressive tumor. Our goals were 1) to establish the feasibility and safety of initial chemotherapy for ambulatory patients; 2) to compare the objective response rate of initial chemotherapy with the response rate of the same or a similar regimen given only after progression on hormonal treatment; 3) to determine if hormonal sensitivity is retained by prostatic cancer after progression on initial chemotherapy and 4) to identify a suitable drug regimen for use as an adjuvant to hormonal manipulation in a prospective randomized trial of hormones with immediate chemotherapy versus hormones with chemotherapy delayed until disease progression in Stage D-2 disease.

Cyclophosphamide 600 mg/m² + doxorubicin 50 mg/m² + cisplatin 60 mg/m² (CAP) given IV every 3-4 weeks was the regimen employed. Among 25 previously untreated patients, CAP produced 33% partial responses, 29% stable disease for four months or more, and 38% non-responders in 21 evaluable patients. Four patients refused therapy after 1-2 cycles of CAP and were not evaluated for response, but did not have inferior survival. After disease progression on CAP, 68% of 19 patients thus far evaluated had partial responses to hormonal manipulation with orchiectomy or diethylstilbestrol 1 mg/day. Median survival of all 25 patients receiving CAP as initial treatment for prostatic cancer will be approximately 24 months. Among 11 patients given CAP only after failing hormonal therapy, 18% had partial response and 45% had stable disease for at least four months. No unanticipated or fatal toxicities were observed in either group of patients.

We concluded that CAP could be safely given to ambulatory Stage D-2 patients with prostatic cancer as initial treatment. The objective response rate does not appear to be substantially higher than in our experience with CAP and CA in men treated after hormonal failure. Sensitivity to hormonal manipulation is retained after disease progression on combination chemotherapy. In part due to the lack of any major progress in systemic treatment of prostatic cancer for the past 40 years, we believe a prospective randomized trial investigating the value of immediate chemotherapy in conjunction with hormones at the diagnosis of distant metastatic prostatic cancer should be performed. Due to constraints on available patients with our Branch's move to Naval Hospital Bethesda, we do not intend to pursue studies in metastatic prostatic carcinoma in the near future.

B. Staging Studies

The clinically dominant manifestation of metastatic prostatic cancer is osteoblastic bone metastases in most patients, leading to considerable difficulty in assessing objective tumor response in this disease. For this reason, most clinical trials in prostatic cancer have used survival as an endpoint. This is a very inefficient way to evaluate new forms of treatment, because of great variability in survival even in patients with bony metastases. Methods of determining objective tumor response would allow traditional single arm Phase II trials to be conducted in prostatic cancer with greater confidence.

Over the past several years we have performed numerous staging procedures prior to and every four months during chemotherapeutic treatment of 55 patients with Stage D-2 prostatic cancer. No patient was considered to have bony metastases solely on the basis of a positive radionuclide bone scan. Abnormal bone films or a positive bone marrow biopsy were required. Tests performed included bone X-rays, intravenous pyelograms, lymph-angiography, computed tomography of the pelvis and abdomen and physical examination of the prostate and peripheral lymph nodes. Substantial changes in any of these tests were considered to represent objective tumor response or progression. Other studies evaluated concurrently were radionuclide bone and liver scans, bone marrow biopsy, serum acid phosphatase determinations by enzymatic and radioimmunoassays (RIA), plasma carcinoembryonic antigen (CEA), creatine kinase-BB (CK-BB) by RIA, performance status, and weight.

We found that radionuclide bone scans showed improvement in approximately half of patients with objective response, although this improvement sometimes lagged several months behind other evidence of response. In some responding patients, bone scan and particularly bone x-rays worsened, presumably due to remodeling of normal bone around the tumor (1). In a small number of patients, a radioimmunoassay for acid phosphatase developed at Walter Reed Army Medical Center was no better than standard enzymatic acid phosphatase in documenting tumor response and progression (2). In a much larger number of patients, neither of two commercially available RIA's for acid phosphatase nor an RIA for CK-BB was superior to the alpha-naphthyl phosphate enzymatic acid phosphatase assay for

monitoring the patients' clinical course. Lymphangiography detected evaluable tumor in lymph nodes in 50% of patients but was of limited value in response assessment because of elution of contrast. CT scan of the abdomen and pelvis was less often positive (32% abnormal nodal enlargement) but proved quite useful in the documentation of tumor response and progression. A combination of prostatic and physical examination, bone X-rays and bone scan, plasma CEA, serum acid phosphatase, CT scan of the abdomen and pelvis, and weight and performance status can document or strongly suggest tumor status in most patients.

II. Hepatocellular Carcinoma

A. Completed Therapeutic Study

The prognosis of most patients with hepatocellular carcinoma (HC) is dismal, with median survival from diagnosis of 1-5 months in most large series. Doxorubicin was the first chemotherapeutic agent identified by ourselves and others which had reproducible, though very modest, anti-tumor activity in HC. In a trial of the Eastern Cooperative Oncology Group streptozotocin and oral 5-fluorouracil produced similar response rates and survival to doxorubicin, while oral 5-fluorouracil alone had no activity and inferior survival. Since doxorubicin (ADR) and streptozotocin (STZ) have virtually non-overlapping toxicities and both are probably active in HC, we initiated a Phase II trial of ADR/STZ in HC (3).

Because of their extremely poor prognosis, severely jaundiced and fully bedridden patients were excluded. ADR 60 mg/m² Day 1 and STZ 0.5 g/m² Days 1-5 were given IV every three weeks to 23 patients. Partial responses lasting 10 and 14 months occurred in two patients (9%), while one additional patient had stable disease for 15 months. Median survival was only 3 months, but eight patients (35%) lived over 12 months. No drug-related deaths were observed. Physical examination and radionuclide liver scan together documented all tumor responses and progressions, although CT scan of the liver and serum alpha-fetoprotein (AFP) often provided confirmatory information. We concluded that ADR/STZ has only modest activity in HC and appears no more active than ADR alone. Because of constraints on patient accrual with the Branch's move to Naval Hospital Bethesda, further therapeutic studies in HC are not planned.

B. Prognostic Factors

Over a six-year period, we treated 35 HC patients with systemic chemotherapy with either ADR or ADR/STZ. Median survival of all patients was 3-4 months, a typical result. However, 11 patients (31%) lived over 12 months. These patients with longer survival fell into two groups. The first had "typical" HC, with one or more of the features of cirrhosis, elevated levels of AFP in the serum, or serologic markers of hepatitis B infection. All had good performance status without jaundice initially, and most responded to chemotherapy. Caucasian patients under the age of 25 comprised the second group; none had cirrhosis, elevated AFP, or hepatitis B markers, and few responded to chemotherapy. All but one,

however, appeared to have fibrolamellar carcinoma pathologically, with broad bands of fibrous tissue within the tumor, and all but one had distant metastases at diagnosis. Median survival of all six young Caucasian patients was 24 months, compared to 3 months for the 29 cases of "typical" HC. Fibrolamellar carcinoma appears to be an indolent form of HC, as previously described. We have documented that this relatively prolonged clinical course occurs despite the frequent presence of distant metastases and the lack of response to chemotherapy.

C. Review Article

A review article on clinical features, staging, and treatment of HC has been completed (4).

III. Carcinoma of the Stomach

A. Completed Therapeutic Study

We have completed a Phase II trial of ICRF-159 in advanced gastric cancer (5). No responses were seen in 10 previously untreated patients and 11 patients who had failed combination chemotherapy. ICRF-159 has been incorporated into several combination regimens for gastric cancer, despite the absence of any data indicating single-agent activity in this tumor. Our negative results perhaps partially explain why combination programs including ICRF-159 have been unsuccessful in the treatment of stomach cancer. No further studies in gastric cancer have been planned since our Branch's move to Naval Hospital Bethesda.

IV. Testicular and Germ Cell Carcinoma

A. Therapeutic Study in Progress

The prognosis of patients with metastatic testicular and germ cell tumors when treated with aggressive chemotherapy regimens such as PVB (cisplatin + vinblastine + bleomycin) is excellent, with the great majority of patients attaining prolonged disease-free survival. However, results are less encouraging in patients with larger tumor bulk, defined as massive pulmonary or abdominal disease or both. Figures are not precise because of varying definitions of poor-risk disease, but only 25-50% of such patients are cured with current treatment. We are participating in a prospective randomized trial originated by the Medicine Branch, in which poor risk patients receive either standard PVB or PVBV, a new regimen with a doubled dose of cisplatin (40 mg/m² IV qd x 5 given in hypertonic saline with 250 ml/hour normal saline hydration for six days) and the newly identified active drug VP-16 (100 mg/m² IV qd x 5). The NCI-Navy MOB has contributed 20% of 25 patients, all but one in recent months. Nephrotoxicity has not been a problem on PVBV, probably due to vigorous hydration and administration of cisplatin in hypertonic saline, a maneuver which prevents cisplatin nephrotoxicity in rodents. There have been three toxic deaths (sepsis in two, pulmonary fibrosis in one) on PVBV and none on PVB. Complete response rate is

higher (86% vs. 70%) on PVBV, as is the number of patients alive without evidence of tumor (79% vs. 57%). Although these results are certainly not yet significantly different, the experience with PVBV is very encouraging.

V. Phase I and Phase II studies with investigational agents

A. Completed studies

Three Phase I studies have been completed and published in the past two years in collaboration with the Clinical Pharmacology and Medicine Branches. Pentamethylmelamine (PMM) was studied in 34 patients (60% contributed by NCI-VA MOB), because of the desire to test a water-soluble analog of hexamethylmelamine suitable for parenteral administration. Eight dose levels were given as a weekly 1-hour IV infusion. Nausea and vomiting were dose-limiting, with 1500 mg/m² being the maximum dose administered and 1000 mg/m² the maximum tolerated dose. Depressed levels of consciousness were also observed, but consistent myelosuppression was not. Pharmacokinetic studies were performed at all dose levels. Emesis associated with PMM on this schedule was so severe that the drug did not enter Phase II trials on this schedule (6). Aziridinylbenzoquinone (AZQ) is a quinone derivative with high lipid solubility designed as a drug which should achieve high dose levels in the central nervous system. Intracerebrally inoculated murine tumors can be cured with IP injections of AZQ. AZQ was given as an IV infusion on Days 1 and 8 every four weeks to 40 patients (one-third contributed by NCI-VA MOB) (7). Myelosuppression was dose-limiting, with 20 mg/m² on Days 1 and 8 being the maximum tolerated dose. Pharmacokinetic studies were performed, and the drug was shown to enter the cerebrospinal fluid in three patients. AZQ has since been found to have some activity in primary brain tumors. Dichloromethotrexate (DCM) was administered to 21 patients at the NCI-VA MOB as a weekly 6-hr IV infusion, with escalating doses every other week (8). Myelosuppression and mucositis were dose-limiting, with 400 mg/m² x 2 weeks, 800 mg/m² x 2 weeks, and then 1200 mg/m² weekly being the maximum tolerated dose-escalation schedule. Plasma concentrations were measured during and after 61 infusions, and pharmacokinetics appear similar to the parent drug methotrexate. Of note, three of seven patients with hepatocellular carcinoma who were treated had partial responses lasting 22-67 weeks. DCM or methotrexate should be evaluated further in this tumor.

B. Planned Studies

Two Phase II studies with CBDCA, a cisplatin analog with little or no renal toxicity in Phase I trials, have been submitted to the Investigational Review Boards of the Naval Hospital Bethesda. The drug will be given as a 24-hour infusion to patients with non-small cell lung and breast cancer. The schedule was chosen because of the short terminal half life of CBDCA which was documented by the Clinical Pharmacology Branch in a recent Phase I trial. The first agent to be studied in

a Phase I trial by the NCI-Navy MOB will be tiazofurin, a false nucleoside structurally related to the antiviral agent ribavirin. The drug inhibits synthesis of guanine nucleotides through its nicotinamide adenine dinucleotide conjugate (TAD). Because of superior results with frequent dose administration in rodent tumors, tiazofurin will be given as a 5-day continuous infusion. It is of interest that tiazofurin produces striking inhibition of clone formation in soft agarose of 3/6 of our lung cancer cell lines which were tested recently, and TAD levels are much higher in the lines which were relatively more sensitive to the drug.

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TITLE: PROTOCOL 82-2 "TREATMENT OF CLINICAL STAGE I AND II CARCINOMA OF THE BREAST WITH MASTECTOMY AND AXILLARY DISSECTION VERSUS EXCISIONAL BIOPSY AXILLARY DISSECTION AND DEFINITIVE IRRADIATION"

I. Personnel

A. Permanent Senior Staff: Steven R. Veach, M.D., Daniel C. Ihde, M.D.; Richard Whittington, M.D. (Radiation Oncology, NHBETH); and Robert Cochran, M.D. (Surgical Service, NHBETH)

B. Fellows: Jeffrey M. Crane, M.D.

II. Collaborators: Allen Lichter, M.D., and Marc Lippman, M.D.

III. Introduction

A. Objectives and Rationale

This study compares conservative and cosmetically superior treatment with removal of the malignant breast mass followed by radiation therapy with the more traditional therapy of modified radical mastectomy. Local control, survival, upper extremity function and cosmesis will be analysed. This is a study from the Radiation Oncology Branch of the National Cancer Institute in combination with the Surgery and Medicine Branches which has been submitted for use at this institution.

IV. Progress Report

This study has not accessioned patients to date (although at this institution patients are being offered a choice between the two alternatives) because of continuing discussions and reservations of the surgery department. The concern is that the surgery can be carried out in the exact manner that the protocol prescribes in section 7.22, 7.3 and 7.4 in an institution that needs to have several different surgeons and surgical residents during the breast biopsies and mastectomies.

V. Future Plans

This concern of the Naval Hospital Surgery Department is currently being addressed in an attempt to have the responsible persons at the National Cancer Institute Surgery Branch and Radiation Oncology Branch work out the details to meet the satisfaction of the department of surgery. If this cannot be resolved in a successful manner the study may be dropped at this institution.

TITLE: PROTOCOL 82-4 "EVALUATION OF CHEMOHORMONAL CHEMOTHERAPY AND BREAST CARCINOMA PATIENTS WITH NO EVIDENCE OF DISEASE FOLLOWING AN EXCISED OR CURATIVELY IRRADIATED RECURRENCE"

I. Personnel

A. Permanent Senior Staff: Steven R. Veach, M.D.

B. Other Professional Staff: Joyce Eddy, R.N.

II. Collaborators: Marc Lippman, M.D., Jane Cassidy, R.N. and Margaret Wesley, R.N. (Medicine Branch, NCI)

III. Introduction

A. Objectives and Rationale

All patients with breast cancer who have had no prior chemotherapy or hormonal therapy have had the first recurrence completely treated with radiation or surgery will be randomized between immediate systemic treatment with CMF/Tamoxifen for one year vs observation and treatment with the same regimen at the second relapse. The objective would be to determine which approach will most positively impact survival with the idea that a few patients may be cured even after a single relapse.

IV. Progress Report

The branch has placed only three patients on this study since its approval in October, 1982 and this is expected since this is primarily a group of stage I patients of which only approximately 20% are expected to relapse and a single site of relapse will be even a smaller percentage of stage I patients. I would anticipate only 3-5 patients would be entered on this study developed at the Medicine Branch of the National Cancer Institute and has been submitted for accessioning patients at this institution.

V. Future Plans

We anticipate the continued accessioning of patients on this study with perhaps the inclusion of patients from other nearby Naval facilities.

Title: PROTOCOL 82/83-10 "A MULTIMODALITY PROGRAM TO INCREASE THE COMPLETE RESPONSE RATE IN METASTATIC BREAST CANCER"

I. Personnel

- A. Permanent Senior Staff: CDR S. Veach, MC, USN, Dr. P.A. Bunn, Jr.
(Principal Investigators)
Associate Investigators: Drs. D.C. Ihde, D.N. Carney, J.D. Minna,
R. Whittington (MC, USN, Radiotherapy)
- B. Clinical Associate/Medical Staff Fellows: Dr. C. Winkler
- C. Other Professional Staff: None
- D. Technical Staff: None

II. Collaborating Units/Branches:

Dr. M.E. Lippman (MB, NCI), C.S. Bagley, R.N. (NCI)
Dr. P. Findlay (ROB, NCI), Dr. D. Danforth (SB, NCI)
Dr. R. Young (MB, NCI), Dr. A. Lichter (ROB, NCI)
Dr. H. Smith (Peralta Cancer Research Inst.)

III. Introduction

A. Objectives:

This protocol is a pilot study to attempt to improve the response rate and duration in patients with metastatic breast cancer designed by the Medical Breast Cancer Section (Dr. M.E. Lippman), MB, NCI.

The objectives are:

1. To evaluate a new and substantially different approach to chemotherapy of metastatic breast cancer using intensive near continuous anti-tumor therapy;
2. To increase response to S phase specific anti-metabolites with the stimulatory hormone, premarin;
3. To test the notion that some patients achieving less than a complete response can be rendered free of disease by combined local and systemic therapy;
4. To help assess the usefulness of a new tumor cell culturing technique to predict drug sensitivity;
5. To evaluate prospectively the optimal timing of hormone stimulation of breast cancer cells prior to drug administration.

B. Rationale and Background:

The proposed study is based on a variety of assumptions with respect to the management of breast cancer and they are summarized in the introduction to the protocol.

C. Specific Aims and Outline of Methodology:

This protocol examines a new approach to the management of patients with metastatic breast cancer. Patients will receive intensive induction chemotherapy with premarin (P), cytoxan (C), adriamycin (A), high dose methotrexate and leucovorin rescue (M), and vincristine (V) (Cycles of PCAMF alternating with PMFV) for 24 weeks. Patients achieving either a complete remission or having limited residual macroscopic disease ("convertible PR") will receive local treatment (surgery or radiotherapy) to prior or residual local bulk disease. The goal of this latter therapy is to prevent relapse in sites of previous bulk disease (for complete responders) or to render the patient free of residual tumor in the case of some partial responders. Patients will then receive maintenance chemotherapy (PCAMP alternating with PMFV for 4 weeks, maintenance cycle A, and then premarin, mitomycin C (M:), vindesine (Vd), PMiVd for 4 weeks, maintenance cycle B). In addition, this protocol will include ancillary receptor, kinetic, and drug sensitivity assays to help assess those subsets of patients most likely to benefit from this approach and to guide further studies.

IV. Progress Report:

The protocol is being submitted to both NCI and Navy protocol review boards.

V. Future Plans:

Protocol activation and assessment of 20-25 patients. At the end of 2 years an assessment of the success of this approach can be made with respect to the cost-benefit to the patient of this more aggressive style of systemic therapy. At that time (if warranted) consideration will be given to a follow-up randomized trial.

VI. Publications:

None.

Title: CLINICAL SEROTHERAPY TRIALS

I. Personnel:

- A. Permanent Senior Staff: Drs. P.A. Bunn, Jr., J.D. Minna, M.J. Matthews, A.F. Gazdar, D.C. Ihde, D.N. Carney.
- B. Clinical Associates: Drs. C.F. Winkler, J. Mulshine, A. Doyle.
- C. Other Professional Staff: Dr. F. Cuttitta, Dr.S. Fargione, J. Eddy, R.N.
- D. Technical Staff: P. Jewett, J. Fedorko, R. Hubert (Med Tech).

Collaborating Units: K.Foon, R. Oldham, R. Schroff (BRMP); E. Glatstein, A.S. Lichter, G. Morstyn (ROB,NCI); T. Waldmann (Metab.B., NCI); S. Broder (AD COP, NCI); S.M. Hsu (Path., NCI); S. Larson (Nuc. Med, NIH).

Background:A. Serotherapy

The antitumor effects of the passive administration of heteroantisera have been well documented in animal systems. In man, while antitumor effects of heterologous antisera have been reported, the responses have generally been short in duration. We have had considerable experience with the treatment of patients with T-cell lymphomas and other lymphomas using heterologous anti-thymocyte globulin (ATG) prepared by Upjohn. We treated 8 patients with 1 gm of ATG daily for 14 days. While 5 of the patients had objective antitumor effects, only one patient had a sustained response lasting more than one month. There was considerable toxicity including development of fever in all patients, serum sickness in 2 patients and severe infections in 2 patients. Six patients with unfavorable histology lymphoma were also treated at the NCI, 3 of whom had objective antitumor response which lasted 1, 2 and 6 weeks respectively. Similarly, Raafat et al reported short lasting responses in 3/4 patients with cutaneous T-cell lymphomas treated with ATG. The major limitations of this approach were the severe allergic reactions due to the large amounts of foreign protein administered, reactions with other cells due to the presence of a wide variety of antibodies, the extensive absorptions required, the variability from lot to lot with low titer of activity and the short duration of the responses.

The hybridoma technique developed by Kohler and Milstein circumvented many of the problems by allowing for nearly unlimited quantities of relatively pure monoclonal antibody. Monoclonal antibodies developed by this method have been shown to inhibit the growth of a variety of animal tumors. More recently, in vivo anti-tumor effects of monoclonal antibody have been demonstrated in humans. Nadler et al treated a patient with lymphosarcoma with a monoclonal antibody directed against a lymphoma associated antigen; Miller and Levy treated several patients with T-cell malignancies with Leu 1 (L17F12) a pan T-cell antibody. Ritz et al treated 4 patients with acute lymphoblastic leukemia with a monoclonal antibody (J5) which reacts with the common ALL antigen; Dillman and Royston treated several patients with CLL and T-cell lymphoma with T101 a pan T-cell antibody similar to Leu 1; and Levy et al treated a lymphoma patient with an anti-idiotypic monoclonal antibody. Preliminary conclusions to be drawn from these studies include: (1) doses from 1 mg to 1500 mg can be administered safely but should be given slowly over 1 hour or more to prevent immediate side effects; (2) patients with circulating

tumor cells usually have a rapid drop in leukemic cell count within hours but this usually returns to pretreatment levels within 4 to 24 hours. However, when antibody is infused every 3-4 days over weeks, a sustained decrease in peripheral cell count may be observed in some patients; (3) skin lesions and lymph nodes can regress following monoclonal serotherapy; (4) leukemic cells in bone marrow may be coated with antibody but may not decrease in number suggesting that cell lysis may occur in the reticuloendothelial system and may not be mediated by complement; (5) malignant cells may undergo antigenic modulation after in vivo or in vitro antibody administration; (6) free antigen may be detected in the serum after antibody administration and may be cleared within 2 days and (7) antimurine antibodies will develop in some patients given murine monoclonal antibodies.

B. Tumor Imaging

The concept of using radioactive antibodies for tumor detection and localization dates back to the 1940's when Pressman et al showed that rabbit antirat kidney antibodies could be labelled with ^{131}I and localized in the kidney after intravenous injection. In subsequent studies using radiolabeled heterologous antibodies it was possible to show specific tumor uptake in a variety of in vitro and in vivo models and in human tumors. For example, Goldenberg and coworkers demonstrated that heterotransplanted human tumors could be localized by external scintigraphy using ^{131}I labeled anti-CEA antibody. These studies were extended to humans where the same workers were able to detect 50-91% of tumor bearing areas using this technique. Unfortunately, other investigators using similar techniques have been less successful in tumor imaging. Mach et al were able to obtain positive scans using ^{131}I anti CEA in only 42% of 53 patients with CEA producing tumors.

More recently, tumor imaging employing monoclonal antibodies has been successfully applied in animal models and in humans. Xenografts of human osteosarcomas and human germ cell tumors were successfully imaged using ^{125}I or ^{131}I labeled antibodies. Mach et al radiolocalized human tumors in 14 of 28 patients using ^{131}I labeled anti-CEA monoclonal antibodies. Epenetos et al obtained positive scans in 15 of 19 patients with ovarian, breast and GI tumors using ^{123}I labeled HM FGI or HMF G2 monoclonal antibodies. Larson et al were able to show tumor specific localization of in 22 of 28 metastatic lesions in patients with metastatic malignant melanoma using ^{131}I labeled anti p 97 monoclonal antibody with no false positives.

C. Therapeutic Applications of Radiolabeled Antibodies.

Antitumor effects of radiolabeled antibodies have been demonstrated in animal models and more recently in humans using both heterologous and monoclonal antibodies. The use of heterologous radiolabeled antibodies in humans has been pioneered by Order and coworkers at Johns Hopkins. They have treated patients with hepatoma and biliary tract cancers with ^{131}I radiolabeled anti-CEA and anti-ferritin. Radiolabeled ^{131}I anti-ferritin was given to 5 patients with hepatoma with objective tumor response in 4 patients lasting 3+, 5+, 7 and 9 months. The patients received 100-150 mCi of ^{131}I labeled antiferritin IgG. Dosimetric studies revealed a 3 day effective half life, low dose rate isotopic radiation to tumors at a rate of 5 rad per hour, a total dose of 2000-

3000 rad to the tumor and a tumor half life of 7.7 days. Toxicity was primarily due to total body irradiation of 1 to 1.5 rad per mCi manifest by leukopenia and thrombocytopenia which was marked in only one patient. This group has treated at least 5 other patients with colon cancer, lung cancer, head and neck cancer and intrahepatic biliary cancer with radiolabeled anti-CEA. Tumor localization was observed by scanning in 4 of the 5 patients. No significant therapeutic effect was noted in 4 extrahepatic cancers at doses up to 100 mCi. One of the difficulties in assessing studies from this group has been that most patients have received systemic chemotherapy and external beam irradiation in addition to the radiolabeled antibody.

Larson et al administered therapeutic doses of radiotherapy using ^{131}I anti-p 97, a monoclonal antibody directed against a melanoma antigen. The $\text{F}(\text{ab})_2$ fragments are utilized in his studies. The major toxicity to date has been myelosuppression from the whole body dose of irradiation. To date there have been no objective tumor regressions although dosimetric calculations suggest doses as high as 6000 rad have been delivered to the tumor.

II. Objectives

1. To evaluate the clinical antitumor effects, dose-response relationships and toxicity of murine monoclonal antibodies in patients with cutaneous T-cell lymphomas and chronic lymphocytic leukemia using T101 and in patients with lung cancer using antibombesin or other monoclonal antibodies.

2. To monitor for biologic effects of monoclonal antibodies including antibody binding in various tissues, free antibody, antigenic modulation, development of antimurine antibody formation and changes in free antigen levels.

3. To evaluate the biodistribution of monoclonal antibodies and assess the usefulness of tumor imaging with radiolabeled monoclonal antibodies.

4. To evaluate the antitumor effects, and toxicity of radiolabeled monoclonal antibodies including dosimetric calculations.

5. To determine the ability of in vitro models to predict the effectiveness of labeled and unlabeled monoclonal antibodies.

III. Progress Report

A. Serotherapy with T101

Seven patients with cutaneous T cell lymphomas have been entered to date (4/83). Two patients were treated with semi-weekly doses of 1 mg; neither had objective tumor response, neither had toxicity and neither had detectable binding of in vivo administered antibody in cutaneous lesions. Both patients also received the 10 mg dose. One patient had improvement in cutaneous lesions and a 50% reduction in circulating cells but no change in lymphadenopathy, hypercalcemia or abnormal liver function tests. The

other patient had no improvement in skin lesions. No toxicity was noted in either patient.

Three patients were entered at 10 mg semiweekly, and 2 of these were increased to 50 mg after 4 weeks when no improvement and no toxicity had occurred. Skin lesions improved in all 3 patients but did not clear entirely and there were no changes in palpable lymphadenopathy. Circulating tumor cells were present in 2 of the 3 patients and were unchanged in one and decreased by less than 50% in the other.

Two patients were entered at the 50 mg dose level, one of whom received 100 mg after 4 weeks at 50 mg. Neither patient had objective anti-tumor response.

Toxicity: Fevers ranging from 99^o to 102^o F occurred during or after the antibody infusion in 5/7 patients but was generally mild and relieved by Tylenol. Three of the patients (2 at 50mg and one at 10 mg) developed chest tightness and shortness of breath during the antibody infusions, none during the first infusion. The symptoms cleared within one minute of discontinuing the antibody in all patients. There were no wheezes. One of these patients developed a small pulmonary infiltrate on chest x-ray which gradually resolved over 2 weeks. There were no other toxicities.

Skin biopsies were obtained 2 to 24 hours after the T101 infusions and examined for in vivo labeling by an ABC immunoperoxidase technique. No staining was observed after the 1 mg dose; weak staining was observed in some instances after 10 mg, and more intense staining was observed after 50 mg. In vitro addition of excess antibody after 10 mg increased the intensity of staining, suggesting that all the antigenic sites were not saturated. Staining was more intense in the upper dermis than the lower dermis after the 50 mg dose suggesting an uneven distribution of antibody, most likely due to the vascular supply rather than heterogeneity of expression since prior to therapy all areas were equally stained when excess antibody was added in vitro. There was evidence of in vivo modulation after the 50 mg dose since the intensity of stain in her upper dermis was less after addition of excess in vitro antibody compared to pretherapy staining while the staining in the lower dermis was the same as pretherapy.

Ten patients with CLL have been treated with T101. Three patients were treated with 1 mg of T101; one had no change in circulating blood counts and 2 had transient reductions in circulating CLL cells which rose to baseline levels over 6-12 hours. None had toxicity.

Three patients were entered at the 10 mg dose level; 2 of the 3 had a greater than 50% reduction in circulating CLL counts but none had a change in lymphadenopathy or organomegaly. There was little toxicity at this dose level except mild fever and transient urticaria in 2 patients.

Four patients were entered at the 50 mg dose. Two patients were removed from study because of the acute onset of chest tightness and shortness of breath during the first and third infusion respectively. One of these had a perfusion abnormality on lung scan which resolved within 2 days and which was not associated with a change in PO₂. The other 2 patients were treated

with prolonged infusions of T101 over 50 hours without toxicity except for mild fever in both and transient urticaria in one. There were transient decreases in circulating CLL counts in these patients but none of the patients had changes in adenopathy or organomegaly.

Serial measurements of antigen expression and on circulating and bone marrow cells showed striking evidence of profound antigen modulation as represented by a decrease in mean fluorescence intensity. The antigenic modulation was more marked with higher doses and with prolonged antibody infusions. The severe antigenic modulation observed with the 50 mg dose given over 50 hours presumably contributed to the ineffectiveness of the antibody. In vivo binding to lymph node cells was not observed in one patient following the 10 mg dose.

The proportion of circulating cells labeled by in vivo antibody administration was dose dependent. At the 1 mg dose only 25% of the circulating cells were labeled, 50-75% were labeled after 10 mg and 75-90% were labeled after 50-100 mg doses. The inability to label all cells at higher doses can be explained by heterogeneity of receptor density and modulation.

None of the CLL patients developed detectable antimurine IgG presumably due to the defects in humoral immunity in CLL patients. None of the patients with shortness of breath developed an IgE antimurine response.

We have drawn several preliminary conclusions from these studies: (1) Circulating CLL cells are stained with in vivo administered T101 in a dose dependent manner are cleared rapidly from the circulation but reappear after antibody is discontinued; (2) Some patients had sustained reductions in circulating CLL counts but none had improvement in adenopathy or organomegaly; (3) Higher doses of antibody are required for CLL cells in lymph nodes or bone marrow and for CTCL cells in the skin. (4) Antigen modulation is a dose and schedule dependent phenomenon which has been demonstrated for circulating cells, bone marrow cells and cutaneous cells; (5) While some improvement in skin lesions were noted there have been no sustained partial remissions to date; (6) A potentially serious side effect of shortness of breath was frequent and appeared to be related in part to the number of circulating cells and the dose. It could be reduced by lengthening the duration of antibody therapy. It appeared to be due to leukoagglutination leading to microemboli in the lungs rather than anaphylaxis or allergic reactions.

B. Radioimaging with T101

A protocol for scanning patients with radiolabel T101 with ^{111}In and ^{131}I has been submitted. A contract has been established with Hybritech for production of the monoclonal antibodies, and labeled antibody has been produced and delivered. Preliminary experiments have shown no loss of antibody affinity from the labeling process.

C) In Vitro Models

We have established in vitro models for testing labeled and unlabeled monoclonal antibodies against T-cell lymphomas and lung cancers in vitro. Serum free conditions have been established for the growth of T-cell lymphomas in liquid culture and in cloning assays in soft agar, and for the growth of lung cancers in liquid culture and soft agar. Preliminary experiments in other NCI laboratories and in our laboratory have shown that differentiation antigens such as T101 have no effect on the in vitro growth or cloning of normal or neoplastic T cells. On the contrary, monoclonal antibodies against the TCGF receptor (anti-Tac) or transferrin receptor have profound effects on both. Dose response relationships are under investigation. Similarly, we have shown that monoclonal antibodies against growth factors for lung cancer may cause significantly more growth inhibition than antibodies against differentiation antigens (vide infra).

We have shown that some antibodies (e.g., T101) produce significant modulation in vitro, that this also occurs in vivo and may limit the in vivo usefulness of some antibodies.

IV. Future Plans

A. Serotherapy. We plan to complete the T101 trial using the highest antibody doses (100 mg). The protocol for Anti-Tac is approved and one patient has been treated. More patients will be entered. The anti idiotypic monoclonal antibody protocol has been submitted and samples from several patients have been obtained for attempts at production of anti idiotypic antibody at the BRMP. Protocols for the use of anti-lung cancer monoclonal antibodies are under development. It appears likely that monoclonal antibodies without conjugated toxins, drugs or particles are unlikely to provide major therapeutic effects against human cancers with large tumor burdens (with the possible exception of anti-idiotypic monoclonal antibodies). We plan to conduct small pilot studies of new monoclonals to insure their safety and to provide some evidence for specificity of binding, then proceed rapidly to tumor imaging and therapy with radiolabeled antibody.

B. Tumor imaging. We plan to radiolabel each monoclonal antibody and image patients in collaboration with the Nuclear Medicine Department and the Radiation Oncology Branch. This will be greatly facilitated by the arrival of Dr. S. Larson and his group from Seattle Washington in July, 1983 when he becomes the head of nuclear medicine at NIH.

C. Therapy with radiolabeled monoclonal antibodies. After we have demonstrated that radiolabeled antibodies have no loss of affinity and can be safely administered in tracer doses for imaging we plan to conduct therapeutic trials. These will begin with radiolabeled T101 and proceed to the other monoclonal antibodies. Dose response relationships will be determined for normal and malignant tissues in vitro prior to testing. Dosimetric calculations will be performed on tumor, normal tissue and whole body in patients given therapeutic radiation doses with monoclonal antibodies.

D. In Vitro Models

T-cell lymphomas: We plan to determine the dose-response relationships for several radioisotopes conjugated to T101 (and other monoclonal antibodies) on normal and malignant T-cells. We plan to determine whether modulation is useful or detrimental in the delivery of drugs or radioactive particles. We plan to study whether more than one antibody will provide greater cell kill when heterogeneity of antigen expression exists.

Lung Cancers: We plan to continue our studies on inhibition of in vitro growth by differentiatial and anti-growth factor monoclonal antibodies. We plan to determine whether modulation occurs in vitro using these monoclonals. We plan to determine dose response relationships for several radioisotopes conjugated to these monoclonal antibodies. We plan to study the effects of antibody combinations when considerable heterogeneity exists.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06575-08 NMOB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tumor Cell Biology

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Dr. A. F. Gazdar, Chief, Section of Human Tumor Cell Biology, NCI-NMOB

COOPERATING UNITS (if any)

See Attached Sheet

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI-Navy Medical Oncology Branch Bethesda, MD 20814

TOTAL MANYEARS:

3.5

PROFESSIONAL:

1.5

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project uses a multidisciplinary approach to study tumor cell biology so as to understand the basic nature of human malignancy and to develop methods for the diagnosis and control of human cancer. Particular emphasis is placed on lung cancer and cutaneous T-cell lymphomas. Our major efforts are in the growth of human tumors in vitro and in the nude mouse to study the differentiation, cell kinetics, immunology, experimental therapy, biochemistry, growth factor requirements, tumor markers, amplification and expression of oncogenes, and ectopic hormone secretion in these model systems. The human tumor colony forming and nude mouse xenograft assays are used to study tumor biology and to test tumor sensitivity in vitro. Another major area is the use of somatic cell hybrids and DNA transfection to study tumor cell biology, genetics and drug-radiation resistance. These include production of monoclonal antibodies by hybridomas against tumor antigens and defined proteins, comparative gene mapping, human hormone production, and genes controlling expression of the malignant phenotype. Other areas studied include tumor cell kinetics, flow cytometric analysis of human tumors, and DNA content of tumor samples. A major new development has been the establishment of a molecular genetics section to study the role of oncogenes in the origins and biology of cancers.

NCI-Navy MOB Senior Staff:

John D. Minna, M.D.
 Adi F. Gazdar, M.D.
 Paul A. Bunn, M.D.
 Mary J. Matthews, M.D.
 Desmond N. Carney, M.D.

Chief
 Deputy Chief (Lab)
 Senior Investigator
 Senior Investigator
 Senior Investigator

(USPHS)

Clinical Associates:

Cameron Little, M.D.
 Martin Brower, M.D.
 James Mulshine, M.D.

NCI-Navy MOB NHBETH
 Medicine-NCI NHBETH
 Medicine-NCI NHBETH

Other Professionals:

Herbert Oie, Ph.D.
 Frank Cuttitta, Ph.D.

NCI-Navy MOB NHBETH
 NCI-Navy MOB NHBETH

Cooperating UnitsNCI and Other NIH

P. Marangos, M.D.
 J. Whang-Peng, M.D.
 J. Peng, M.D.
 M. Zweig, M.D.
 J. Schlom, Ph.D.

M LCS
 C MD
 C MD
 CC CP
 VO

D. Cooney, M.D.
 G. Curt
 J. Mitchell
 A. Lichter
 D. Johns

C LT
 C DCT
 C RD
 C RD
 C LMCB

Others Cooperating

K. Becker, M.D.
 S. Bhatena, M.D.
 G. Schechter, M.D.

VAMC, Washington, DC
 VAMC, Washington, DC
 VAMC, Washington, DC

S. Baylin, M.D.

Johns Hopkins Hospital, Baltimore, MD

T. Moody, Ph.D.

George Washington Univ., Washington, DC

J. Shaper, Ph.D.

Johns Hopkins Hospital, Baltimore, MD

A. Wolfson, M.D.

UCLA, Los Angeles, CA

S. Cohen, M.D.

Stanford University, Palo Alto, CA

L. Deftos, M.D.

VAMC, San Diego, CA

1983

LABORATORY

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I. Personnel

- A. Senior Staff: A. Gazdar, D. Carney, M. Matthews
- B. Clinical Associates: None
- C. Other Professional Staff: I. Linnoila, M.D. (will commence 7/1/83)
- D. Technical Staff: E. Russell, V. Bertness (part time); H. Sims (part time); A. Simmons (part time)

II. Collaborators

S. Baylin, Johns Hopkins University; T. Moody, George Washington Univ.; P. Marangos, NIMH; L. Deftos, VA Hospital, San Diego; A. Wolfson, UCLA; S. Schlegel, and C. Harris, NCI

III. Introduction

A. Objective

A primary objective of this Branch is to develop improved methods for the diagnosis and treatment of lung cancer. To further these aims, and to provide a rational basis for them, we have developed a program to study, in detail, the biology of lung cancer in vitro and in vivo. The first aim of this program was to establish and fully characterize lung cancer cell lines from well studied and documented patients. As described in another section, this aim has been achieved for one major type, small cell lung cancer (SCLC). In addition, several lines have been established from non-small cell lung cancers (NSCLC). These lines, and corresponding nude mouse xenografts have been (and will continue to be) intensely studied, and have led to major advances in our understanding the biology of lung cancer. Clinical applications of some of these studies are underway. Also, the cell lines form the basic tools for the other laboratory studies described elsewhere. We have also embarked on a study of the normal precursor cells of SCLC, so as to understand the origin of this tumor type and its relationship to other lung cancers.

B. Rationale and Background

The WHO classification (1981) divides lung cancers into four major types and several relatively rare types. The four major types account for over 98% of all lung cancers (excluding mesotheliomas) and consist of two "differentiated" tumors (squamous cell and adenocarcinoma), and two "undifferentiated" types (large cell carcinoma and SCLC)(14-16). Each major type has two or more subtypes. SCLC has three subtypes (14): 1) oat cell (cells with scant cytoplasm and hyperchromatic nuclei); 2) intermediate (somewhat larger cells with moderate amounts of cytoplasm and nuclei with finely stippled chromatin and inconspicuous nucleoli); and 3) combined tumors (SCLC plus squamous cell or adenocarcinoma). Tumors with SCLC and large cell (LC) carcinoma are classified as intermediate subtype. As discussed below, this is unfortunate, as SCLC with LC elements belongs in the combined tumor category.

During the last 15 years, it has gradually been realized that for clinical, therapeutic, ultrastructural and biochemical reasons SCLC was very different from other non-SCLC lung cancers (NSCLC). While NSCLC is a convenient concept, it reflects our relative lack of knowledge of these tumors, as well as their resistance to cytotoxic therapy (28). In the late 1960s, Pearse identified a network of related cell types present in many organ systems that shared many common properties. He named it the APUD cell system, an acronym for the cell's chief properties (amine precursor uptake and decarboxylation). APUD cells concentrate amine precursors and convert them to dopamine or serotonin by means of a specific enzyme, L-dopa decarboxylase (DDC, also known as L-aromatic amino acid decarboxylase). In addition to amines, most APUD cells also make one or more polypeptide hormones. The APUD cell products are stored in cytoplasmic membrane bound dense core granules (DCG). Further, it has recently been discovered that all APUD cells expressed a specific highly acidic form of the enzyme enolase, previously thought to be limited to neuronal cells, and hence called neuron-specific enolase (NSE). While Pearse suggested that APUD cells shared a common neuroectodermal embryological origin, evidence from many sources (and our own data presented below) indicate that this hypothesis is untenable (18). The APUD cells of the pulmonary and GI tracts must share a common endodermal origin with the other mucosal cells.

Polypeptide hormone secretion by SCLC is its APUD cell property that has generated the most clinical and biological interest. SCLC is the human tumor that is most often associated with syndromes of "ectopic" hormone secretion and other paraneoplastic syndromes. In addition, "silent" hormone secretion can be detected in the majority of SCLC patients. SCLC cell lines may simultaneously secrete up to 10 polypeptide hormones.

In culture, SCLC cells, unlike other epithelial tumors, lack substrate adhesion and grow as floating cell aggregates (see below). These highly unusual properties suggest that the surface membranes, and perhaps the cytoskeletal proteins, of SCLC cells may differ from those of other lung cancers.

SCLC is a relatively well studied and common human tumor. However, it lacks suitable animal models, and we know remarkably little about its origin and premalignant changes. Cells with APUD cell properties are present throughout the mucosa of tracheo-bronchial tree, occurring singly or in small clusters. These cells have been given many different names (22), but we prefer the term pulmonary endocrine cell (PEC). PEC are relatively common in late fetuses and neonates, where at least three morphological types occur. In adults they are relatively rare, and only one type has been described. PEC are usually triangular in shape, with their base along the basement membrane, and the apex pointing towards the lumen. They express all of the classic APUD cell properties, and elaborate serotonin. Of the eight or so peptide hormones found in the lung, three (bombesin, calcitonin, and leu-enkephalin) have been localized to PEC. For over 15 years it has been presumed that SCLC and bronchial carcinoids arise from malignant transformation of PEC. Certain pathological conditions (anoxia, bronchiectasis) and some carcinogens (nitrosamines and asbestos) induce a

proliferation of these cells, and the latter may lead to the development of endocrine tumors. However, PEC are not known to be able to undergo mitosis in the adult, and are probably terminally differentiated. Presumably these stimuli cause division of precursor cells which subsequently differentiate into PEC.

The establishment of many well studied SCLC lines has resulted in an explosion of knowledge about the biology of this tumor (30). Unfortunately, our knowledge of the biology of NSCLC is relatively primitive (28). NSCLC is a much more heterogeneous group of tumors than SCLC, and few well studied and characterized cell lines exist. While pulmonary adenocarcinomas are relatively easy to culture (see below), squamous cell--the commonest form of lung cancer--is exceedingly difficult and usually undergoes terminal differentiation (see below). Major advances in our knowledge of NSCLC will require adequate in vitro models of all types of lung cancer.

IV. Progress Report

1. Morphological Studies

In a study with Dr. Mary Matthews, we examined nearly four hundred pretreatment SCLC surgical specimens and biopsies. Of these, 94% were "classic" SCLC tumors i.e., they did not contain NSCLC elements and consisted of oat cell or intermediate subtypes or a mixture of the two. In addition, multiple specimens from several patients frequently varied in the subtype. In well preserved excisional surgical specimens and in most effusions, the intermediate subtype predominated. The oat cell subtype predominated in small crushed biopsies, in ischemic areas, in autopsy specimens, and in poorly fixed tumors. Examination of SCLC xenografts and cell cultures always revealed the intermediate subtype, irrespective of the subtype of the original tumor.

In 6% of pretreatment specimens, combined elements were present, usually SCLC and LC carcinoma. We did not include the SCLC/LC tumors in the intermediate subtype, as recommended by the WHO classification, but regarded them as combined tumors and analyzed them as such. There were no significant differences between the clinical presentation, response to therapy or survival of the oat cell and intermediate subtypes. However, there were significant differences between the response to therapy and survival times of the combined tumors compared to "classic" SCLC tumors. These studies are described in greater detail elsewhere. Our studies indicate that the intermediate subtype is the true morphological expression of SCLC, while the oat cell subtype is a degenerative or ischemic artifact. Thus, from a morphological and clinical viewpoint, SCLC can be classified into two subtypes: 1) "classic" SCLC tumors (whether oat cell or intermediate); and 2) combined tumors, including SCLC/LC tumors. We did a detailed autopsy study of 87 patients who had presented with "classic" SCLC tumors (14,16). Of these tumors, five had completely converted to some other type of lung cancer, while 30 were combined SCLC/NSCLC tumors. Thus, nearly 40% of classic SCLC tumors had undergone morphological alteration between the time of diagnosis and

death. It is not known whether therapy induced these alterations, or whether it selectively destroyed the more sensitive SCLC elements, followed by selective proliferation of a previously unrecognized minor NSCLC component. These studies have major clinical and biological importance. Selective proliferation of NSCLC elements may be one method by which SCLC tumors become resistant to further therapy. Also, the frequent occurrence of combined tumors is further evidence for a unitarian theory of origin of all bronchial carcinomas (18).

2. Heterotransplantation of Human Lung Cancers

We have inoculated over 100 lung cancers into athymic nude mice, BALB/c background. We also attempted to use nude mice of NIH Swiss background which are bigger, healthier, and have a longer life span. However, the latter were not suitable, as few tumors took or could be transplanted into them. The reason may be the much higher NK cell activity present in NIH Swiss nude mice. In BALB/c nude mice, approximately 40% of SCLC and 30% of NSCLC tumors inoculated subcutaneously (sc) induced progressively growing tumors at the inoculation site. The tumors grew progressively but did not metastasize (1). Most could be passaged indefinitely.

In an effort to increase the xenograft success rate, we compared the sc and intracranial (ic) inoculation routes (1). The ic route was much more sensitive, yielding higher take rates, requiring 10-1000 fold fewer cells, and having shorter latent times. The ic tumors always localized in the meninges, but also invaded and destroyed the adjacent brain tissue. In contrast to sc tumors, ic tumors were invariably fatal within a few days of the onset of physical signs.

Xenografts provide gram quantities of reproducible tumor material and are useful for many biochemical and immunological studies. They provide additional sources of cell culture material - we successfully cultured 10/12 sc and 9/10 ic SCLC xenografts. The overall culture success rate (86%) from xenografts was higher than for fresh tumors (presented elsewhere). Xenografts also serve as models for testing newer diagnostic and therapeutic methods. However, xenografts have certain disadvantages - they are almost always contaminated with xenotropic strains of murine oncornaviruses, and they consist of mixtures of human tumor cells and mouse stromal cells.

3. APUD cell and other characteristics of SCLC cells

SCLC cells express all of the classic APUD cell properties. Almost all cell lines express high concentrations of the key APUD cell enzyme DDC (2,3) and neuron specific enolase (NSE) (4). All cell lines with these properties also have cytoplasmic DCG (2). In fact, in a survey of over 200 human tumors of nearly 40 different types, we found that concentrations of DDC were the highest in SCLC tumors than in any other APUD tumor. In fresh SCLC tumors, APUD cell properties are expressed at lower frequencies and concentrations than in cell lines and mean DDC concentrations were 2 to 3 fold lower in fresh tumors. The reasons for this include problems of tumor sampling, tumor necrosis, and admixtures of varying proportions of stromal

cells in tumor samples. In contrast, cell lines consist of viable, rapidly dividing, uniform tumor cell populations. Another method of demonstrating the APUD cell properties is formaldehyde induced fluorescence (FIF) (2). All cell lines with DDC also demonstrate FIF. However, FIF can only be demonstrated if the SCLC cells are preincubated with amine precursors, indicating lack of significant amounts of preformed, stored amines under in vitro growth conditions. While DDC concentrations are a more sensitive and quantitative method of demonstrating APUD cell properties, FIF is useful for demonstrating the APUD cells in combined tumors, and also has been used by surgical pathologists to aid in the differential diagnosis of small round cell tumors.

We investigated the expression of 16 peptide hormones in 34 SCLC and 15 NSCLC cell lines. Of the many peptides made by SCLC cells, the most consistent is bombesin (BN) (5). BN is expressed in all SCLC lines having APUD cell properties. The next commonest peptide expressed in vitro is calcitonin (CT). An interesting difference in expression of these 2 peptides is that CT, but not BN, incidence and concentrations decrease with in vitro culture time. The other peptides were either seldom expressed (usually at low concentrations) or were not detected. Of interest, 2 peptides, ACTH and arginine vasopressin, frequently hypersecreted clinically in SCLC patients, were relatively rarely expressed in vitro (less than 35 and 15% respectively). While Pearse has suggested that any APUD cell product may be expressed in any APUD tumor, peptide expression in SCLC is not random. Thus BN and CT are frequently expressed, insulin and glucagon seldom if ever.

All SCLC lines elaborating CT and BN constantly released these products into the supernatant fluids (6,7). Physiological stimuli, such as high K^+ concentrations in the presence of Ca^{++} stimulate release. The frequent finding of BN and CT in SCLC cultures must lead us to re-evaluate the concept of ectopic hormone secretion. These peptides are products of the normal PEC, which presumably shares a common precursor cell with SCLC. Thus, elaboration of these products by SCLC cells should be regarded as eutopic instead of ectopic. We originally considered SCLC to be related to a subpopulation of the PEC that were selectively programmed to differentiate into bombesinergic cells. However, the recent finding that BN may function as an autocrine growth factor for SCLC (see below) suggests that bombesinergic SCLC cells may have a selective growth advantage. There is remarkable uniformity of expression of APUD cell markers by SCLC cell lines. Thus 32/34 lines expressed all 3 markers (DDC, NSE, and BN). The concentrations of these products varied up to 1200 fold, but the important point is that almost all SCLC lines expressed all the markers, while they were absent in all 15 NSCLC lines tested. While rare NSCLC tumors express APUD cell properties (3, 19, 20), these tumors may either contain occult endocrine elements, or if not, provide further evidence for a unitarian theory.

Occasional NSCLC tumors may express any of several APUD cell peptides even in the absence of expression of the other classic APUD cell properties. We have not detected BN in NSCLC tumors or cultures, although other reports describe it in some pulmonary adenocarcinomas. We, and our collaborators, have tested several antisera to CT. A CT antiserum prepared by Dr.

Leonard Defetos appears highly specific for SCLC, with no positives found in NSCLC cell lines and supernatant fluids.

Working with Dr. Mark Zweig, we have discovered that SCLC tumors and cultures express very high levels of the enzyme creatine kinase (CK), predominantly in the form of its brain isoenzyme (CK-BB) (8). Enzymatic CK levels are about 30 fold greater, and CK-BB concentrations more than 70 fold greater in SCLC than in NSCLC and normal lung.

As described in another section, all SCLC lines are aneuploid, with a wide range of chromosome number and many structural abnormalities. However, a specific chromosomal abnormality, deletion 3p, is present in all cell lines examined.

4. Clonal Heterogeneity and Homogeneity of SCLC

We studied expression of APUD markers and peptides in clonal derivatives of SCLC line NCI-H128 which has 2 distinct aneuploid peaks (both of which were present in the original tumor sample). All 15 clones expressed one or other of the aneuploid peaks, but not both, confirming their clonal origin. All clones expressed SCLC morphology, all of the classic APUD cell properties, and BN, indicating that expression of these markers was clonally conserved. In contrast to BN, expression of other peptides demonstrated clonal variation. For a review of other examples of tumor cell heterogeneity found in SCLC see reference 27. These include, chromosome number, cloning efficiency, tumorigenicity, and surface antigen expression.

5. Morphological Variants of SCLC

While most SCLC cell lines and xenografts morphologically resemble the intermediate subtype, some express other morphological features (8,14, 18,21). The latter consist of several types: 1) combined tumors and lines consisting of distinct SCLC and NSCLC elements; 2) convertor tumors and lines in which the SCLC cells have been completely replaced by NSCLC elements; and 3) transitional tumors and cultures, whose individual cells have cytological features of both SCLC and NSCLC. We refer to all of these types as morphological variants of SCLC. Obviously, they reflect similar changes occurring in vivo. Their incidence increases with culture and transplantation time, but some exhibit variant morphology from the start (most of the latter were originated from patient tumors that had combined morphology). Their overall incidence is approximately 20% of all SCLC cultures.

As discussed elsewhere, variant lines have increased growth and cloning efficiencies. They discordantly lose SCLC markers, especially DDC and BN, but often retain high CK-BB and NSE concentrations. All variant lines retain the characteristic SCLC cytogenic marker 3p- (see below), providing evidence of their SCLC lineage. Variant cultures provide important models to study the inter-relationships and conversions between SCLC and NSCLC. It is also possible that the LC convertors represent a less differentiated or 'stem cell' precursor of SCLC, which is a classic endocrine tumor cell.

6. Clinical Applications

Many peptides and other tumor markers such as CEA, AFP, and HCG have been studied as clinical markers in SCLC patients. No single marker or combination has proven useful in the majority of patients. Many excellent intracellular tumor markers exist for SCLC, but some are not actively secreted, thus limiting their clinical application. DDC, NSE and CK-BB are not actively secreted, but are released from dead and dying cells. Thus increased blood concentrations would only be expected in cases with extensive tumor burden. DDC is measured enzymatically as no specific antibody exists. We have not detected it in culture supernatant fluids, or in the blood or effusions of SCLC patients. We measured serum NSE concentrations in 94 untreated patients with SCLC (13) and the data are presented in another section. We also measured CK-BB levels in 106 untreated SCLC patients. Only 2% of the patients with limited disease, and 40% of the patients with extensive disease had elevated levels, and there was an excellent correlation between elevated levels and the number of metastatic sites, and also with response to therapy. Thus serum NSE and CK-BB levels may serve as clinical markers for tumor burden and response to therapy. BN and CT are actively secreted SCLC products, and offer greater promise as markers for identification of small SCLC tumors. Unfortunately, BN is not stable in blood and is difficult to extract. Our preliminary results with Dr. Terry Moody are not very encouraging, with only a small percentage of patients with extensive disease having elevated levels. CT, while not expressed as frequently by SCLC tumors as BN, offers greater promise as a marker. It is stable in the blood, and Dr. Deftos' results suggest that it may be elevated in up to 84% of patients with extensive stage disease.

We are exploring the use of markers in the CSF of SCLC patients with leptomeningeal disease. The markers, especially BN are easier to extract and measure in CSF than in blood, and are stable for longer periods. In a preliminary study, CSF samples from Danish patients with SCLC were sent to us by Drs. Hansen and Vindelov, and analyzed for BN and NSE. Very high levels of both of these markers were present in some of the samples, but we have not correlated the results with the cytological findings.

7. Studies with other lung cancers

We have established, in long term culture, 15 NSCLC cell lines. These consist of 7 adenocarcinomas, 3 LC carcinomas, 1 mucopidermoid carcinoma, and 4 mesotheliomas. Several other tumors are currently being cultured, but cannot be considered to be fully established cell lines. In addition, another 3 adenocarcinomas and 2 squamous cell carcinomas are serially passaged as xenografts in nude mice. While mesotheliomas, strictly speaking, are not bronchial carcinomas, they are a related form of cancer, share some of the same etiological factors, and have to be distinguished from pleural metastases of adenocarcinomas. The cell lines have been established and maintained in conventional serum supplemented media as well as in fully defined media (discussed in another section).

Adenocarcinomas are relatively easy to establish in culture, as are mesotheliomas. We have had moderate success with large cell carcinomas,

and none with moderate or well differentiated squamous cell carcinomas. NSCLC lines (but not mesotheliomas) demonstrate substrate adhesion, have an epitheloid morphology, have relatively short doubling times, clone relatively efficiently, and induce tumors in nude mice closely resembling the original human tumor (28). The mesotheliomas, which have long branching processes, and other typical ultrastructural characteristics, clone inefficiently in semi-solid media, and are nontumorigenic. The NSCLC lines do not express the biochemical or cytogenetic markers characteristic of SCLC.

In culture, well and moderately differentiated squamous cell tumors usually keratinize and undergo terminal differentiation. We are currently attempting to prevent this by a number of different techniques. So called squamous cell carcinomas established by other laboratories have also been studied. These lines lack the characteristic ultrastructural features of squamous cells, and the tumors they induce in nude mice resemble large cell or adenocarcinomas. However, the mucoepidermoid carcinoma we have established, has (as did the original tumor) two distinct cell components - a cell filled with small mucin vacuoles, and another containing many dense bundles of tonofilaments. The latter may be regarded as a type of squamous cell.

As with most human epithelial tumors, NSCLC cell lines have receptors for epidermal growth factor (11), and require it for growth in defined media. In contrast, SCLC lack receptors for this factor, and do not require it for growth in defined media.

8. The Cell Surface and Cytoskeletal Proteins of Lung Cancers

The unusual, perhaps unique, in vitro growth characteristics of SCLC suggest that its cell surface, and perhaps cytoskeletal proteins, are very different from those of other lung cancers. We have studied these proteins in collaboration with Drs. Stephen Baylin and Joel Shaper and have conducted a more detailed study of the cytoskeleton keratins in conjunction with Drs. Harris and Schlegel.

The cell surface protein phenotypes of lung cancer cell lines and other appropriate human lines were studied by radioiodination followed by two dimensional polyacrylamide gel electrophoresis (9). The findings were confirmed by metabolic labelling studies, using [³⁵S] methionine.

The major findings may be summarized as: about 25 surface proteins are consistently present in SCLC cells, having molecular weights of 35 - 100kD. Of these, 12 (35 - 70kD) are not present in NSCLC tumors. While six of these 12 SCLC proteins are also present on the surface of neuroblastomas, three may be unique to SCLC as they have not been identified on other normal or malignant human cells. In NSCLC lines (all of those phenotypes are similar), the most characteristic feature is intense iodination of a group of large neutral proteins (> 100kD). In addition, they have 10 smaller proteins (35 - 70kD) not present in SCLC cells. Dr. Baylin has also studied a SCLC/LC variant isolated by him, which had a unique phenotype--it had a complete SCLC surface phenotype as well as several proteins typical of NSCLC. Thus,

For study of the cytoskeletal proteins, cells were labelled with [³⁵S] methionine, homogenized, exposed to Triton X-100 and the cytoskeletal proteins, then extracted with special buffers and analyzed on 2-D gels. SCLC cells have four distinguishing proteins (also present in neuroblastomas) 140,000 - 190,000kD in size. NSCLC cells have three distinguishing proteins--48,000 - 80,000kD. One of these three proteins (55,000kD) was identified as vimentin. Vimentin, an intermediate filament expressed in mesenchymal cells, including mesothelial cells, is not found in epithelial cells in vivo. However, most cells in culture express vimentin. Of great interest, the attached NSCLC cells expressed vimentin in vitro, while the floating SCLC cells did not. It is not certain whether absence of vimentin expression is the cause of the SCLC cells to lack substrate adhesion, or whether floating cell lines fail to express vimentin.

For the study of keratins, the extracted cytoskeletal proteins were immunoprecipitated selectively with antisera to human keratin and analyzed on a 1-D gel. All lung cancers expressed some keratin proteins. The adenocarcinomas expressed predominantly low molecular weight keratins, as did the LC carcinomas. Mesotheliomas had varying and distinct patterns. SCLC lines varied in their keratin profiles. All contained the low molecular weight keratins (48 - 50kD) present in other lung cancers. They frequently contained small amounts of 46kD keratin. A few SCLC lines contained intermediate weight (54 - 59kD) keratins, and these may represent SCLC tumors undergoing squamous differentiation. In the mucoepidermoid cell line, intermediate weight keratins predominated, similar to fresh squamous cell carcinomas.

9. Culture of Pulmonary Endocrine Cells

Obviously, if SCLC shares a common precursor cell with the PEC, a study of the latter is of great biological interest. As the PEC are present in very small numbers in the adult, it is difficult to study them in vivo. We have attempted to culture rodent PEC. We cultured bronchial cells from late fetal and neonatal hamsters and rats and from nitrosamine treated adult hamsters. We used conventional serum-supplemented media, defined HITES medium, and mixtures of the two. Rat cultures did not replicate significantly, and postnatal hamster cultures became contaminated with fungi after 4-6 weeks. However, fetal hamster cultures could be maintained for up to 12 weeks. Several different cell types were propagated in the various media, including epithelial cells, ciliated cells and a polygonal cell lacking substrate adhesion. The latter grew as single irregular shaped cells which could readily be separated from the other cell types. These cells replicated rapidly for 4-6 weeks, and then slowly for another 3-6 weeks. Immunocytochemical studies and radioimmunoassays of supernatant fluids indicated that they elaborated BN, CT and NSE. Electron microscopy demonstrated numerous large DCGs. These cells are probably a form of PEC, although more definitive studies have to be performed.

V. Future Studies

1. Continued study of SCLC panel for biochemical, immunologic and other markers

We have established and characterized a large bank of SCLC cultures having classic and variant morphologies. These lines, and their NSCLC counterparts, form the basis of practically all of the laboratory work performed in the Branch in the field of lung cancer. They have provided us with major insights into the biology of SCLC, and will continue to fulfill this role. Further studies with small cell lines include completion of the hormone secretion patterns, clonal heterogeneity, and the search for new markers. For instance, the amphibian peptide physalamin has recently been described in a very limited number of SCLC tumors and heterotransplants. A survey of a large number of lung cancer lines, including NSCLC lines, could be undertaken relatively rapidly, and the relationship between expression of physalamin and the related peptide bombesin determined. The cell lines also provide sources for cloning the genes of the various markers, and some of these studies are underway with collaborators. The lines provide rich sources of some of the products (CK-BB constitutes up to 4% of the soluble proteins in SCLC cells). They are being used as a source of mammalian BN, the sequencing of which is being attempted for the first time.

2. Study of "Variant" lines

The large number of morphological variants of SCLC available offers this laboratory unique tool to study the origin of SCLC and its relationship to other lung cancers. Do the large cell convertor and transitional lines contain a less differentiated precursor cell of SCLC? For instance, do they represent the stem cell of SCLC, or, perhaps, of all lung cancer cell types? We are attempting to induce 'differentiation' in these cells, using phorbol esters and retinoids, and comparing the effects of these substances on the variant and classic lines. The cell surface and cytoskeletal protein phenotypes of the variant lines have to be determined, to see if they express features common to SCLC and NSCLC.

3. Development of NSCLC panel

A large representative bank of all the major (and some of the minor) lung cancer types would be of great benefit in studying NSCLC which constitutes 75% of all lung cancers. Our current bank is not large, nor is it representative. The major deficiency has been our failure to grow continuous lines from squamous cell carcinomas. Another major problem that has hindered these studies has been lack of a source of a large number of suitable specimens. We will be correcting the latter problem shortly. Approximately 50 lung cancers are resected at the Naval Hospital every year. In the past, we have had limited access to this material. Recently, we have had approved a protocol (82-12, 'In Vitro Culture and Drug Testing of Human Tumors') that will provide us with lung cancers, as well as other tumor types. These tumors will be utilized for drug testing, for developing new methods to grow NSCLC tumors, for heterotransplantation, and for molecular genetic and monoclonal antibody studies. New types of defined

media will have to be developed for the various lung cancer types. In addition, squamous cell terminal differentiation will have to be retarded by agents such as T₃ and retinoids. We will test defined media developed for human keratinocytes. Xenografts will be used as additional sources of tumor material for culture. Whenever possible, non-transformed cell lines (B lymphoblastoid, fibroblastoid) will be established simultaneously from the patients. We regard this project as our highest priority.

4. Isolation and culture of precursor cells of the PEC

An effort is being planned to reproducibly grow PEC in long or intermediate term culture. These efforts include the use of improved defined media and the use of media conditioned by rapidly growing SCLC cultures. In addition to rodents, we will attempt to grow PEC from neonatal and adult humans and primates, as well as from lung cancer patients. Once adequate culture conditions have been established, attempts will be made to transform these cultures with the use of nitrosamines and asbestos.

5. Clinical applications of lung cancer markers

We have identified a panel of markers (DDC, NSE, BN, CT, and the immunologic markers discussed elsewhere) that reproducibly distinguish SCLC from NSCLC. A major clinical application will be a prospective immunocytochemical study of lung cancers, to determine whether the markers aid diagnosis, and help predict the clinical course and response to therapy. This study is described in another section.

We will complete the study of markers in the CSF of SCLC patients (outlined above), and investigate with Dr. Deftos the use of his highly specific CT assay in the staging of SCLC.

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Title: DEVELOPMENT OF NEW METHODS FOR GROWING HUMAN LUNG CANCER AND OTHER TUMOR CELLS IN VITRO

I. Personnel

- A. Senior Staff: Dr. D.N. Carney, Dr. A.F. Gazdar, Dr. J.D. Minna
- B. Clinical Associates: Dr. M. Brower
- C. Other Professional Staff: F. Cuttitta, S. Fargion, F. Calvo
- D. Technical Staff: Ms. V. Bertness

II. Collaborators

- Dr. T. Moody (George Washington University); Dr. C. Pert (NIMH);
- Dr. G. Morstyn, Dr. J. Mitchell (ROB/NCI);
- Dr. L. Korman (VA Medical Center, Washington, D.C.)

III. Introduction

A. Objective

While we, and other investigators, have had moderate success in establishing lung cancer cells in continuous culture, the success rate is far from optimal. Further, we have been able to successfully clone, in soft agarose, the majority of lung cancer tumor specimens, but the mean number of colonies per 10^5 cells plated is seldom large enough to permit drug testing. Following the lead of G. Sato, we have developed improved methods for the culture and cloning of lung cancers, and other tumors, using serum-free defined media. Because some tumors release factors required for their own growth, we also investigated the role of autocrine factors in tumor growth.

B. Rationale and Background

Use of Serum-Free, Hormone Supplemented Media:

At the present time the vast majority of human tumor cell lines are cultured and maintained in serum-supplemented media (SSM). While supporting the growth of some tumor cells, the use of serum has many disadvantages. Most notably, its use supports the growth of both normal cells and tumor cells such that in many instances the growth of normal cells is favored and overgrowth of the tumor cells occurs and they are lost to culture. In addition, the use of SSM is nonspecific such that the successful establishment of a tumor cell line is dependent more on the biologic behavior of the cell rather than the specific nutrients provided by serum. In addition, in SSM, growth factor and receptor studies may be inaccurate because of the effects of the many known and unknown proteins in serum. It is also possible that for some cells, serum proteins may be stimulatory but be inhibitory for others. For these reasons, and more importantly, because with SSM, the successful establishment of cell lines is unpredictable, a study was undertaken to identify the specific serum-free growth requirements of the major histologic types of lung cancer. The design of the study was in 2 parts: (1) to identify the set of hormones and growth factors

essential to maintain the growth of established cell lines in serum-free media (SFM) and (2) to evaluate the efficacy and selectivity of these media on the growth and establishment of cell lines of the same histologic tumor type from fresh biopsy specimens. Although considerable success has been achieved in the establishment of continuous cell lines of small cell lung cancer in serum-free defined medium (HITES), major difficulties still exist in the soft agarose cloning of clinical specimens of SCLC. Both in serum-supplemented medium, and in HITES medium, adequate cloning to permit drug testing can be achieved in only 22% of all specimens received. In addition, in HITES medium, colony forming efficiency (CFE) for both cell lines, and fresh specimens is approximately only 1% of that observed in serum-supplemented medium.

Clonogenic Assays of Tumor Cells:

Among assay systems developed to selectively grow human tumor cells *in vitro*, the soft agarose clonogenic assay [clonogenic assay (CA), human tumor stem cell assay] has emerged as one technique to achieve this goal. In recent years following the initial publication of Salmon and Hamburger of the use of the CA for the growth of tumor cells from fresh biopsy specimens and their subsequent demonstration that this assay could be used to select patients' chemotherapy, we and a large number of laboratories have evaluated this assay for the growth of fresh specimens of lung cancer, in particular, SCLC. In addition, we have modified the clonogenic assay to use serum-free medium with added growth factors to look for new factors stimulating tumor cell growth.

Autocrine Growth Factors:

Two approaches can be used to identify factors which may improve the growth of human tumors in culture: 1) screen many different hormones produced by a range of endocrine cells; and 2) screen hormones known to be produced by the tumors under study ("autocrine" factors). Using the first approach, the HITES medium was designed and shown to improve the establishment of continuous cell lines of SCLC in liquid culture. To improve the CFE of SCLC cell lines we have investigated these cells to determine if they produce autocrine growth factors. Factors evaluated included bombesin and arginine vasopressin, two hormones produced *in vitro* by the majority of cell lines of SCLC, and conditioned medium obtained from SCLC cell lines continuously cultured in serum-free HITES medium. Bombesin (BN) a peptide initially isolated from frog skin has been identified in the central nervous system, gastrointestinal tract, and fetal lung. More recently bombesin like peptides have been identified in a number of cell lines of SCLC, but not in non-SCLC lung cancer cell lines. As bombesin may mediate some of its biologic effects in the CNS through the binding with specific bombesin receptors, a study was undertaken to: 1) evaluate BN in a large panel of human tumor cell lines; 2) to determine if specific bombesin receptors were present in these cell lines; and 3) to determine the physical properties of bombesin receptors in human tumor cell lines. Since the intracellular second messenger cAMP mediates hormone secretion in other systems, we also evaluated the action of agents which alter cellular cAMP on bombesin secretion in SCLC cell lines.

Growth Factor Requirements of NSCLC:

Although there are approximately 90-100 cell lines of human small cell lung cancer in the world at the present time, there are fewer than 20 well characterized cell lines of human NSCLC. Our understanding of the biology of SCLC increased in parallel with our improving ability to establish cell lines of SCLC. One key to this improvement was the development of the serum-free defined medium HITES, which selectively supports the growth of tumor cells but not stromal cells from fresh clinical specimens of SCLC (Carney et al., PNAS 1981). In 1981, Barnes et al reported on the development of a serum-free medium that supported the growth of a single established cell line of human adenocarcinoma of the lung (Methods in Enzymology 79: 368, 1981). This medium consisted of insulin, transferrin, selenium, hydrocortisone, epidermal growth factor, and albumin, used in culture flasks precoated with fetal calf serum. (LA medium)

Growth Factor Requirements of Breast Cancer:

Although two defined media containing hormone supplementation have been described (Barnes et al., Allegra et al), these media have not been shown either to support the growth of other human breast carcinoma cell lines, or the growth of fresh clinical specimens of breast carcinoma. A study has been undertaken to design a serum-free hormone supplemented medium which will support the growth of multiple cell lines of breast carcinoma, and which may improve both the primary cloning of breast carcinoma, and the establishment of cell lines of this tumor in continuous cell culture.

Tumor Cell Heterogeneity:

Considerable data from a number of different investigators using animal model systems suggest that metastatic lesions are composed of specific subpopulations of cells present in the original tumor (Keller and Hart). In addition, these investigators suggest that metastatic lesions from different sites have different biological properties, including metastatic potential. Small cell lung cancer, unlike other forms of lung cancer, is characterized by a propensity to early widespread dissemination. At diagnosis more than 2/3 of patients will have clinically detectable metastases. For these reasons SCLC is an ideal human model to determine if there is heterogeneity in biological properties among different metastatic lesions.

IV. Progress Report

1. Growth and Characterization of Clinical Biopsy Specimens of SCLC in Serum-Free Defined Media (SFM)

Prior studies with established cell lines of human small cell lung cancer had demonstrated that a SFM containing hydrocortisone (10 nM), insulin (5 g/ml), transferrin (10 g/ml), 17-B-estradiol (10 nM), and selenium (30 nM), when added to RPMI 1640 medium without serum supplementation

(HITES medium), could support the long-term culture of well established cell lines of SCLC. When SCLC tumor cells were transferred from serum supplemented medium (SSM) to HITES medium, after a lag period, the tumor cells in HITES proliferated at a rate similar to that observed in SSM.

Many other hormones tested demonstrated no stimulatory effect on the growth of these cell lines. These included ACTH, blood meal, calcitonin, epidermal growth factor, fibroblast growth factor, growth hormone, glucagon, luteinizing hormone, nerve growth factor, pitressin, progesterone, prolactin, putrescine, testosterone, tri-iodothyronine, and TSH. The influence of these hormones on SCLC growth was tested in liquid culture, measuring cell density at a defined period from plating.

To evaluate the usefulness of HITES medium for the growth of tumor cells from fresh clinical biopsy specimens, a study was carried out in which consecutive specimens obtained from patients with SCLC were plated in HITES medium and in SSM and observed for tumor cell growth. Thus far, a total of 153 specimens from patients with SCLC have been evaluated; 100 specimens were pathologically negative for SCLC tumor cells and 53 specimens contained SCLC tumor cells. When these 53 specimens were plated in SSM and in HITES medium, SCLC tumor cell proliferation was observed in 39 specimens (74%) in HITES medium, while only 26 (49%) of the same specimens demonstrated tumor cell proliferation in SSM. In HITES medium, no difference in successful growth was observed for tumor cells obtained from previously treated or newly diagnosed untreated patients. In addition, in the HITES medium, successful tumor cell growth was observed in specimens obtained from a variety of metastatic sites, including bone marrow, lymph nodes, pleural effusion, and brain. In addition, a single specimen of primary SCLC lung cancer, obtained at surgical resection, also demonstrated proliferation.

The nature of the cells proliferating in HITES medium was confirmed by gross morphological examination of cultures, by cytology examination, by DNA content analysis, and by nude mouse tumorigenicity studies. In comparison with specimens cultured in SSM where, in addition to tumor cell proliferation, growth of normal admixed stromal cells was frequently observed, in the HITES medium only growth and proliferation of tumor cells were observed. By 7-14 days from plating, a pure population of rapidly dividing tumor cells (20-25% of cells in G₂ + M + S phase) was usually obtained in the HITES medium.

In studies with the first 25 specimens, in most instances, after 6-24 weeks culture in HITES medium, tumor cell proliferation slowed or ceased. In these cases, the addition of fetal bovine serum (2.5-10%, v/v) was sufficient to maintain the continual proliferation of the tumor cells and the establishment of a permanent cell line of SCLC. However, over the past 18 months, with 2 changes, continuous cell lines of SCLC have been established with relative ease in serum free HITES medium. The changes include (1) the maintenance of the tumor cells in HITES at a high density and (2) at passage the carry-over of approximately 50% of the culture medium in which the cells had been grown ("conditioned media"). With these changes 14 cell lines have now been permanently

established in HITES medium direct from patient biopsy specimens. Of these 14 cell lines, 10 lines express all the typical morphological and biochemical characteristics of "classic" SCLC cell lines while 4 cell lines, although morphologically similar to SCLC cell lines, lack 2 or more of the classic biomarkers of SCLC. These latter cell lines (variant SCLC cell lines) may represent a subset of SCLC with atypical biological properties (vide infra).

The selectivity of HITES medium for the growth of SCLC specimens was confirmed by the failure of noraml cells to proliferate in this media and the failure of the majority of other human tumor specimens (2/30), including 10 specimens of non-SCLC lung cancer, to grow in this media.

To determine if the growth of SCLC tumor cells in HITES medium favored the selective growth of a specific subpopulation of tumor cells within the clinical biopsy specimen, the biological characteristics of cell lines permanently established in HITES medium were compared with cell lines established from the same biopsy specimen in SSM. Of the 14 cell lines established in HITES medium, 9 of these 14 lines were also established in SSM. The evaluation of a number of properties, including growth rate, nude mouse tumorigenicity, DNA content, and the expression of biomarkers including L-dopa decarboxylase and bombesin, revealed no differences between the populations cultured in HITES or SSM.

2. Autocrine Growth Factors for SCLC

The influence of these factors on the growth of cell lines was tested using a soft agarose clonogenic assay with serum free HITES as the basal medium. When bombesin was added to culture medium (serum-free HITES) stimulation of colony formation was observed for 5 cell lines of SCLC, but was not observed in 4 other cell lines including 2 melanoma and 2 non-SCLC lung cancer cell lines. A dose response was obtained with peak stimulation observed at 50 nM bombesin. Although only a small number of cell lines have thus far been tested, no correlation was observed between the amount of bombesin produced by the cell line and the mitogenic response of the cells to bombesin, or between the presence or absence of significant levels of bombesin receptors on the cells and their response to exogenous bombesin.

When arginine vasopressin was studied in an identical culture system, increased colony formation of SCLC tumor cells was observed. The effects of AVP on non-SCLC lung cancer cells have not yet been evaluated.

It has been postulated that the reduced growth-factor requirements of tumor cells in culture is related to the ability of these cells to produce their own growth factors (autocrine growth factors). The establishment of cell lines in serum-free media should facilitate the identification and characterization of growth factors ("transforming growth factors", Todaro et al.). Conditioned medium (CM) from cell lines in HITES medium was tested for its ability to stimulate colony formation in HITES of SCLC cells, non-SCLC cells, and CFU colonies from normal bone marrow aspirates. In the 4 cell lines tested, CM (25% v/v)

stimulated CFE in HITES medium of SCLC cell lines, but not non-SCLC lung cancer cells, normal NRK cells, or bone marrow stem cells. A dose response was observed with peak stimulation at 25% v/v. CM collected 4 days after a medium change was stable to freeze/thawing, and to heat ($100^{\circ} \times 3$ mins). The addition of CM to either SSM, or to culture medium supplemented with both bombesin and AVP, also stimulated colony formation above control culture plates. With CM, colony formation in serum-free medium approximated that observed in serum-supplemented medium representing a 100-fold increase in cloning above that observed in HITES medium alone. In liquid culture, the addition of CM to cells plated at low density in HITES medium supported their continual proliferation, which was observed at a much lower efficiency in HITES medium alone. At higher density, the addition of CM to cells in liquid culture did not improve their growth rate.

3. Characterization of Bombesin Expression, Receptors and Mechanism of Action

Because of the growth stimulatory role of bombesin we wish to characterize this neuropeptide and its receptors further in lung cancer cells.

Cell lines studied included a panel of 34 cell lines derived from patients with small cell lung cancer; 10 non-SCLC lung cancer cell lines, and 9 other human tumor cell lines (breast (3), melanoma (2), hypernephroma (2), T-cell lymphoma (2)). All cells for study were harvested in log phase growth 2 days after a medium change and bombesin receptor determinations were assayed as previously described using $^{125}\text{I-Tyr}^4$ bombesin (Moody et al. PNAS 75: 5372-5376, 1978).

In a screen of 34 different cell lines derived from patients with SCLC, immunoreactive BN (>0.1 pmol/mg cellular protein) was detected in 32 (94%) of these cell lines. Although a wide range in BN activity (0.1-18.3 pmol/mg protein) was observed in these SCLC cell lines, no correlation between site of origin of the cell line (i.e., bone marrow, lymph node, etc.) or treatment status of the patient at time specimen was obtained and bombesin level was observed. In addition, culture conditions (growth in serum supplemented medium, or serum-free HITES medium) did not influence the expression of bombesin by the cell lines. For cell lines studied at intervals over an 18 month period, bombesin expression did not show any significant change.

Among all other human tumors cell lines studied, bombesin production was not observed (all values less than 0.01 pmol/mg cellular protein). Of interest, bombesin expression was observed in 4 human B lymphoblastoid cell lines, but not in 2 human T-cell lymphoma cell lines. Specific binding receptors were detected (>10 fmol/mg protein) in 9/34 SCLC cell lines, but not in any other human cell lines studied. As for bombesin expression in SCLC cells, a wide range in receptors was noted (14-48 fmol/mg protein). No correlation was observed between bombesin production by the cell line and the amount of receptor detected on the cell membrane. The level of high affinity binding sites in SCLC cell lines was comparable to levels previously detected in rat brain.

In cell line NCI H446, highest levels of specific receptor sites were detected. Of interest, although this cell line was derived from a patient with classic small cell lung cancer, in continuous culture the cell line does not express either bombesin or L-DOPA decarboxylase. The properties of the receptors for bombesin were investigated in this cell line. Kinetic and equilibrium studies indicated that the K_d was 0.5 nM. In addition, approximately 3,000 high affinity binding sites were demonstrated per cell. This high affinity binding was trypsin sensitive. In addition, pharmacological studies indicated that the C-terminal octapeptide of bombesin is essential for high affinity binding. These data suggest that the bombesin receptors in SCLC cell lines are very similar to those receptors on forebrain or exocrine pancreas.

Preliminary studies with cAMP regulation of bombesin secretion have been carried out on two cell lines. In NCI H345 which was established in serum-free medium, and which expresses high levels of bombesin (4.5 pmol/mg cellular protein), the addition of theophylline (10 nM), vasoactive intestinal peptide (VIP, 1 μ M), and glucagon (1 μ M), caused a 3-7 fold increase in cellular cAMP and a 2-3 fold increase in bombesin secretion. Both bombesin secretion and cellular cAMP reached a maximum within 5 minutes of adding the agents. Theophylline augmented the VIP and glucagon stimulated cAMP but did not increase bombesin secretion. Of interest, the addition of bombesin to this cell line, which also has high levels of bombesin receptors, did not alter cAMP cellular levels. As we have shown that exogenous bombesin can stimulate the soft agarose cloning of SCLC cell lines, these data suggest that the receptors that recognize bombesin and mediate its mitogenic effect in SCLC cells are not linked to adenylate cyclase.

In studies of cell lines NCI H446, which has high levels of bombesin binding receptors but which does not express bombesin, the addition of the same agents to this line was also associated with a marked increase in cellular cAMP but was not associated with any increase in either cellular bombesin or bombesin secretion.

4. The Biology of Metastatic Lesions and Clonal Lines of SCLC

Three approaches have been used to evaluate this question: (1) to compare the properties of cell lines derived from different patients and from different metastatic sites; (2) to establish and compare cell lines derived from multiple different sites in the same patient; and (3) to establish and compare clonal cell lines from (a) clinical specimens and (b) established cell lines of SCLC.

All cell lines studied were derived from patients with SCLC and were evaluated for growth properties in vitro and in athymic nude mice; expression of a variety of biomarkers, including L-dopa decarboxylase, bombesin, neuron-specific enolase and creatine-kinase-BB; DNA content analysis by flow cytometry; and antigenic expression as determined by monoclonal antibody testing.

In a study of 27 cell lines of SCLC established from bone marrow (10 cell lines), pleural effusions (9), lymph nodes (5), and other sites (3), although a wide range in expression of biomarkers was observed, no consistent differences were detected between cell lines derived from different metastatic sites. In all instances, levels of biomarkers were higher than those observed in non-SCLC lung cancer cell lines. In addition, no differences were observed in the ability of these cell lines to form tumors in nude mice; gross morphology in culture; or DNA content. In addition, no differences were observed between cell lines derived from untreated or heavily pretreated patients.

Multiple cell lines from different metastatic sites have been established from 3 patients with SCLC: 5 cell lines (1 from lymph node, 4 from different subcutaneous nodules) have been established from 1 patient and 4 cell lines (1 from bone marrow and 1 from lymph nodes; and 2 from different bone marrow aspirates) from 2 other patients. Among these cell lines, for the phenotypes examined, no major differences were observed among cell lines derived from the same patient.

Twenty clonal cell lines have been established and grown to mass culture from a fresh bone marrow aspirate specimen of a SCLC patient who had relapsed from intensive combination chemotherapy. As for the above cell lines tested, these clonal lines demonstrated no significant variation in growth, nude mouse tumorigenicity, or expression of a variety of SCLC biomarkers. In addition, no variation was observed in the DNA content of these clonal lines and all demonstrated the deletion of chromosome 3, consistent with other cell lines of SCLC origin.

5. In Vitro Culture of Non-SCLC Lung Cancer in Serum-Free Defined Medium

We initially tested the ability of LA medium to support the growth of 3 NSCLC lines, NCI-H23, NCI-H125, and A549. All three lines showed doubling times comparable in serum and serum-free media, but saturation density was less in serum-free medium, suggesting that some factor(s) were missing from LA medium.

Approximately 20 hormones, nutrients, and growth factors have been screened for their ability to further support the growth of NSCLC. It was found that the addition of triiodothyronine slightly stimulated growth, and that the optimal concentrations of insulin, EGF, and albumin were higher than used in Barnes' LA medium. In addition, the serum-precoating requirement was removed by coating culture flasks with fibronectin and collagen.

The medium defined by these changes, ACL-3, has been demonstrated to support the continuous replication of 10 NSCLC cell lines (including both adenocarcinoma and large cell carcinoma), in liquid culture. The cell lines can also clone in this medium in soft agarose without serum at an efficiency approximating that observed in serum-supplemented medium. One fresh specimen of lung adenocarcinoma, NCI-H441, has been maintained in suspension culture in ACL-3 medium for 11 months, and a fresh specimen of squamous cell carcinoma, NCI-H520, has been maintained for 5 months.

6. Primary Cloning in Soft Agarose of Clinical Specimens of Lung Cancer

Initial studies using the media designed by Hamburger and Salmon revealed no growth of fresh specimens and very poor cloning of established cell lines of lung cancer compared to growth in RPMI 1640 medium supplemented with 10-20% fetal bovine serum. Subsequent studies of fresh specimens were performed using this simple culture media. Thus far, a total of 475 specimens have been received from patients with SCLC undergoing approved protocol staging procedures. The specimens include bone marrow aspirates and biopsies (390); pleural effusions (42); lymph node aspirates and biopsies (28); liver biopsies (12); and single biopsies of brain, adrenal mass, and subcutaneous nodule. After specimens were processed for culture, a cytospin preparation of each was prepared, stained with H&E, and examined microscopically to determine if tumor cells were present. SCLC tumor cells were identified in 116 specimens (24 %), including 6 specimens (5 bone marrow and 1 pleural effusion) in which tumor cells were not identified by routine pathological examination.

Tumor cell colony formation was observed in 84% of the 90 SCLC positive specimens plated in agarose. The median number of colonies per 10^5 viable mononuclear cells plated was 23 (mean 66; range 3-350). The tumor cell origin of the colonies was confirmed by cytological examination in every case; by the ability of colonies to form tumors in athymic nude mice (10/12 attempts yielded a typical SCLC tumor); by DNA content analysis which revealed the selective growth of tumor cells (using aneuploid DNA content as a marker); and by electron microscopy examination of colonies (2 specimens) which revealed dense core granules consistent with SCLC diagnosis.

Among the 359 specimens in which tumor cells were not identified either by routine cytopathological techniques or by cytospin preparation, tumor cell colony formation was not observed in any instances. However, in 4 bone marrow specimens, typical hematopoietic colony formation was observed, in the absence of added growth factors required for CFU formation. In addition to clinical specimens of SCLC, in vitro soft agarose cloning has been evaluated in 72 other clinical specimens of human tumors including 25 specimens of nonsmall cell lung cancer specimens. For the 25 non-SCLC lung cancer specimens, tumor cell colony formation was observed in 15 (60%) with a median number of 17 colonies/ 10^5 cells. Tumor cell colony formation of these specimens was confirmed as for SCLC specimens. In the remaining 47 specimens, obtained from a variety of tumors, colony formation was observed in 16 (33%) specimens.

7. In Vitro Culture of Human Breast Cancer in a Serum-Free Defined Medium

Using the well characterized breast carcinoma cell line, MDA-MB231, a large number of different hormones and growth factors were tested for their ability to stimulate the growth of these cells in liquid culture. Seven factors, including insulin, transferrin, dexamthasone, tri-iodothyronine, 17 B-estradiol, prostaglandin F2a, and fibronectin were

shown to be mitogenic. Together, these 7 factors in a mixture of DMEM/F12 media were shown to support the long term growth of both this cell line, and 4 other cell lines of breast carcinoma. In the serum-free medium, the growth rate of the cells was similar to that observed for the cells cultured in serum-free medium. In addition to supporting the growth of breast carcinoma cell line in liquid culture, this serum-free medium, with the addition of 0.5% bovine serum albumin, supported the soft agarose cloning of the cells at an efficiency 2-3 fold greater than that observed in plain serum-supplemented medium.

Because the growth of breast cancer *in vivo* can be modified by hormone supplementation, the influence of hormone addition to the growth of these cells and their response *in vitro* to chemosensitivity agents was tested using a clonogenic assay. Cells, cultured in both serum-supplemented medium and serum-free hormone supplemented medium were tested in parallel for their sensitivity to 8 different cytotoxic agents including adriamycin, vincristine, 5-fluorouracil, and methotrexate, agents commonly used in the therapy of breast cancer. Although the hormone supplementation markedly improved the colony forming efficiency of the cell lines, no alteration in the chemosensitivity pattern of the cell lines were observed with these additions.

V. Future Studies

The use of SFM for the growth of SCLC clinical specimens has greatly improved our ability to establish cell lines of SCLC and has aided the recognition of a subset of SCLC which, although morphologically similar to other cell lines of SCLC, lack many of the biochemical markers of SCLC. In addition, the rapid expansion of tumor cells in HITES medium should facilitate our efforts to determine *in vitro* drug sensitivity of tumor cells within a short period of obtaining them from patients. The ability to establish continuous cultures in SFM should also facilitate the identification and characterization of factors which are both produced by the cells and which function as autocrine factors for their growth.

Future studies for growth of SCLC include the isolation and characterization of the factors in the conditioned medium, the generation of monoclonal antibodies to these factors, and the use of these factors in the cloning of fresh specimens of SCLC.

Future studies with bombesin include 1) the characterization of receptors in small cell lung cancer cell lines; 2) the correlation of receptors with both bombesin production and the response of cells to exogenous bombesin; and 3) the generation of monoclonal antibodies to bombesin receptors which may be used to regulate growth of SCLC cells both *in vivo* and *in vitro*. Also, we will screen other SCLC cell lines which have both bombesin receptors and which secrete bombesin. If mechanisms can be determined which regulate the secretion of bombesin they have potential use in the differential diagnosis of patients with lung cancer (bombesin stimulation similar to calcitonin stimulation in medullary

carcinoma of the thyroid), and in regulating the growth of these cells both in vivo and in vitro. Future studies regarding metastatic lesions include (1) evaluation of surface protein phenotypes among different metastatic lesions; (2) evaluation of the in vitro radiation biology and chemosensitivity of cell lines; and (3) evaluation of the oncogene profile of lesions from different sites.

For culture of NSCLC, we are particularly interested in improving the substrate on which attached NSCLC cells grow, using such newly discovered factors such as laminin. Further efforts to improve the growth of fresh specimens of human NSCLC will be undertaken.

In an attempt to improve the CFE of fresh tumor specimens, enzymatic methods for obtaining single cell suspensions and the addition of hormones and growth factors to culture media will be further evaluated.

Future studies on breast cancer culture will include the evaluation of our medium for the primary cloning of breast carcinoma specimens, and the establishment of breast carcinoma cell lines in continuous culture.

A project to clone the gene for bombesin is just getting underway. However, Dr. Moody has been able to extract mRNA from one of our lines producing large amounts of bombesin (NCI-H209) and has obtained in vitro translation of immunoreactive bombesin as detected by a radioimmunoassay. Because of the important role of bombesin in the differentiation of small cell lung cancer and its role as an "autocrine" growth factor, we feel the ability to study the bombesin gene directly warrants the effort necessary to clone the bombesin gene. The direct input of our laboratory in the cloning procedures will depend on how smoothly the work goes at George Washington University. If progress is not being made at an appropriate rate, we will become fully involved.

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Title: IN VITRO STUDIES OF DRUG AND RADIATION SENSITIVITY AND RESISTANCE OF HUMAN LUNG CANCER CELLS

I. Personnel

- A. Senior Staff: Dr. D. N. Carney
- B. Clinical Associates: Dr. G. Morstyn, Dr. M. Brower, Dr. R. Knop
- C. Other Professional Staff: None
- D. Technical Staff: Ms. V. Bertness

II. Collaborators

Dr. J. Mitchell (ROB/NCI); Dr. T. Kinsella (ROB/NCI); Dr. G. Curt (CPB/NCI); Dr. B. Chabner (CPB/NCI); Dr. K. Cowan (MB/NCI); Dr. G. Batist, Dr. C. Myers, (CP/NCI); Dr. D.A. Cooney and Dr. D.G. Johns (LMCP/DTP/NCI)

III. Introduction

A. Objective

In vitro cell culture of human tumor cells, either in liquid or in semi-solid media, offers important systems to study drug and radiation biology and also have major clinical implications. We have utilized both culture methods for these purposes and have also performed in vivo-in vitro comparisons of tumor sensitivity.

B. Rationale and Background

Data from two major groups of investigators (Salmon et al., Tucson and Van Hoff et al., San Antonio) have suggested that the clonogenic assay may be of value in predicting patients' response to chemotherapeutic agents. As part of the evaluation of the clonogenic assay for the growth of primary specimens of lung cancer, the in vitro chemosensitivity of fresh specimens was determined and results obtained correlated with clinical responses. The assay was determined in a manner very similar to that described by Salmon et al. (NEJM, 1978). In addition to clinical specimens, in vitro chemosensitivity was also determined on established cell lines of lung cancer to evaluate its potential for predicting patients' responsiveness to therapy, and its use as a tool for screening new agents prior to their entry into Phase I-II clinical trials.

Established cell lines of human tumors are a useful model to evaluate the radiation biologic properties of histologic subtypes within a given tumor. Using cell lines of SCLC and non-SCLC, a study was carried out to determine the in vitro radiation sensitivity of lung cancer cells and to correlate the observed responses with (1) the known clinical responses of these tumors to radiation and (2) the sensitivity of cell lines to a variety of cytotoxic agents used in the treatment of patients with lung cancer.

Histologic variants of SCLC have long been recognized, including the lymphocyte-like type, the fusiform, polygonal and other subtypes. We and others have shown that among these subtypes no significant differences have been observed in the clinical presentation, extent of disease, sites of metastases or response to therapy. However, in approximately 6% of newly diagnosed patients with SCLC, a mixture of SCLC with other types of lung cancer, including large cell (LC), has been observed. Of interest, in these patients, the prognosis is significantly worse than for those patients with "classic" SCLC. These mixed histology patients have a significantly lower complete response rate to cytotoxic therapy and a shorter median survival (4).

In a large number of autopsy studies of patients treated for SCLC, a mixed histology (mainly SCLC/LC) has been observed in up to 39% of all patients. It is unclear whether this observation represents the evolution of 2 separate lung cancers or the transformation of the initial pure SCLC to a large cell variant cell type. In addition, tumor biochemical markers (including L-dopa decarboxylase), usually elevated in classic SCLC, were markedly lower in tumors with mixed histology. As patients with SCLC who relapse from initial intensive combination are usually resistant to further chemotherapy and radiation, it is possible that this "resistance" may be a reflection in the change in the phenotype of the tumor cells from classic SCLC with APUD properties to a large cell variant with loss of these properties.

In the establishment of a large number (51) of cell lines from patients with SCLC, 3 subclasses of cell lines have been identified: (1) cell lines with typical morphologic and biochemical characteristics of SCLC including elevated levels of L-DDC and bombesin, classic APUD cell markers; (2) cell lines which have both gross and light microscopic characteristics of SCLC but which lack APUD cell properties (biochemical variant cell lines); and (3) cell lines which either in vivo (patient or nude mouse) or in culture undergo transformation from a small cell to a large cell morphology with loss of cell APUD characteristics (morphologic variants of small cell). Unlike non-SCLC cell lines which demonstrate substrate adherence, SCLC cell lines and their variants grow as floating cell aggregates and have elevated levels of neuron-specific enolase and the BB isozyme of creatine kinase, CK-BB.

Studies of the in vitro chemosensitivity of SCLC cell lines have demonstrated that lines from previously treated patients are resistant in vitro to most cytotoxic agents, while cell lines from newly diagnosed untreated patients are sensitive. Because sensitive cell lines and resistant cell lines are not distinguishable from each other with respect to a number of phenotypes including gross morphology, doubling time, and expression of APUD cell markers, the cell lines are a useful model to evaluate mechanisms of drug resistance in human tumors. Because most patients with small cell lung cancer receive therapy with regimens which usually include methotrexate, initial studies have evaluated why some cell lines are resistant to this drug and others sensitive.

Glutathione peroxidase (GSH-Px) is a selenium dependent enzyme that is

essential for quenching lipid peroxidation and minimizing free radical formation from molecular oxygen. Radiation and some chemotherapeutic agents (adriamycin, bleomycin) may mediate their cytotoxicity via the generation of free oxygen radicals. Small cell lung cancer cell lines have been cultured in a serum-free media containing hydrocortisone, insulin, transferrin, estradiol and selenium (HITES). To evaluate GSH-Px dependence on selenium and its role in adriamycin toxicity a variant of NCI H345, a SCLC line permanently established in HITES medium was developed. This variant line, NCI H345H has been adapted to grow in, and has now been established in serum-free medium without selenium supplementation (HITE medium). Although the growth rate of the cells cultured in the HITE medium is slower than those cultured in HITES, no change has been observed in a number of other biological properties of these cells including the expression of a variety of APUD cell characteristics.

IV. Progress Report

1. In Vitro chemosensitivity studies of fresh clinical specimens and established cell lines of small cell lung cancer

As previously indicated, because of the low CFE of clinical specimens of SCLC, in vitro drug sensitivity studies were possible in only 22% of 80 specimens evaluated which contained SCLC tumor cells (or in only 7% of all SCLC specimens received--both positive and negative specimens). In these specimens, at least 30 colonies were present in control plates, and at least 1 drug could be evaluated in triplicate at 3 different concentrations. Drugs evaluated included those drugs currently used in our SCLC protocols and agents undergoing Phase I-II evaluation. In 17 clinical specimens, a total of 75 in vitro drug assays were carried out. In vitro results of sensitivity or resistance were correlated with the observed patient responses. (It should be noted that most patients were treated with combination therapy while in vitro tests evaluated each drug separately.) In this retrospective analysis, the accuracy of the in vitro assay for predicting clinical sensitivity was 77% and for predicting in vivo resistance, 100%. In the latter tests, all specimens were obtained from patients who had relapsed from prior intensive multiple drug combination therapy.

Because most clinical specimens of SCLC are inevaluable for in vitro drug sensitivity responses, well established cell lines of SCLC were also tested for in vitro sensitivity. Nine cell lines, 5 established from patients who had relapsed from multiple drug combination therapy and 4 cell lines established from newly diagnosed patients, were tested. Results were compared with the observed in vivo patient responses as for fresh specimens. Although the cell lines were in culture for periods ranging from 4-48+ months at the time of testing, the in vitro drug sensitivity assay accurately predicted the patient behavior (i.e., response/no response) similar to that observed for fresh clinical specimens. Several cell lines, evaluated at 12 month intervals, revealed no significant changes in their in vitro chemosensitivity patterns.

These data suggest cell lines of human SCLC retain their "predicted" pattern of in vitro chemosensitivity in continuous culture, in that cell lines established from heavily pretreated, unresponsive patients remain resistant in vitro, while cell lines established from newly diagnosed patients who subsequently respond to chemotherapy remain sensitive. Thus, cell lines may be used to predict for in vivo chemosensitivity, to evaluate mechanisms of drug resistance, and to identify new drugs with potential activity in the treatment of patients with SCLC.

In a study of the in vitro chemosensitivity of 5 cell lines of non-SCLC established from previously untreated patients, in vitro sensitivity was observed in only 4 of 45 tests with standard doses of 9 drugs commonly used in the treatment of non-SCLC. This response rate (10%) is similar to that observed in the treatment of patients with this tumor with single agent chemotherapy. Thus, as for SCLC, cell lines of non-SCLC predict the in vivo-observed clinical responses of these tumors to chemotherapeutic agents and potentially could be tested to select patients' chemotherapy, evaluate mechanisms of drug resistance, and screen new agents prior to their use in clinical trials. Because of the poor CFE of fresh clinical specimens and because in vitro sensitivity studies with established cell lines appear correlated with clinical responses, subsequent studies of in vitro sensitivity will be carried out in most instances using cell lines which have been in culture for short periods of time. The use of cell lines, in addition to the above, has the advantage of permitting the testing of many more agents than is possible with fresh clinical specimens.

2. In Vitro Radiation Biology of Human Lung Cancer Cell Lines

Using a soft agarose clonogenic assay, the in vitro radiation sensitivity of 7 "classic" cell lines of SCLC and 8 cell lines of non-SCLC lung cancer, including adenocarcinoma (4 cell lines), large cell (2 cell lines), and mesothelioma (2 cell lines), was evaluated in collaboration with Drs. Mitchell, Morstyn, and Kinsella (ROB/NCI). For radiation studies, a single cell suspension of cells in log phase growth was irradiated and plated at varying densities in soft agarose. Cell samples were irradiated at room temperature using a 6 MeV photon beam from a Mevatron VI linear accelerator at a dose rate of 200 rad/min. Cells were irradiated over a dose range of 0-1,000 rad. In vitro chemosensitivity was also determined using a soft agarose clonogenic assay. Radiation survival curves for the 7 classic cell lines of SCLC were very similar and were characterized by D_0 values ranging from 51-140 rad. With the exception of a single SCLC cell line (NCI-H146), the extrapolation numbers (n) were small, ranging from 1.0-1.49. For NCI-H146 the n value was 3.3.

Five of the 7 cell lines of SCLC demonstrated in vitro resistance to a variety of cytotoxic agents, including vincristine, adriamycin, BCNU and methotrexate, while 2 cell lines (both established from untreated patients) were sensitive to these drugs. No correlation was observed between in vitro radiation sensitivity (D_0 , n) and in vitro sensitivity of SCLC cell lines to chemotherapy agents.

The radiation biological properties of 8 non-SCLC cell lines demonstrated considerable heterogeneity. For these cell lines D_{05} s ranged from 97-180 and extrapolation numbers (n) ranged from 1.1-14. The lowest n value was observed in an adenocarcinoma cell line (NCI-H324) while the highest value (14) was observed in a large cell lung cancer cell line (NCIH157). Although the cloning efficiency (CE) varied from 0.3-65% among the non-SCLC lung cancer cell lines, no correlation was observed between cloning efficiency and radiation sensitivity. Because the majority of cell lines of non-SCLC lung cancer grow as attached monolayer cultures, a comparison of the radiosensitivity of the cell lines plated in soft agarose or in liquid culture was determined. Although the cloning efficiency of cell lines in agarose was 10-fold less than in liquid culture, the radiobiological properties were identical.

In comparing results obtained with both SCLC and non-SCLC cell lines, while there was considerable overlap in D_{05} s, a major difference was observed in the percentage of cells surviving 200 rad fraction. For SCLC, this percentage ranged from 2-32%; while for the 8 non-SCLC cell lines, the surviving percentage ranged from 27-95%.

3. In Vitro Chemosensitivity and Radiation Sensitivity of Variant Cell Lines of SCLC

The in vitro growth properties of 4 variant cell lines (NCI-H437, H446, H524, and H526) and 2 large cell variant cell lines (NCI-H82 and N417) were compared and contrasted with "classic" SCLC cell lines. In contrast to SCLC cell lines, which in vitro demonstrate an initial slow growth rate and which rarely reach a steady state of proliferation until approximately 2-4 months after initial plating, these variant cell lines were characterized by an early rapid and sustained tumor cell proliferation. These cell lines do not have a crisis in growth and are established as permanent cell lines with relative ease. The relative ease in the establishment of a cell line from a patient with SCLC is highly suggestive of a variant cell line.

In contrast to SCLC cell lines which have a colony-forming efficiency in soft agarose of 1-5% and a relatively long doubling time (36-96 hr), the variant cell lines have a much higher colony-forming efficiency (10-40%), a shorter doubling time in liquid culture (< 24 hr) and a shorter latent period to tumor formation in athymic nude mice compared to "classic" SCLC cell lines. Of interest, cell line NCI-H526, lacking APUD characteristics, but which morphologically resembles SCLC, was established from a newly diagnosed untreated patient suggesting that this "transformation," at least in some instances, is not an effect of cytotoxic therapy. In addition, and perhaps of greater significance, the patient from whom this cell line (NCI-H526) was established did not respond to induction cytotoxic therapy, a feature observed in less than 10% of our newly diagnosed patients with SCLC.

In collaboration with Drs. Mitchell and Kinsella (ROB) the radiation biology of 2 morphologic variant cell lines (NCI-H82 and NCI-N417) was determined and contrasted with classic SCLC cell lines. Methodology was

as previously described. While the D_{05} of the large cell variant cell lines (80 and 91 rad) were similar to 7 classic SCLC cell lines (51-140 rad), the extrapolation numbers (n) were 5.6 and 11.1 in contrast to 1-1.5 for 6/7 SCLC cell lines. No correlation was observed between in vitro chemosensitivity and radiation response. The surviving fraction of cells at 200 rad was 2- to 5-fold greater for the large cell variants compared to the SCLC lines. These data suggest that transformation of SCLC to variant morphology and loss of APUD characteristics is associated with a marked increase in radiation resistance (n) in vitro. The observation of a significant increase in survival in large cell variants following 200 rad suggests that the use of large daily radiation fractions and/or radiation sensitivity drugs might lead to a greater clinical response in patients with large cell morphology.

4. Mechanisms of Drug Resistance in Human Tumor Cell Lines

Initial studies utilized a cell line NCI H249 which was established in vitro from a patient who had relapsed from high dose methotrexate chemotherapy, received as a single agent. In vitro sensitivity of the cells direct from the patient revealed that the cells were indeed resistant to methotrexate. This resistance persisted in vitro from 5-10 passages. Subsequent testing revealed that the cells were now sensitive to methotrexate in vitro. In collaboration with G. Curt and B. Chabner, CPB, early passage cells and late passage cells were studied in detail with respect to methotrexate metabolism.

In early passage the cell line displayed amplification for the gene coding for dihydrofolate reductase. Studies with 3H uptake revealed no impairment of drug accumulation. Cytogenetic analysis of the cells at this time revealed a large number of double minute chromosomes. In late passage cells, with loss of resistance to methotrexate, dihydrofolate reductase levels fell to that observed in other sensitive cell lines. In association with this, there was a marked decrease in double minute chromosomes. These data would suggest that in some SCLC cell lines, instability of drug resistance may be related to gene amplification on double minute chromosomes.

Further studies on MTX metabolism in this cell line (NCI H249, early passage) have shown that this SCLC rapidly forms MTX polyglutamate species with 2-5 glutamyl groups. Those metabolites with more than 3 glutamyl groups are preferentially retained in the absence of extracellular drug and can cause delayed cytotoxicity when this retention exceeds the intracellular binding capacity.

Methotrexate studies have now been extended to a large number of SCLC cell lines. Comparison of cell lines (sensitive and resistant) include: 1) growth rate; 2) MTX uptake and intracellular binding; 3) DHFR specific activity and affinity studies; 4) Thymidylate synthetase activity and 5) synthesis and retention of MTX polyglutamates. Preliminary data indicates that retention of drug above intracellular binding capacity is the most important determinant of MTX responsiveness. This retention is in turn dependent on the degree of synthesis of higher molecular weight

polyglutamates. Drug metabolism is both time and concentration dependent. Together these results suggest that high-dose MTX may be clinically useful, not because of circumvention of transport deficits as has been previously considered; rather, MTX in high doses may allow accumulation of sufficient MTX polyglutamates for prolonged retention above intracellular binding capacity.

In collaboration with Drs. Cooney and Johns, the biochemical correlates of clonal growth inhibition in human lung tumor cell lines by tiazofurin has been evaluated. 2- β -D-Ribofuranosylthiazole 4-carboxamide (tiazofurin; NSC-286193; TR) is an IMP-dehydrogenase (IMPD) inhibitor which shows unusual activity in the murine Lewis lung tumor test system, and which will shortly be entering Phase I clinical trial. The active form of the drug appears to be its dinucleotide anabolite TR-phosphate-phosphate-ribose-adenine (TAD) (D.A. Cooney *et al.*, *Biochem. Pharmacol.* 31: 2133, 1982). We have examined the correlation between colony survival, IMPD inhibition and TAD formation in 6 human lung cancer cell lines, at a TR concentration in the therapeutic range (10^{-4} M). Three of four non-small cell tumor lines were sensitive to TR in a soft agarose clonogenic assay (colony survival <1%), whereas two small cell lines were resistant (colony survival >50%). Sensitive lines formed a greater amount of TAD (1110-2100 pmoles/mg protein) than did resistant lines (47-370 pmoles/mg protein). IMPD inhibition ranged from 73 to 98% in the sensitive lines and from 15 to 74% in the resistant lines, while IMP pools increased by an average of 33-fold in the sensitive lines vs 6-fold in the resistant lines. Guanine nucleotide pools showed a greater average decrease in the sensitive lines. The data indicate a correlation between growth inhibition by TR and biochemical parameters of TR action, and suggest a greater therapeutic potential for TR in non-small cell as compared to small-cell lung cancer.

5. The Role of Selenium Glutathione Peroxidase in Cellular Sensitivity to Adriamycin

Preliminary studies of the variant cell line have shown that whole cell specific activity of GSH-Px declined by 50% in the absence of selenium supplementation. Subcellular distribution studies demonstrated the decline of the cytosolic selenium dependent enzyme from 35% to 0%, with remaining GSH-Px present only in membranes and subcellular organelle fractions.

V. Future Studies

Although efforts, through the use of growth hormones, etc., will be made to improve the CFE of primary specimens, because of our success in establishing cultures from approximately 75% of clinical specimens of SCLC, and because cell lines appear not to alter their *in vitro* chemosensitivity pattern in continuous culture, most studies of *in vitro* chemosensitivity will be carried out on short-term culture cell lines (see Protocol NCI-83-13). In addition, cell lines will be used to study mechanisms of drug resistance, in particular using paired

cell lines (i.e., lines established from the same patient prior to receiving therapy and following relapse from therapy) and for screening all new Phase I-II agents used in clinical trials.

Future radiobiology studies include (1) the determination of the radio-sensitivity of fresh clinical specimens of lung cancer and (2) the correlation of in vitro radiation sensitivity of cell lines with glutathione levels and synthesis by these cells.

Changes in GSH-Px have been demonstrated in tumor cell lines after exposure to various concentrations of adriamycin. These changes appear to be greatest in the cytosolic GSH-Px pool. Studies will be undertaken to determine the effects of adriamycin on the GSH-Px pool in the selenium independent cell line and the biologic significance of these findings regarding sensitivity of the cells to this compound.

Nuclear Magnetic Resonance (NMR) offers a unique non-invasive system for evaluating intracellular metabolic pathways. An NMR group, headed by Dr. J. Cohen, has developed techniques allowing for detection of intracellular metabolites in cell culture systems using NMR spectroscopy techniques. NMR studies will be carried out on different histologic types of lung cancer cell lines to determine if differences can be identified which will separate one histologic type from another. These studies will be done using phosphorus (^{31}P). In addition, adenosine triphosphate cellular levels will be measured and changes in these levels in response to a variety of agents, including cytotoxic agents will be assayed. Detection of cellular creatine phosphate as a marker for small cell lung cancer, in contrast to non-SCLC lung cancer cells, is being reviewed.

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T A B L E 1

PRIMARY HETEROTRANSPLANTATION OF HUMAN LUNG CANCERS FROM PATIENTS
INTO ATHYMIC NUDE MICE (1977 - 1983)

<u>Lung Cancer Type</u>	<u>No. Tumors Innoculated/ No. of Takes</u>
Small cell	34/86 (40%)
Non small cell	12/40 (30%)

T A B L E 2

ESTABLISHMENT OF LUNG CANCER CELL LINES (1977 - MARCH 1983)

<u>Tumor Type</u>	Cell Line Establishment	
	<u>(1977-1983)</u>	<u>(1982-1983)</u>
SCLC	51/124 (41%)	17/23 (75%)
NSCLC	15/42 (36%)	5/13 (38%)
Mesothelioma	4/6 (50%)	1/2 (50%)

T A B L E 3

TYPES OF LUNG CANCER CELL LINES

<u>Lung Cancer Type</u>	<u>No. of Cell Lines</u>
Small cell lung cancer	51*
Adenocarcinoma	7
Large cell carcinoma	3
Mucoepidermoid	1
Mesothelioma	4

*Includes SCLC lines with 'classic' and variant morphologies.

T A B L E 4

ORIGINS OF 51 CONTINUOUSLY GROWING SCLC LINES (1977 - MARCH 1983)

<u>Sex</u>	
Male	37
Female	14
<u>Therapy Status</u>	
Prior therapy	35
No prior therapy	16
<u>Sites of Origin</u>	
Bone marrow	15
Pleural effusion	13
Lymph node	10
Subcutaneous nodule	4
Lung (primary tumor)	5
Liver	1
Brain	1
Unknown (outside source)	2

T A B L E 5

SUMMARY OF PROPERTIES OF SCLC CELL LINES*

	<u>Fraction Positive</u>
L-dopa decarboxylase	34/39
Bombesin	32/34
Neuron specific enolase	29/30
CK - BB	27/27

*All lines had classic SCLC morphology. "Variant" lines are excluded from this table.

T A B L E 6

INFLUENCE OF PRIOR TREATMENT STATUS OF PATIENT ON GROWTH OF
CLINICAL SPECIMENS IN DEFINED HITES MEDIUM

<u>Treatment Status</u>	<u>Fraction with Growth</u>
Prior therapy	19/26 (73%)
No prior therapy	20/27 (74%)

T A B L E 7

INFLUENCE OF SITE OF ORIGIN OF SCLC TUMOR CELLS AND GROWTH IN
DEFINED HITES MEDIUM

<u>Specimen Source</u>	<u>Fraction With Growth</u>
Bone marrow	22/25 (88%)
Pleural effusion	7/13 (54%)
Lymph node	9/14 (64%)
Brain	<u>1/1 (100%)</u>
TOTAL	39/53 (74%)

T A B L E 8

SELECTIVITY OF HITES MEDIUM FOR THE GROWTH OF CLINICAL SPECIMENS OF SCLC

<u>Histology</u>	<u>Fraction With Growth</u>
SCLC	39/53 (74%)
Non-SCLC	2/30 (7%)
Nonmalignant	0/100 (0%)

T A B L E 9

NUDE MOUSE TUMOURIGENICITY OF SCLC LUNG CANCER AGAROSE COLONIES.

<u>Prior Chemo- therapy</u>	<u>Specimen Source</u>	<u>No. of Colonies per 10⁵ Cells^a</u>	<u>No. of Agarose Colonies Inoculated</u>	<u>Tumour Forma- tion</u>	<u>Latent Period (Weeks)</u>
Yes	Bone marrow	22	8	Yes	9
Yes	Bone marrow	90	4	Yes	15
Yes	Bone marrow	320	12	Yes	9
No	Bone marrow	16	6	Yes	12
No	Bone marrow	26	6	Yes	15
No	Lymph node	16	2	Yes	16
No	Lymph node	90	6	Yes	18
No	Pleural effusion	34	5	Yes	15

^aMean no. colonies per 3 plates. Agarose colonies were harvested 14-21 days after plating, pooled and inoculated intracranially into athymic nude mice.

T A B L E 10

IN VITRO AGAROSE CLONING OF CLINICAL SPECIMENS OF LUNG CANCER

	<u>SCLC</u>	<u>NSCLC</u>
No. of tumor positive samples	90	25
No. of tumor colony growth	76 (84%)	15 (60%)
No. of colonies/10 ⁵ cells	3-350	16-86
Median no. of colonies	22	17
Percentage suitable for in vitro drug testing	22%	20%

T A B L E 11

IN VITRO/IN VIVO SENSITIVITY CORRELATION FOR CLINICAL SPECIMENS AND ESTABLISHED CELL LINES OF SMALL CELL LUNG CANCER

<u>Specimen Source</u>	<u>No.</u>	<u>No. Tests</u>	$\frac{R^*}{R}$	$\frac{R}{S}$	$\frac{S^*}{S}$	$\frac{S}{R}$
Clinical specimens	17	75	48	0	21	6
Cell lines	<u>9</u>	<u>72</u>	<u>41</u>	<u>0</u>	<u>28</u>	<u>3</u>
TOTAL	26	147	89	0	49	9

Predictive Accuracy

	<u>True Positive Rate (Sensitivity)</u>	<u>True Negative Rate (Resistance)</u>
Clinical specimens	77%	100%
Cell lines	<u>92%</u>	<u>100%</u>
TOTAL	85%	100%

*R = resistance, S = sensitivity.

Title: LABORATORY STUDIES OF THE BIOLOGY OF MALIGNANT T CELLS

I. Personnel

- A. Permanent Senior Staff: Dr. P.A. Bunn, Jr.
- B. Clinical Associates: Dr. C.F. Winkler
- C. Other Professional Staff: Two visiting scientists to arrive July 1983 (Drs. Boven and Lindmo)
- D. Technical Staff: P. Jewett

II. Collaborating Units

Drs. R.C. Gallo and F. Wong-Staal (Laboratory of Tumor Cell Biology, NCI); Dr. S. Broder (Laboratory of the Director of the COP, NCI); Dr. J. Whang-Peng (Cytogenetics Section, Medicine Branch, NCI); Dr. K. Gray (Division of Endocrinology, University of North Carolina); Dr. G. Mundy (University of Texas, San Antonio)

III. Introduction

A. Objectives

1. To establish malignant cell lines from patients with T cell malignancies.
2. To optimize the methods for serum-free growth of normal and malignant T cells and to compare the growth requirements.
3. To define the exogenous and endogenous (autocrine) growth factors for malignant T cells.
4. To evaluate growth factor receptors and to assess the ability of antibodies to growth factors and growth factor receptors to inhibit cell proliferation.
5. To survey malignant T cells for the presence of HTLV RNA or DNA sequences and other oncogenes.
6. To determine whether HTLV⁺ and HTLV⁻ mature T cell lines have specific chromosomal translocations or deletions.
7. To isolate and purify growth factors produced by the malignant T cells.
8. To identify, isolate and purify osteoclast activating factors produced by malignant T cells.
9. To determine the effects of monoclonal antibodies, biologicals and drugs on malignant T cells.

B. Background

Recent advances in cell biology, molecular genetics and immunology have made it possible to identify subsets of normal and malignant cells, to define their growth requirements in chemically defined serum-free media, to isolate, purify, and sequence cell produced autocrine growth factors and growth factor receptors; to clone the genes for these factors, to probe malignant cells for related transforming genes and retrovirus nucleic acid sequences, to determine specific chromosomal aberrations and translocations; and to determine whether these abnormalities correspond to the location of oncogenes or genes responsible for growth regulation and differentiation.

Tumor Growth Factors: Todaro and Sporn have postulated that many tumors produce factors which are autostimulatory. These same tumors have certain receptors specific for their own growth factors. Consequently, growth regulation is uncontrolled in this "autocrine" system. These growth factors (TGF's) may be responsible for transformation and may be the products of oncogenes or of retroviral nucleic acids. For example, rat embryo fibroblasts transformed by Abelson Murine leukemia virus produce and release a TGF. The major virus gene product, P120, is associated with a functional tyrosine specific protein kinase which in turn is correlated with production of the TGF. This TGF has been purified and studies of its nucleic sequence are in progress. Recent studies suggest that a wide variety of human tumors produce their own TGF's and have specific receptors for these TGF's. For example, in our own laboratories we have preliminary evidence that human T cell lymphomas produce at least two different TGF's and have separate receptors for these TGF's. It is likely that production of one of these TGF's is associated with the human T cell lymphoma virus (HTLV), while the other is not associated with this virus. Small cell lung cancer cells also produce their own growth factor(s). Isolation, and characterization of these growth factors, their receptors, associated viruses and transforming genes is dependent on the ability to grow and clone malignant human cells.

Serum-Free Media: Sato and coworkers reported that human tumors can be cultivated in vitro in serum-free media supplemented with defined chemicals and hormones. Growth factors appeared to be specific for individual cell types and vary between different cell types. In many instances, growth factors required for growth of normal and malignant cells of the same type are similar. The ability to grow cells in defined media allows analysis and purification of factors (tumor produced and exogenous) which stimulate and inhibit tumor growth. Defined media may also increase our ability to establish permanent cell lines from fresh tumor samples. For example, we have defined a hormonally supplemented media which specifically supports the growth of human small cell lung cancer. This media supports the growth of established cell lines, and has increased our ability to establish permanent cell lines from fresh patient specimens. The observation that conditioned media from mitogen stimulated normal lymphocytes may contain specific growth factors

aided in the subsequent development of permanent malignant human T-cell lymphoma cells in our laboratory.

T-cell Growth Factor or Interleukin II: Gallo, Ruscetti and co-workers demonstrated that normal human T-lymphocytes produce a specific protein (MW 13,000) which allows for the continuous propagation of normal T-cells. This factor is produced only by T-cells which have been activated by mitogen or antigen and which have been in contact with lymphocyte activating factor (LAF, interleukin I) produced by macrophages. Normal resting T-cells lack TCGF receptors and do not respond to TCGF. In contrast, antigen/mitogen activated T-cells develop TCGF receptors and undergo active proliferation in response to TCGF. After initial activation T-cells can often be maintained indefinitely in vitro with addition of exogenous TCGF.

The permanent malignant T-cell lines which were established in our laboratory are independent of exogenous TCGF. Gootenberg, et al. demonstrated that one of these cell lines (Hut 102) constitutively produces large quantities of TCGF which is biologically active but which has minor biochemical difference from the TCGF produced by normal lymphocytes. The same group reported that these malignant cells have TCGF receptors which are present without mitogen/antigen stimulation and which may be associated with transformation.

Growth Factor Receptors: Waldmann and coworkers developed a monoclonal antibody, anti-Tac, which reacts with mitogen/antigen activated lymphocytes. This monoclonal antibody was later shown to react directly with the TCGF receptor, is not present on resting lymphocytes but develops in response to mitogens or antigens. Its development precedes the onset of DNA synthesis and DNA synthesis can be blocked by using the antibody. We and others have demonstrated that this antibody reacts strongly with 100% of Hut 102 cells and cells from all HTLV related malignant T-cell lymphomas. In contrast, this antibody does not react with the malignant cells from most patients with cutaneous T-cell lymphoma in the U.S.

The transferrin receptor is also not present on resting lymphocytes, develops within 8 hours of mitogen activation before DNA synthesis, and is present on all HTLV⁺ malignant cell lines without activation. Treatment with anti-transferrin receptor antibody inhibits cell proliferation. Treatment with anti-Tac 6 hours after mitogen stimulation inhibits the development of transferrin receptors whereas treatment with anti-transferrin receptor does not block development of TCGF receptors.

Human T-cell Lymphoma Virus: The establishment of Hut 102 led to the identification of HTLV by Poiesz, et al. Subsequent studies demonstrated that HTLV is associated with unique T-cell disorder found primarily in Japan, the Caribbean basin, and to a limited extent in the Southeast U. S. While some clinical features

(hypercalcemia, lytic bone lesion, aggressive clinical course) of this syndrome are unique, there is considerable overlap of the clinical pathological and immunological cellular profile (mature helper inducer phenotype) with other malignant T-cell neoplasms.

Oncogenes: Some retroviruses are highly oncogenic, transform cells in culture and induce neoplastic disease in infected animals. Approximately 20 such transforming retroviruses have been characterized. The viral transforming genes have homologous, conserved sequences, cellular oncogenes in normal cells. These retroviruses thus appear to be recombinants in which a transforming gene, derived from a homologous gene of normal cells, has been inserted into a retrovirus genome. As one mechanism, it has been postulated that integration of viral cDNA in the vicinity of a cellular oncogene may result in abnormal gene expression. Studies of the MC-29 virus induced B-cell lymphomas, for example, have shown that 80% of such lymphomas have viral DNA sequences, including a viral transcriptional promoter, integrated in the vicinity of the cellular gene homologous to the myc transforming virus.

DNA from human tumors can, in some cases, induce transformation by transfection into NIH 3T3 cells. Such transformation has been demonstrated in one human T-cell mycosis fungoides/Sezary tumor by Lane and Cooper.

Since some human T-cell lymphomas contain a retrovirus and since one malignant T-cell lymphoma has been shown to have transforming genes, additional studies are needed.

Cytogenetic Studies: Specific chromosomal abnormalities have been associated with several human malignancies. It has been postulated that specific translocations associated with certain tumors may cause activation of genes involved in the transformation process. For example, in B-cell malignancies the translocations of chromosomes 2, 14, and 22 into 8 result in rearrangements and/or expression of the c-myc cellular oncogenes.

The chromosomal locations of many oncogenes have been mapped. With Dr. J. Whang-Peng, we have conducted extensive cytogenetic studies on patients with cutaneous T-cell lymphomas (1,8). If cytogenetic changes are found, we can evaluate these cells for possible rearrangement of oncogene loci.

IV. Progress Report

1. Establishing malignant T cell lines.

We have shown that malignant T cell cultures are rarely, if ever, occasionally provide adequate stimulation, but the response of the malignant cells varies considerably to different mitogens. Their response is also often less than that of normal lymphocytes. Thus, when TCGF is added in addition to mitogen growth of normal T cells invariably ensues. Establishing permanent cell

lines from CTCL (HTLV⁻) patients has proven exceedingly difficult though we have developed multiple single cell clones of one such line (Hut 78). The data suggest that the response of CTCL cells and the development of TCGF receptors in response to mitogen is suboptimal when compared to normal T cells.

In contrast, the malignant T cells from HTLV⁺ patients invariably have de novo TCGF receptors without prior mitogen activation, and in most instances cells can be maintained indefinitely in lectin free TCGF. In some instances these HTLV⁺ cultured cells developed their own constitutive production of TCGF and exogenous TCGF can be gradually withdrawn. We have maintained malignant cells in culture from patients in excess of 5 months. Characterization of these lines by nude mouse tumorigenicity, cytogenetics, lymphokine production, histochemical staining, monoclonal phenotype, etc., is in progress.

2. Determination of serum-free growth conditions for malignant T cells. We have demonstrated that a serum-free media (bovine serum albumin, insulin, transferrin, ethanolamine, selenium [BITES]) will support the growth of established malignant T-cell lines. These malignant T-cell lines can be cloned in soft agarose using this defined media. Fresh lymphocyte specimens from HTLV⁺ patients grow preferentially in this defined media in comparison to serum-supplemented media. Our established cell lines have been maintained in this medium for more than 8 months. In addition, we have been more successful in establishing new lines from clinical samples using this medium than serum containing medium. The ability to grow the malignant HTLV⁺ cells in serum-free media has allowed us to assay for the presence of other growth substances which may be present in serum or be inhibited by serum.
3. We have shown that the malignant cells can be cloned in soft agar. While our present serum-free medium supports the growth of the malignant cells in mass culture, it is not optimal since the cloning efficiency is still extremely low and is improved by addition of lectin-free TCGF, as well as other factors.
4. We have shown that clonal lines from at least one line (Hut 78)--previously thought to be HTLV⁻ by virtue of antibody analysis, reverse transcriptase assay, and electron microscopy--actually contains portions of the HTLV envelope gene as determined by Southern blot analysis by Dr. Gallo's lab using a cloned HTLV probe. Analysis of further samples is being performed.
5. We have made preparations for cytogenetic analysis of seven HTLV⁺ patients for chromosome banding studies, and these are being analyzed by Dr. Whang-Peng.

V. Future Plans

1. Growth studies will be performed on HTLV⁺ and HTLV⁻ established cell lines using serum-free ITES media as a baseline. Hormones and growth factors including phorbol esters, glucagon, GH, T₃, TSH, ACTH, estrogens, androgens, progesterones, prostaglandins, mercaptoethanol, Beta endorphin, epinephrine, and other amines, bombesin, EGF, NGF, PDGF, and lithium will be studied for growth stimulating and inhibitory effects. Conditioned (CM) media from cells grown in serum-free media will be collected, and concentrated. Various fractions of the CM will be evaluated for their effects on proliferation. Clonogenic soft agar assays will be performed in parallel using the same factors.
2. Monoclonal antibodies which react with a variety of T-cell antigens may influence T cell growth. For example, anti-T3 is mitogenic for normal human T cells whereas anti-Tac and T9 which bind to the TCGF receptor and transferrin receptors, respectively, may inhibit proliferation. The effects of these antibodies on in vitro cloning and growth will be investigated. We will also study the effects of antigen density and modulation on the ability of monoclonal antibodies to inhibit proliferation.
3. Isolation, Purification, and Sequencing of Growth Factors Produced by Malignant T-Cells: Preliminary experiments show that Hut 102 produces a TCGF which stimulates the growth and cloning efficiency of other virus positive malignant T cell lines but which inhibit growth of virus negative lines. The CM from Hut 78, on the other hand, contains factor(s) which stimulates the growth and cloning efficiency of virus negative and positive cell lines. Biochemical concentration, characterization and purification of these growth factors will be performed in this laboratory. Collaborative studies to sequence the factors will be performed after purification. We will assay cell lines for receptors for isolated growth factors. These studies may be expanded to evaluate immature malignant T cells and malignant B-cells.
4. Lymphokine Identification: In collaboration with Drs. Mundy, K. Gray, the conditioned media from established cell lines will also be analyzed for lymphokine production. It is well known that HTLV⁺ patients have a high frequency of hypercalcemia and lytic bone lesions. We have demonstrated that supernatants from these cultures contain an osteoclast activating factor (OAF) which is not prostaglandin nor PTH. If such factors are produced they will be concentrated, characterized and purified. T cells from patients with CTCL and ATL have mature phenotypes and produce a number of other lymphokines. Established cell lines will be assayed for interferon production, CSF activity, production of IL2 and IL3, and production of immunoregulatory substances.

5. HTLV Genetic Studies: In collaboration with Dr. Gallo's lab, established clonal cell lines will be assayed for the presence of retrovirus particles, reverse transcriptase, viral antigens and retrovirus nucleic acid sequences.
6. Cellular Oncogenes: In collaboration with Drs. Kuehl and Minna, we will survey the cell lines for the presence of oncogenes and determine whether these oncogenes are associated with growth factor production. We will determine whether the cell lines have transforming genes and whether these transforming genes, if present, are similar to known oncogenes or retrovirus genes.
7. Cytogenetics:
In collaboration with the cytogenetics section of Dr. J. Whang-Peng, we will determine whether HTLV⁺ and HTLV⁻-cell lines have specific chromosomal translocations or deletions. If specific abnormalities are identified we will seek to determine whether these are located near known transforming genes, the genes for TGF production or retrovirus genes.

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Title: LABORATORY STUDIES OF CELLULAR KINETICS OF HUMAN MALIGNANCIES

I. Personnel

- A. Permanent Senior Staff: P.A. Bunn, M.D.
- B. Clinical Associate: C.F. Winkler, M.D.
- C. Other Professional Staff: None
- D. Technical Staff: P. Jewitt

II. Collaborators: M. Lippman, M.D. (Medicine Branch, NCI)

III. Introduction

A. Objectives

1. To study the cellular kinetics of human malignancies before and after therapy.
2. To determine the effects of biological response modifiers (hormones, monoclonal antibodies, interferon) on cell cycle kinetics.
3. To determine whether expression of a variety of tumor cell associated antigens is cell cycle related.

B. Background and Rationale

There are extensive data on the cell cycle kinetics of a variety of animal model tumor systems. The data in human tumors are scanty, but available information suggests human tumors proliferate at much slower rates and with considerably more heterogeneity than the animal models. There are few data on the biologic and prognostic implication of kinetic measurements in human tumors.

Rational design of treatments for human tumors has been hampered by the lack of kinetic data and the heterogeneity observed in the available studies. There are few studies of cell cycle perturbation caused by biologics in human tumors. For example, in vitro studies have shown an increase in cell kill of breast cancer by anti-metabolites and stimulation with estrogens. Preliminary studies suggest the same may occur in humans in vivo, but there are few data. Also, the expression of a number of antigens as assessed by monoclonal antibody binding is cell cycle related. When considering the potential therapeutic uses of monoclonals, binding of the antibodies to proliferating cells is desired. If, however, the fraction of proliferating cells is low, few cells would be killed and resting cells would be recruited into the cycle after antibody administration. Even with prolonged antibody infusion (>3 weeks) many cells might not proliferate. It is possible that combinations of monoclonal antibodies directed against differentiation and proliferation antigens may be advantageous therapeutically. These hypotheses can be tested in laboratory experiments in vitro.

IV. Aims and Methodology

We will study the DNA content and via it the cell cycle characteristics of tumor cells (primarily CTCL, lung cancer, and breast cancer) taken directly from patients or after short and long term culture. We will also study the binding of a panel of defined monoclonal antibodies to the lymphoma and lung cancer cells. The anti-T cell antibodies are commercially available or obtained from other investigators (e.g. anti-TAC) while the lung cancer antibodies are prepared by Dr. Minna's group.

The cell sorter methodology we use is standard and has been published. The equipment is a Coulter Epics V cell sorter capable of dual parameter analysis. Also see report on monoclonal antibodies by Dr. Minna.

V. Progress Report

A. Cutaneous T-Cell Lymphomas

We have shown that circulating lymphoma cells in the Sezary syndrome do not proliferate and do not express the T cell activation antigens Tac and T9. The cutaneous malignant cells do proliferate but at a rate slower than in other sites, presumably the lymph nodes. While there is circulation of cells between the three compartments (lymph node, blood, and skin), the primary flux appears to be from node--> blood-->skin. This implies that any treatment designed with curative intent must treat nodal as well as cutaneous sites. We have shown that a minority of patients have tumor aneuploidy by DNA content analysis at diagnosis and these patients have a worse prognosis. In addition, with relapse and disease progression diploid tumors became aneuploid suggesting genetic mechanisms in this conversion. DNA content changes were associated with cytogenetic changes and eventual clone formation.

The T-cell differentiation antigens (e.g., T101, Leu 1, Leu 3a, etc.) are not cell cycle related whereas the expression of T9 and anti Tac are cell cycle related. DNA content analysis of aneuploid specimens coupled with measurements of TCGF receptors (by anti Tac) has shown that the malignant CTCL cells have no TCGF receptors when circulating and resting. Their development of TCGF receptors is suboptimal when compared to normal lymphocytes after mitogen and IL 2 stimulation.

B. Lung Cancer

We have shown that the doubling times in small cell lung cancer (median 67 days) are longer than previously published. These doubling times are shorter than in the other lung cancer cell types but there is considerable overlap. These long doubling times suggest that relapses after complete response may be seen through 5 years. Clinical data have, in fact, confirmed this prediction. DNA content analysis has shown relatively high S fractions (mean 21%) which are about 5% higher than the labelling indices. This is presumably due to non-dividing or slowly dividing S phase cells. There is also

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considerable overlap in S fraction between cell types, though the highest was found in small cell lung cancer.

C. Myeloma

The fraction of cells in S phase is extremely low in myeloma. The majority of myelomas have aneuploid DNA content and these aneuploid tumors tend to have a worse prognosis than near-diploid tumors. We are beginning studies with simultaneous staining of paraprotein and DNA content to allow computation of the proliferating fractions of normal and myeloma cells which is impossible using DNA content alone.

VI. Future Plans

A. Cutaneous T-Cell Lymphoma

To complement our clinical trial we plan to continue to measure DNA content and a series of monoclonal antibody defined cell surface antigens in CTCL patients to determine the diagnostic and prognostic implications of such measurements. We plan to study the effects of biologicals (monoclonal antibodies to differentiation antigens, and growth factors receptors; and interferon) on the cell cycle of cultured normal and malignant T lymphocytes to complement the clinical treatment trials with monoclonal antibodies and interferon in CTCL.

B. Lung Cancer

To complement the tumor cell culture and monoclonal antibody work by other investigators in our lab we will continue to measure DNA content and a panel of monoclonal antibody on fresh tumor samples. This use of dual labeling should allow for estimation of the S fraction in more cases. The biologic and prognostic implications of S phase calculations can then be assessed more fully and will show the relationship of antigen expression to the cell cycle.

C. Breast Cancer

In collaboration with the Medicine Branch we are undertaking a clinical study of hormonal therapy with premarin followed by chemotherapy in advanced breast cancer. We will be measuring the effects of premarin on the tumor cell cycle in these patients to directly show whether or not an increase in the S fraction occurs prior to treatment with antimetabolites.

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Title: MOLECULAR GENETICS OF B-LYMPHOCYTE DEVELOPMENT

I. Personnel

- A. Permanent Senior Staff: W. Michael Kuehl
- B. Research Associates: Richard Currie; Timothy P. Bender
- C. Other Professional Staff: None
- D. Technical Staff: None presently

- II. Collaborators: Stanley J. Korsmeyer, Metabolism Branch (NCI); Dan L. Longo, Medicine Branch (NCI); Herbert C. Morse, Laboratory of Microbial Immunology, and Ronald Germain, Laboratory of Immunology (NIAID); M. Nau, John Minna, et al., NCI-Navy Medical Oncology Branch (NCI)

III. Introduction

A. Objectives

1. To clarify the molecular bases for regulation of immunoglobulin gene expression.
2. To better understand the cellular and molecular bases for B lymphocyte development (differentiation).
3. To construct B cell lines representing different stages of B cell development which can undergo maturation in appropriate in vivo or in vitro microenvironments.
4. To gain insight into the relationship between B lymphocyte transformation (including malignant transformation) and B-lymphocyte differentiation.

B. Rationale and Background

Immunoglobulin (Ig) gene expression, which is the most valuable marker of B-lymphocyte development and represents perhaps the best studied gene(s) in higher eukaryotes, includes a number of interesting features. First, multiple developmentally regulated gene rearrangements are necessary for gene formation and alteration of Ig gene structure and function in somatic cells (i.e., H-chain switching). Second, H chain gene expression is subject to allelic exclusion, and L chain gene expression is subject to allelic and isotypic exclusion. Third, developmentally regulated RNA processing accounts for coexpression of secreted and membrane forms of Ig, or of 2 classes of Ig (i.e., IgM and IgD) from a single H chain gene. Fourth, H and L chain gene expression are coordinately amplified about 10-100 fold during B cell development. Fifth, selective somatic mutation of a portion of H and L chain genes (i.e., V_HD_JH and V_LJ_L segments, respectively) apparently occurs during or after H chain switching. Cellular intermediates in the B-lymphocyte developmental pathway are distinguished by differences in the organization of H and L chain genes, the kinds of Ig chains expressed, and the level of Ig gene expression as well as by other differentiation markers. (See Figure 1 below)

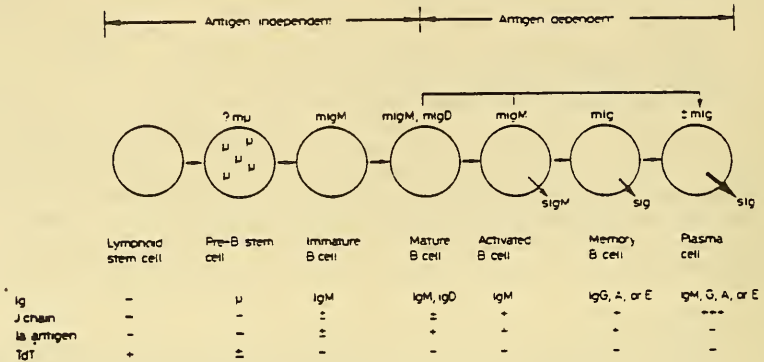


Fig. 1 Murine B lymphocyte developmental pathway. The scheme shown depicts a minimum number of cell intermediates, and indicates only a few of the known differentiation markers. See text for further details.

mIg = Membrane immunoglobulin
 sIg = Secreted immunoglobulin
 TdT = Terminal deoxynucleotidyl transferase

It is thought that a pluripotential hematopoietic stem cell generates lymphoid stem cells which subsequently generate pre-B stem cells, i.e., the first stage of lymphocyte differentiation in which it is clear that a commitment to the B-cell lineage has been made. As a lymphoid stem cell differentiates to a pre-B cell, there is H-chain gene formation, transcription, translation, and expression of cytoplasmic μ H chain but no expression of L chain. Progeny of this pre-B stem cell are thought to be permanently committed to the $V_H(D)-J_H$ gene sequence formed at this stage of development (except for somatic mutation as cited above). Although most pre-B cells express only cytoplasmic μ H chain, several groups have reported that some pre-B cell lines can express μ H chain on the external cell membrane despite the absence of L-chain expression. This provocative result, which requires further validation, has not yet been found in normal pre-B cells. Early pre-B cells have not rearranged their L chain genes, but are proliferating cells which are presumed to eventually rearrange and express different L chain genes together with the H chain to which the pre-B cell is already committed.

A possible late pre-B cell developmental stage is suggested by several recent examples of pre-B cell lines (e.g., 70Z/3) which have functionally rearranged K L chain genes, but do not express L chain until the cells are

stimulated by bacterial lipopolysaccharide. This late pre-B cell developmental stage (i.e., L chain DNA rearrangement but no L chain expression) has not been clearly identified in normal cell populations, but may represent a transient stage of normal B cell development.

As the pre-B cell develops to an immature B cell, L chains are expressed and IgM monomers are displayed on the cell surface as antigen receptors. Progeny of this cell are thought to be permanently committed to the V_H gene sequence and L chain gene which are expressed at this time. The immature B cell develops into a mature B cell which co-expresses IgM and IgD on the cell surface. Up to the mature B-cell stage of development, B-lymphocyte maturation is antigen independent, although immature B cells which express surface IgM are presumably capable of being affected by contact with antigen or anti-idiotypic antibody. Development beyond the mature B-cell stage is thought to require interaction with an appropriate combination of mitogens, antigen, T-cell factors, T cells and macrophages. The stimulated mature B cell develops into either: (1) an activated B cell which secretes significant amounts of IgM; (2) a terminally differentiated plasma cell which no longer expresses surface I_a antigens, but expresses large quantities of secreted IgM, IgG, IgE, or IgA, but little, if any, surface Ig; or (3) a memory B cell which continues to express surface I_a antigens but expresses a new isotype of surface immunoglobulin (IgG, IgE, or IgA), and perhaps some secreted Ig of the same isotype. Appropriate stimulation of memory B cells generates plasma cells secreting IgG, IgE, or IgA. There is no evidence for memory B cells generating an enhanced secondary response of IgM. Whether plasma cells secreting IgG, IgE, or IgA in a primary response must pass through an intermediate similar to or identical to the memory B cell is unclear.

Although human B cell development is similar, in general, to murine B cell development, specific markers (e.g., J chain, surface I_a antigen equivalent) may not be expressed at the same developmental stages.

Murine tumors of the B cell lineage corresponding to various developmental stages have proven to be of great value in the analysis of immunoglobulin gene expression. The pre-B cell is represented by the carcinogen-induced 70Z cell line, as well as by Abelson virus-transformed lymphoid cell lines. Immature B cells, mature B cells, activated B cells, and memory B cells are represented by spontaneous and carcinogen-induced B cell lymphomas. Plasma cells are represented by plasmacytoma (myeloma) tumors. Needless to say, it is critical to study normal cells from different B lymphocyte developmental stages to verify that cell lines and tumors represent valid models for B lymphocyte development. Although some studies of normal cells have been possible, isolation of pure populations of cells representing a particular B lymphocyte developmental stage previously has been difficult. The recent development of continuous cloned lines of normal mouse lymphoid cells and their precursors may represent an extremely important breakthrough for future studies on B cell development.

Corresponding human tumors of the B cell lineage have also been identified, but those adapted to cell culture may represent a slightly more narrow spectrum of B cell development than murine tumor cell lines. For example, it has not been possible to adapt human myeloma cells to culture, except for a line called U266 which is phenotypically closer to a preplasma cell. A major problem of human B cell lines is that they have generally not been characterized as fully as murine B cell lines.

In addition to providing homogeneous cells "arrested" at a particular stage of B lymphoid development, in some instances lymphoid cell lines or tumors can be induced to differentiate to the next developmental stage by treatment with B-cell specific mitogens (e.g., lipopolysaccharide or dextran sulfate). Thus, they are not always strictly "frozen" at one developmental stage. A table listing examples of murine and human B cell lines available in my laboratory or from Dr. Stan Korsmeyer, together with some of their properties, is given below.

Table 1. B LYMPHOCYTE CELL LINES

<u>Cell Line</u>	<u>Develop- mental Stage</u>	<u>Ig</u>	<u>Ia</u>	<u>J Chain</u>	<u>TK</u>	<u>HPRT</u>	<u>OUA</u>	<u>Remarks</u>
A. <u>MOUSE</u>								
* ABL58	Pre-B	-	-	-	+	-	S	H & L genes rearranged abberantly
* 18-81-T13B	Pre-B	cyto- plasmic u only	-	<u>+</u>	+	-	S	L genes not rearranged
* 18-81-1H6A	Pre-B	mIgM	-	<u>+</u>	+	-	S	
x 70Z/3B	Late Pre-B	cyto- plasmic u only	-	-	-	+	S	K L gene re-arranged but not transcribed
B17-10	Immature B	mIgM	<u>+</u>	<u>+</u>	+	-	R	
A202J	Memory B	secIgG2a mIgG2a	++	++	+	-	S	Presents antigen
A20-1.11	Memory B	secIgG2a mIgG2a	++	++	-	+	S	Presents antigen
4T00	Myeloma	secIgG2b	-	++	+	-	R	
PBOU	Myeloma	secIgG1	-	++	-	+	R	
o CBO	Myeloma	secIgG2a	-	++	-	+	R	

* Abelson murine leukemia virus induced

x (DBA2 X C57/B6)F, Mouse

o C3H mouse

TABLE I. B LYMPHOCYTE CELL LINES (Continued)

<u>Cell Line</u>	<u>Develop- mental Stage</u>	<u>Ig</u>	<u>Ia</u>	<u>J Chain</u>	<u>TK</u>	<u>HPRT</u>	<u>OUA</u>	<u>Remarks</u>
B. <u>HUMAN</u>								
GM1500	lympho- blastoid	mIgG2 secIgG2	+	+	+	-	S	EBV trans- formed but non-product- ive
8392	lympho- blastoid	mIgM secIgM	+	+	+	+	S	EBV trans- formed but non-product- ive
U266	pre-plasma cell	mIgE secIgE	+	+	+	+	S	Derived from human tumor
HPB-Na11	pre-B (CALL)	u only	+	?	+	+	S	Germline L genes
Na1m-6	pre-B (CALL)	u only	+	?	+	+	S	Deleted and rearranged L genes

Two kinds of abnormal genes have been identified in DNA derived from tumors of B cell lineage. The first kind is exemplified by altered c-myc or integrated v-abl, both of which are classified as oncogenes by their ability to transform fibroblasts or other cell types when in an appropriate context. The best evidence for a critical role of these genes in generating B cell neoplasms includes: 1) correlation of v-abl gene modification with ability to transform fibroblasts or pre-B cells; 2) correlation of integration of avian leukosis virus near c-myc gene in avian bursal cells which grow into a bursal B cell tumor; and 3) correlation of changes in gene organization (e.g., chromosomal translocation) or expression of c-myc gene in various spontaneous human (e.g., Burkitt's lymphoma) and murine (e.g., plasmacytoma) tumors. The relationship, if any, of an altered c-myc or v-abl gene to the stage of development (i.e., pre-B versus intermediate B versus plasmacytoma) of a B cell tumor is unclear. The altered c-myc and integrated v-abl genes are alleged not to transform NIH 3T3 cells in a transfection assay. The second kind of abnormal gene is classified as a stage-specific transforming gene. G. Cooper and colleagues (Cell 28, 873-880, 1982) have identified this class of genes based on the ability of tumor DNA to transform NIH 3T3 cells in a transfection assay. They have identified at least 3 B cell stage-specific transforming

genes which are uniquely present in pre-B cell, intermediate B cell, and plasma cell-like (Ig secreting) B cell tumors, respectively. Somatic cell hybridization of B lymphoid cell lines with each other, with T lymphoid cell lines, with fibroblastic cell lines, and with normal spleen cells has been used in analyzing B cell differentiation and regulation of Ig expression. As is true for most cell lines expressing differentiated functions, when B lymphoid cell lines are fused to fibroblastic cell lines (e.g., L cells or NIH 3T3), expression of differentiated functions (e.g., Ig) are extinguished. More informative results have been obtained by fusing together different kinds of lymphoid cells. Fusion of mouse myeloma cells to all mouse, human, or rat B lymphoid cell lines, to mouse T lymphoid cell lines, and to mouse, human, or rat normal lymphoid cells results in somatic cell hybrids which have a myeloma phenotype (e.g., secretion of large amounts of Ig, plasma cell morphology), and coexpress both parental Igs (if both parents contain functionally rearranged Ig genes). Fusion of surface Ig positive murine B lymphoma cells to normal murine spleen B cells results in hybrids having a phenotype similar to that of the B lymphoma parent; whereas fusion of murine B lymphoma cells or normal murine B cells to a murine T lymphoma results in hybrids which extinguish Ig expression and induce expression of Thy 1 antigen encoded by the B cell genome. Fusion of a human "myeloma" cell line (i.e., U266), or a human EBV-transformed lymphoblastoid cell line (e.g., GM 1500) to normal human lymphocytes or to EBV transformed lymphoid cells results in hybrids with a myeloma or pre-plasma cell phenotype, i.e., secretion of large amounts of both parental Igs.

Based on these various studies, we hypothesized that hybrids formed between B-lymphocytes representing different developmental stages have: 1) codominant Ig expression, and 2) a phenotype of Ig expression, as well as an overall phenotype, which corresponds to the most differentiated parental cell.

C. Specific Aims

1. Analysis of somatic cell hybrids constructed from murine pre-B lymphoma cell lines and murine spleen B cells.
 - a. Since adult mouse spleen contains predominantly immature and mature B cells but few pre-B or plasma cells, our hypothesis predicts a surface Ig positive phenotype for these hybrids. Is this predicted result verified by experiment?
 - b. If pre-B lymphoma or B lymphoma X normal spleen hybrids express surface Ig, can we isolate hybrids expressing antigen specific surface Ig?
2. Analysis of somatic cell hybrids constructed from two murine B cell lines representing different stages of B cell development.

- a. Is surface Ia antigen expression extinguished in B lymphoma X myeloma hybrids, as predicted by our hypothesis?
- b. Do pre-B lymphoma X B lymphoma hybrids have the phenotype of the more differentiated B lymphoma parent as predicted by our hypothesis?

IV. Progress Report

1. Analysis of somatic cell hybrids constructed from murine pre-B lymphoma cell lines and murine spleen B cells.

An Abelson murine leukemia virus transformed Balb/c pre-B cell line (ABLS8) was fused to spleen cells (depleted of T cells and macrophages) of immunized Balb/c, A/J, and C57/B6 mice. Surprisingly, about 50% of the hybrids were Ig negative, about 50% of the hybrids were mu H chain positive and L chain negative, and only a few percent of the hybrids expressed mu H chain plus an L chain. Southern blots confirmed that fusion had occurred to spleen B cells in the few putative hybrids examined by this method. This result suggests either that fusion of the ABLS8 pre-B cell line to mature or immature spleen B cells results in hybrids having a pre-B cell phenotype, or that there is selective fusion of ABLS8 to spleen pre-B cells. Additional studies in which other pre-B lymphoma lines or B lymphoma lines were fused to spleen cells have been technically inadequate.

2. Analysis of somatic cell hybrids constructed from two murine B cell lines representing different stages of B cell development.
 - a. Fusion of myeloma cells to either pre-B lymphoma or B lymphoma lines resulted in hybrids which coexpress both parental Igs and have the phenotype of the myeloma parent, including extinction of surface Ia antigen expression contributed by the B lymphoma parent.
 - b. The 70Z/3B thymidine kinaseless pre-B lymphoma cell line (cytoplasmic mu H chain positive, L chain negative, surface Ia antigen negative, J chain negative) was fused to the A202J HGPRT negative B lymphoma cell line (expresses membrane and secreted IgG_{2a}, surface Ia-A and Ia-E antigens, and cytoplasmic J chain). All 20 hybrids analyzed express cytoplasmic mu H chains, no surface Ia antigen, no J chain cytoplasmic RNA, and no secreted immunoglobulin. Some hybrids express no detectable L chain, but most express low levels of L chain. Only a few hybrids express very low levels of cytoplasmic gamma H chains. To our surprise, then, all 20 hybrids analyzed have a phenotype more like that of the pre-B lymphoma parent than the more differentiated B lymphoma parent. This result disproves our hypothesis (see above), and supports the conclusion that fusion of a pre-B cell line to normal B cells results in hybrids having a pre-B lymphoma phenotype (see #1 above).

V. Future Plans

A. Rationale

Our recent somatic cell hybridization studies to analyze regulation of Ig gene expression and B cell differentiation (see IV Progress Report) have caused us to revise our hypothesis regarding phenotypes of somatic cell hybrids between B cells. First, somatic cell hybrids constructed from B cell lines and normal B cells have the phenotype of the parental B cell lines. Second, somatic cell hybrids constructed from B cell lines representing two different B cell developmental stages may have the phenotype of either the more differentiated B cell line (e.g., pre-B lymphoma X myeloma or B lymphoma X myeloma), or the less differentiated B cell line (e.g., pre-B lymphoma X B lymphoma). It is possible that the B cell developmental stage-specific transforming genes are, in fact, normal or abnormal mediators of the state of B cell development. B cell lines may be relatively fixed in a specific stage of development as a result of expression of an abnormal transforming gene product or of an excessive expression of a "normal" transforming gene product. The phenotypes of somatic cell hybrids constructed from two cell lines (e.g., pre-B lymphoma X myeloma) may reflect the net effect of the simultaneous expression of the two respective stage-specific transforming genes. Similarly, the phenotype of somatic cell hybrids constructed from a cell line (e.g., myeloma) and a normal spleen B cell may be determined by the stage-specific transforming gene contributed by the cell line. The role of oncogenes (cf. c-myc, v-abl) is unclear, but may serve to facilitate the transformation process (e.g., amplified or over-expressed c-myc gene in large cell variants of small cell lung cancer). Our goal is to continue somatic cell hybridization studies--both intra- and interspecies--and to set up DNA transfection experiments to test these ideas.

B. Specific aims and explanations

1. Mouse - mouse B cell line X B cell line hybrids

- a. Is our result for pre-B lymphoma X B lymphoma hybrids a general finding?

We will test other combinations of pre-B lymphoma and B lymphoma cell lines, i.e., 18-812E5A X A20-1.11, LS8T2XA20-1.11. We will assay for Ig biosynthesis and secretion, Ia antigen mRNA and surface expression, J chain mRNA, and other markers (e.g., terminal deoxynucleotidyl transferase), as appropriate reagents (e.g., differentiation specific monoclonal antibodies or DNA clones) become available. By studying other markers we may find, for example, that different groups of markers behave differently with respect to phenotypic expression. In addition, it will be of particular interest to determine

if IgG--as well as IgM--is not secreted in such hybrids since the lack of J chain would not be predicted to affect IgG secretion.

- b. Can we isolate segregants that alter the phenotype of the hybrids (e.g., re-expression of J chain or surface Ia antigen)? For example, it may be possible to select for revertants by panning or FACS using anti-Ia reagents.
- c. What would be the phenotype of B lymphoma X B lymphoma hybrids constructed from parental cell lines that differ slightly in phenotype? For example, mIgM⁺, B lymphoma X mIgM⁺, mIgD⁺ lymphoma. G. Cooper's data on stage-specific transforming genes apparently does not distinguish B lymphomas with minor phenotypic differences.

2. Mouse-mouse B cell line X normal cell hybrids

- a. By fractionating spleen B cells or fetal liver cells (e.g., by panning or FACS based on surface Ig expression, we hope to determine whether B lymphoma X normal pre-B cell hybrids have a pre-B cell phenotype as predicted by our modified hypothesis.
- b. Can we isolate hybrids expressing antigen-specific mIg by fusing B lymphoma cells to normal spleen B cells derived from immunized mice?

If so, can these hybrids be induced to differentiate in an appropriate in vivo or in vitro micro environment? Isolation of a line that can undergo heavy chain switching would be useful in helping us to study this important step in B cell development.

- c. What is the phenotype of B cell line X normal fibroblast hybrids? Fusion of a B cell line to a fibroblastic cell line generally results in extinction of Ig expression, but our hypothesis predicts that this may not occur in fusions to normal fibroblasts.

3. Human cell line X mouse B cell hybrids

- a. Human B cell line X mouse B cell line hybrids

It is known that mouse myeloma X human B lymphoma hybrids have a myeloma phenotype. We plan to fuse human "myeloma" (e.g., U266) cell lines or lymphoblastoid cell lines (e.g., GM 1500) to a mouse pre-B lymphoma or B lymphoma to determine if the hybrid has a myeloma phenotype. If the hybrid does have a myeloma phenotype and if there is selective segregation of human chromosomes, we hope to map the human chromosome(s) necessary for maintaining the myeloma phenotype.

b. Human B cell line X normal mouse spleen cell hybrids

Will a human "myeloma" or lymphoblastoid line X normal mouse spleen cell fusion result in immortalized lines having a myeloma phenotype (as is observed in the reciprocal fusion)? If so, we may be able to map chromosomes responsible for "immortalization" as well as for the "myeloma" phenotype as human chromosomes segregate.

- c. What would be the effect of putting either a large number of human c-myc genes (or an abnormal human c-myc gene which is amplified in expression) in normal mouse spleen cells by somatic cell hybridization? This experiment can be done by fusing human lung tumor cell lines--which have been shown to express excessive c-myc (see section by M. Nau, J. Minna et al)--to normal mouse B cells. Chromosome segregation should eliminate most human chromosomes, including (hopefully) other oncogenes in the human tumors. If necessary, the human cell line can be treated with BUDR or x-irradiation to favor segregation of human chromosomes. This experiment might enable us to ascertain the effect of excessive c-myc expression in the absence of a B lymphoid stage-specific transforming gene. We might observe, for example, murine B cell immortalization or proliferation without "freezing" the cell in a specific developmental stage. Of course, we realize that species differences and/or the presence of other human oncogenes may complicate this experiment, which will be done in collaboration with M. Nau and J. Minna. An alternative approach, i.e., fusion of human lung tumor microcells (Fournier, R. and Ruddle, F., Proc. Nat. Acad. Sci. 74, 319, 1977), to normal mouse spleen cells, might minimize or eliminate the problem of introducing other human oncogenes or transforming genes into the mouse spleen cells.

4. Isolation and characterization of genes responsible for B-lymphocyte differentiation

Our ultimate goal is to identify, clone, and characterize genes which are responsible for the various steps in B-lymphocyte differentiation. Specific aims #1-3 should help us to determine the best approaches, e.g., if a human "myeloma" cell X mouse pre-B lymphoma fusion produces hybrids with a myeloma phenotype, it may be feasible to transfect DNA from a human "myeloma" cell into mouse pre-B lymphoma cells as an assay for the gene responsible for differentiation to a plasma cell phenotype. Nonetheless, we plan to begin work on specific aim #4 as soon as possible, since we are now able to transfect

DNA, albeit at low efficiencies, into pre-B and B-lymphoma cell lines.

- a. Isolation of a myeloma stage-specific transforming gene. G. Cooper and his colleagues have demonstrated that an EcoRI or Bam HI fragment isolated from the GM 1500 human lymphoblastoid line or from a variety of mouse myeloma lines can transform NIH-3T3 cells in a transfection assay. We will attempt to clone this gene by preparing a lambda phage library from myeloma cell (e.g., S107) DNA which can be transfected into NIH-3T3 cells to generate primary transformed foci. We should be able to isolate the gene by sib selection, or by cloning fragments of DNA containing phage lambda sequences from a secondary NIH-3T3 transformed focus (e.g., see Goubin, G. *et al.* Nature 302, 114-119, 1983 for isolation of B_{LYM} gene from "intermediate" B lymphoma cells). John Minna has already used the NIH-3T3 transfection-transformation assay for transfecting NIH-3T3 cells with DNA from human lung tumors; he will help us in setting up this assay. If we are able to isolate this gene, we will attempt to transfect it into murine pre-B lymphoma cells to directly determine if it causes differentiation of this cell to a myeloma phenotype.
- b. Isolation of genes responsible for B cell differentiation using an assay which measures B cell differentiation

We will isolate DNA from a myeloma cell (human and/or mouse) and transfect it into mouse pre-B lymphoma (e.g., 70Z/3B) or B lymphoma cells. We will assay for differentiation of the pre-B lymphoma to a myeloma phenotype by initially using a transiently expressing cell population to detect plaque-forming cells with a reverse plaque assay which can detect Ig secretion from a single cell. If this preliminary experiment demonstrates our ability to cause pre-B lymphoma cells to differentiate to a myeloma phenotype, we will isolate stably transfected cells and attempt to identify a clone which has a myeloma phenotype. Ultimately it may be necessary to use a more sophisticated approach which might involve construction of myeloma DNA libraries in a vector which contains, for example, an immunoglobulin "enhancer" sequence since a transfected gene may not be expressed efficiently in pre-B lymphoma or B lymphoma cells. If we are able to develop an appropriate assay, it should be possible to develop an uncomplicated strategy to clone the gene(s) responsible for this differentiation step.

VI. Publications, 1980-1983

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9. Riley, S.C., Brock, E.J., and W. Michael Kuehl. Rearrangement and Expression of a Pre-B Cell Light Chain Gene in Pre-B Cell X Myeloma Hybrids. Immunoglobulin Idiotypes and Their Expression, ICN-UCLA Symposia, 1981, pp 219-228.
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B. In Press

12. Kuehl, W.M. Analysis of B-Lymphocyte Development by Somatic Cell Hybridization in Mechanisms of B-Cell Neoplasia, Basel Institute of Immunology. Editors: M. Weigert, M. Potter, and F. Melchers.

13. Emorine, L., W.M. Kuehl, L. Weir, E. Max, and P. Leder. Sequence of Homology Region in Mouse, Human, and Rabbit Jk-Ck Introns. Submitted as letter to Nature.

VII. Other relevant research by principal investigator

1. Analysis of variant mouse myeloma cells which no longer express light chains.

Cloned MPC 11 cells express two kappa light chain genes which code respectively for a full length light chain (L) and a fragment light chain (FCL) containing constant region sequences only. By restriction mapping genomic DNA, we have shown that these cells contain only two kappa constant region gene sequences, which correspond to the genes coding for L and FCL. We have obtained five independent variants which no longer express L. Restriction analysis of genomic DNA from these five variants indicates: L gene loss in three variants; rearrangement or insertion resulting in apparent alteration of the coding region at the 5' end of the L gene and lack of detectable L transcription in one variant; and no gross change in the organization of the L gene in one variant. Analysis of nuclear RNA by Northern blotting analysis (in collaboration with Dr. R. Wall, UCLA) indicates the presence of a wild type L primary transcript but incomplete processing (splicing) so that L mRNA is not generated in the latter variant; a mutation in the L gene which prevents normal processing of RNA is a probable explanation. Sequence results on the cloned genomic L gene from this variant demonstrate at least one deletion in the J segment and the subsequent intervening sequence adjacent to the J segment.

2. Ig expression in murine B-lymphoma cell lines.

Six B-lymphoma cell lines are reported to express immunoglobulin (Ig), Fc receptors, and Ia antigens, but not complement receptors on the cell surface (K. Kim et al, J. Immunol. 122: 549, 1979). Several lines have generated interesting clones which will be useful in analyzing the molecular bases for some of the unique aspects of Ig expression. First, clones from one line are unusual in that they synthesize two different intracellular forms of H chain of the γ 2a class with molecular weights of 62,000 and 68,000 daltons. These clones secrete IgG2a containing an H chain of 64,000 daltons, while IgG2a containing an 70,000 dalton form of the γ 2a H chain is expressed on the cell surface. Somatic cell hybrids derived from the fusion of these cells with murine myeloma cells amplify approximately fifty-fold the synthesis and secretion of the lymphoma 64,000 dalton γ 2a H chain as IgG2a. The 70,000 dalton surface form of the lymphoma 2a H chain is not expressed at detectable levels in the hybrid. Our studies indicate that different mRNA species code for the

surface and secreted forms of γ 2a H chain. The molecular basis for expression of the two forms of 2a mRNA by a single gene is being investigated, in collaboration with Dr. R. Wall (UCLA), using recombinant DNA technology. These collaborative studies have resulted in identifying genomic sequences encoding two membrane exons (M1 and M2) for both γ 1 and γ 2b genes. The full DNA sequence of both M1 exons have been determined, as well as the entire DNA sequence of the 2b M2 exon. The M1 exon DNA sequence encodes a hydrophobic peptide sequence which is presumed to anchor the γ H chain in the membrane. The γ 1 and γ 2b M1 sequences are greater than 90% homologous and both γ M1 sequences are about 70% homologous to the mu M1 sequence. The γ 2b M2 exon (which presumably encodes a peptide sequence localized on the cytoplasmic side of the cell membrane as well as a 3' untranslated mRNA sequence) is substantially larger than the mu M2 exon. Appropriate splice signals indicate that the mechanism by which a gene simultaneously expresses secreted and membrane forms of heavy chain is the same as that proposed by Wall, Hood, and collaborators for mu heavy chains. Second, we have isolated a cloned lymphoma cell line which simultaneously expresses surface IgM as well as the secreted and surface forms of IgG2a. This clone provides a model for investigating the molecular basis by which two classes of H chain (mu and γ 2a) are expressed simultaneously in the same cell, a phenomenon observed in normal lymphoid cells, perhaps during or subsequent to H chain switching. Third, clones from three lines simultaneously express kappa and lambda chains (isotype exclusion). We have demonstrated that both L chains are functional.

3. Murine pre-B cell line: A model for L chain selection and allelic exclusion.

Ig is first expressed in pre-B cells as cytoplasmic u chain (u_c), indicating that selection of a specific L chain occurs later in development than H chain expression. An Abelson virus transformed pre-B cell line (18-81) which expresses only u H chain is being studied. Fusion of this cloned line to variant mouse myeloma cells which do not express L chain results in hybrids which express an L chain coded for the pre-B cell genome. We have also observed that occasional subclones of the pre-B cell line spontaneously express L chain. We have demonstrated that the original pre-B cell cloned line contains a single unrearranged (germline) Ck gene. When subclones or hybrids are examined, all those expressing an L chain have rearranged the Ck gene, whereas all those not expressing an L chain have not rearranged the Ck gene. Independent subclones that express L chain do not express the same L chain. No subclones expressing lambda light chain have been found. Studies are continuing to determine the efficiency and mechanism of the DNA rearrangement which generates functional L chain genes.

4. Glycosylation of nascent H chains is a rate-limiting step in H chain translation.

Continued studies on the temporal relationship of glycosylation and translation of H chains, as determined by nascent chain isolation and analysis, indicates that the glycosylation process blocks translation of H chains *in vivo*. The block is alleviated by blocking glycosylation with tunicamycin, or by greatly slowing the rate of peptide elongation with cycloheximide. In the presence of tunicamycin, the relative rate of H chain synthesis, compared to the relative rate of L chain synthesis, is enhanced 15%-20%. This result accounts for the slight molar excess of L chain synthesis, compared to H chain synthesis, which is observed in most normal and malignant lymphoid cells. Since others have shown recently that glycosylation inhibits translation of ovalbumin synthesis, the effect of glycosylation on translation appears to be a general phenomenon.

5. Jk-Ck intron homology region.

We have sequenced the human Jk-Ck intron homology region, including about 500 bp of flanking sequences on each side of the homology region. Currently, we are doing transfection experiments into lymphoid cell lines to determine whether the homology region is important in regulation of kappa light chain expression.

Title: DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES WITH SPECIFICITY FOR HUMAN TUMORS AND OTHER INTERESTING MOLECULES

I. Personnel

- A. Permanent Senior Staff: Drs. J. Minna, A. Gazdar, D. Carney, P. Bunn
- B. Clinical Associates/Medical Staff Fellows: Drs. A. Doyle, J. Mulshine
- C. Other Professional Staff: Dr. F. Cuttitta (Staff Fellow), Dr. S. Fargion (Visiting Fellow).
- D. Technical Staff: Mr. J. Fedorko, Ms. S. Stephenson, Mr. H. Simms, Ms. A. Simmons

II. Collaborators and/or Collaborating Branches

Dr. S. Rosen (Northwestern Medical School); Dr. S. Baylin and J. Shaper (Johns Hopkins Cancer Center); Dr. T. Moody (George Washington Univ.); Dr. P. Marangos (NIMH); Dr. S. Wilson (NCI); Dr. J. Berzofsky (NCI); Dr. I. Berkower (NCI); Dr. V. Ginsburg (NIADDKD), Dr. L. Huang (Univ. Virginia)

III. Introduction

A. Objectives

To produce and characterize monoclonal antibodies with specificity for human tumors and other molecules of biologic and clinical importance.

B. Rationale and Background

The technique for producing monoclonal antibodies using somatic cell fusion methodology has revolutionized immunology and the use of antibodies as reagents for scientific research. Because of this we have invested considerable effort in developing and using monoclonal antibody technology (1-8).

Many laboratories have been making antibodies with varying degrees of specificity for human tumors. Antibodies with sufficient specificity can be used for pathologic typing of tumors, early diagnosis, nuclear medicine scanning, purging tumor contaminated bone marrow, and potentially for treatment either by themselves or after coupling to toxins, radiopharmaceuticals, cells, or drugs.

Monoclonal antibodies against defined molecules of biologic interest can be used to establish assays for such molecules (e.g., radioimmuno or immunohistochemical assays), and to aid in the purification of the molecules, or in the cloning of the genes coding for them. Proteins of interest with respect to human tumors and lung cancer in particular include peptide hormones such as bombesin, other autocrine growth factors produced by lung cancer cells, neuron-specific enolase (an excellent marker of small cell lung cancer as discussed by Dr. Gazdar in another report), DNA synthetic enzymes which are important

in cell replication, and various cellular oncogenes. In addition, it will ultimately be important to know the nature of the antibody binding site to well defined molecules, including the nature of the variable region (idiotype) of the monoclonal antibody molecules.

One approach to generating anti-tumor monoclonal antibodies is to immunize with whole cells or crude preparations of cells (eg, cell membrane preparations). With this approach, mice and rats are confronted with thousands of antigens and a screening technique must be developed to identify rare hybrid cell cultures making antibodies of interest. We have taken the initial approach of identifying antibodies that react with at least two different lung cancer cells but not with B lymphoblastoid cells usually from the same patient donating the lung cancer cells (1,2). This restricts the antigens detected. For the initial screen we usually use "target plates" (96 well microtiter plates) coated with the cell or antigen of interest in a fixed form and perform a radiobinding assay. This allows us to screen thousands of cultures in a short period of time. Further selection criteria can then be made to restrict the number of hybridomas to be carried, cloned, grown to large quantities, and characterized (a very laborious, time consuming process). These restrictions can take the form of searching for certain antibody classes (e.g., IgM or IgG2A), lack of reactivity with other tumor cell types, reaction with cell surface molecules of whole live cells, or lack of reaction with other sources of normal tissue.

Another approach is to immunize with very purified molecules of defined interest. As reported by Dr. Gazdar elsewhere in collaboration with Drs. Baylin and Shaper at the Johns Hopkins Cancer Center, we have been studying the 2-D gel membrane protein phenotype of our lung cancer cell lines (9). We have found that small cell lung cancers have one characteristic membrane protein phenotype while non-small cell lung cancers have another. Using surface iodination, approximately 12 different spots are representative of small cell lung cancer but not of non-small cell lung cancer while 15 spots have the opposite phenotype. However, we recognize that until specific antibodies are prepared against these spots we will not be sure about their distribution in normal tissues or various tumors. Thus, one approach we are just beginning, is to use purified spots cut out of 2-D gels for immunization of rodents prior to monoclonal antibody production. Because we would ultimately like to use the antibodies for therapeutic purposes we are concentrating on cell surface molecules that the antibodies could react with in the patient. We are doing this in collaboration with Drs. Baylin and Shaper.

A related approach is to use other defined molecules of biologic interest for immunization. One set involves cytoskeletal proteins. As described by Dr. Gazdar, collaborative studies by our group with Dr. Bernal at Harvard has revealed a group of cytoskeletal proteins that distinguish lung cancer types (10). We are just beginning to prepare crude cytoskeletal proteins for immunization. Such antibodies will be useful for typing tumors.

Another molecule of great interest is neuron specific enolase also discussed elsewhere in detail by Dr. Gazdar (11). While heteroantisera exist they are limited in amount. Because of the diagnostic importance of neuron specific enolase in typing lung cancers we feel it is worth the effort to make and characterize monoclonal antibodies against this molecule. In collaboration with Dr. Paul Marangos of the NIMH we are making such antibodies. Dr. Marangos purifies neuron specific enolase from human brain and we are using this for immunization. Dr. Marangos has been the world's major, if not only, source for anti-neuron specific enolase heteroantisera.

Dr. Carney has discussed the role of the peptide hormone bombesin. In collaboration with Dr. T. Moody of George Washington University, we have prepared monoclonal antibodies with specificity to bombesin using synthetic amphibian bombesin conjugated to BSA as the immunogen.

Cell replication and DNA synthesis ultimately characterizes tumor cells. A fundamental understanding of the regulation of DNA polymerases in human tumor cells would be of great importance. To approach this we have collaborated with Dr. Samuel Wilson (NCI) in preparing monoclonal antibodies with specificity for DNA polymerases.

Once monoclonal antibodies are in hand, what criteria should be used for their input into early clinical trials. We are using a variety of criteria including: detailed studies of immunohistochemistry with normal tissues (one would like as little reaction as possible); studies of tumor cell heterogeneity (one would like all tumor cells to bind the antibody); studies of antigen localization (the antibody should react with live unfixed tumor cells as they would in the patient); studies of in vitro tumor cell kill. The antibodies either alone or after some manipulation (e.g., conjugation to radioisotopes) should be able to kill tumor cells in a model situation (e.g., in vitro cloning system, or while growing as a heterotransplant in a nude mouse). We are studying all of these as potential criteria to select antibodies to take into clinical trials.

Parts of experiments carried on by this project are discussed in detail in other reports including: immunohistochemistry and cell sorter assays (Dr. Mulshine's report), and the clinical serotherapy trials we are already embarked on with other monoclonal antibodies (Dr. Bunn's report). Ultimately we wish to use the antibodies we have developed for a variety of clinical trials and diagnostic procedures.

We have also made antibodies against myoglobin (4,5,6). While this may have clinical application for cardiologists its usefulness for cancer is small. Potentially they could be used for typing human sarcomas. The reasons for involvement are: first, the antibodies are against a very well defined molecule, myoglobin, and because of the variety of myoglobins available we should be able to pinpoint precisely the binding site of the antibody and ask whether this is related to the primary amino acid sequence or secondary/tertiary structure; second, the idiotypes of the monoclonal antibodies could be studied and

potentially related to the antigenic binding site and immune response; and finally, it provided excellent interaction with a fine protein chemistry-immunology lab (that of J. Berzofsky, NCI).

C. Specific Aims and Outline of Methodology

1. To prepare monoclonal antibodies that would distinguish small cell from non-small cell lung cancer and vice versa.
2. To prepare monoclonal antibodies that would react with lung cancer but not other common tumors.

The approach to these first two objectives is to use both whole cell immunization and rigorous initial screening procedures, and 2-D gel purified membrane protein spots (provided by Drs. S. Baylin and J. Shaper).

3. To prepare monoclonal antibodies against lung cancer that would:
1) react with as few normal adult tissues as possible; 2) react with the live cell surface; 3) detect antigens expressed on lung cancer cells from many different patients; 4) detect antigens that were homogeneously rather than heterogeneously expressed. Many of these approaches are discussed in detail in Dr. Mulshine's report.
4. To prepared monoclonal antibodies that would react selectively with neuron specific enolase compared to other enolases. The approach to this is to use purified human neuron specific enolase (provided by Dr. P. Marangos).
5. To prepared monoclonal antibodies with specificity for human bombesin. The approach here is to immunize with synthetic amphibian bombesin conjugated to bovine serum albumin and characterize monoclonal antibodies using synthetic peptide hormones and small cell carcinoma.
6. To prepare monoclonal antibodies against autocrine growth factors made by human lung cancer cells. The approach here is to immunize with peptide extracts from serum free media of lung cancer cells that are shown to contain clonogenic stimulatory activity.
7. To prepare monoclonal antibodies that would bind to cell surface receptors and stimulate or inhibit tumor cell growth in the cloning assay. Our initial approach here is to test the antibodies generated for other objectives for these features in the cloning assay.
8. To prepare monoclonal antibodies that would react with tumor "stem" cells. Here we have immunized with the large cell variants, our current best approximation of a lung cancer "stem cell," and then testing the antibodies as in objective #7 above.

9. To generate monoclonal antibodies that would distinguish the cytoskeletal proteins from different types of lung cancer. We will partially purify cytoskeletal proteins by the method of Bernal and Chen (10) and use these for immunization.
10. To prepare antibodies with specificity for myoglobin, a defined protein antigen.
11. To prepare monoclonal antibodies with specificity for human DNA polymerase. We have immunized and screened with purified polymerases provided by Dr. S. Wilson, NCI.

Methodology:

Much of the methodology is reported in our publications or discussed in a general way above. Basically mice and rats are hyperimmunized with the desired antigen(s), spleens are removed and standard cell hybridization performed with polyethylene glycol and a non-producing mouse myeloma line (either 653, or NS11). Hybrids are selected in HAT medium with various supplementations and two weeks after fusion, screening for the desired antibodies takes place. This screening is usually initially done with a radiobinding assay using microtiter antigen coated target plates, hybridoma supernatant, connecting antibodies, and ¹²⁵I labeled protein A, or the same type of assay with an enzyme linked detection system. Hybridomas producing antibodies of interest are expanded, cloned in microtiter plates, cryopreserved, and then large amounts of antibodies are grown up as ascites in pristine primed mice. The antibody is purified from ascites using standard techniques and then characterized.

The types of assays used to characterize antibodies include: radio-binding assays to fixed cells; fluorescent activated cell sorter assays for live cells; immunohistochemistry assays using avidin biotin technology with staining for peroxidase on fixed and frozen sections of tumors from patients and nude mouse heterotransplants as well as from normal tissues from autopsies and surgical specimens; immunoprecipitation analysis using iodinated and metabolically labeled (e.g., ³⁵S methionine) tumor cells; immunoblot ("Western Blot") analysis; and tests for biologic activity such as inhibition of cloning in the soft agarose assays and inhibition of binding of a hormone to a receptor in the radioreceptor assay; epitope reactivity by using either pure peptide variants (e.g., synthetic analogues of a peptide hormone such as bombesin) or peptide fragments or mutants (such as for myoglobin) or the use of directly labeled monoclonals which can then be checked for cross competition with one another in a direct binding assay; glycolipid characterization by thin layer chromatography and overlay with a labeled antibody (3).

IV. Progress Report and Future Plans:

1. Projects 1 through 3:

A panel of monoclonal antibodies against small cell carcinoma,

adenocarcinoma, and variants of small cell carcinoma, has been prepared using whole tumor cell lines for immunizations (1-3,7,8). The properties of these monoclonal antibodies are summarized in the accompanying Tables. These antibodies were all selected by virtue of reactivity with the immunizing cell line and lack of reactivity with B lymphoblastoid cell lines. Several patterns are apparent. The small cell lines have nearly always elicited IgM antibodies despite hyperimmunization, while the non-small cell lines have generated predominantly IgG antibodies. Many of the determinants on the small cell carcinoma surface are glycolipids while many on the adenocarcinoma cells are proteins. Although the antibodies were raised against tumor cell lines they react with tumors taken directly from patients.

Most of the antibodies react with many lung cancers from different patients of the same histologic class (e.g., small cell vs. adenocarcinoma). However, whereas monoclonal antibodies against non-small cell lung cancer rarely react with small cell carcinoma, monoclonal antibodies against small cell carcinoma not infrequently react with at least some non-small cell lung cancer lines. Studies of antibody reactions within individual tumors using both cell sorter and immunohistochemical assays have revealed considerable heterogeneity of antigen expression.

Certain antigens are immunodominant, especially the glycolipid antigen, lacto-N-fucopentaose III (3). However, the monoclonal antibodies reacting with LNFP III often recognize different epitopes on this molecule. These glycolipid antigens are frequently preserved after formalin fixation and thus should be of use for clinical work and retrospective tumor review.

Immunohistochemical studies of normal tissues have revealed common patterns of binding with the anti-small cell monoclonals including normal bronchial epithelium, and the proximal tubules of the kidney. This is covered in detail in Dr. Mulshine's report. We plan to continue to characterize the pattern of reactivity of these monoclonals particularly using immunohistochemistry of tumor specimens and normal tissues (7).

Many of the antibodies against both small cell and non-small cell lung cancer react with other tumor types. In the case of small cell antibodies these include human neuroblastoma (but not melanoma, or glioblastoma) and breast cancer. In the case of non-small cell antibodies these include melanoma and some breast cancer lines. We plan to include other tumor types as discriminatory antigen targets in future screening assays to try to detect antigens common to lung cancer but not to these other malignancies.

2. Project 4:

Monoclonal antibodies against neuron specific enolase are being prepared. We plan to characterize them by immunoprecipitation studies, and comparison to immunohistochemical patterns using a defined anti-neuron specific enolase hetero-antiserum.

3. Project 5,6:

We have prepared monoclonal antibodies with specificity for the peptide hormone bombesin. By using a series of synthetic analogues we can pinpoint the binding site of the antibody. This binding site is also the site on the peptide predicted to bind to the bombesin receptor. In collaboration with Dr. T. Moody, we can show that the monoclonal anti-bombesin antibody will inhibit the binding of bombesin to a rat brain receptor for the peptide.

Because of the stimulatory effect on small cell lung cancer cloning efficiency by bombesin, we have introduced the purified monoclonal antibody into a cloning assay using small cell lung cancer. The monoclonal antibody in a dose dependent fashion will inhibit the growth of small cell lung cancer and this inhibition can be reversed by adding exogenous bombesin. We hope to observe an *in vivo* anti-tumor effect by giving the monoclonal antibody to nude mice bearing small cell lung cancer. Bombesin is known to be present in fetal bronchial epithelium and gut and decrease with maturation. Thus, we hope to determine if the antibody can have an effect on mouse fetal development. Finally, because the antibody reacts with human bombesin we can use it in immunohistochemical assays of tumors. With the Hammersmith group (Drs. Polack and Bloom) we have seen immunohistochemical staining of bombesin containing cells in human fetal bronchus, human small cell lung cancer, and the chick foregut. We are continuing these immunohistochemical studies of human tumors and fetal tissues.

4. Projects 7, 8:

We have prepared monoclonal antibodies against variant cell lines of small cell lung cancer which are highly enriched in clonogenic cells (8). Two of these antibodies bind to different epitopes on a cell surface protein (p120). When added to a complement free, tumor cell cloning system, one of them can inhibit the growth of colony formation. Thus, this antibody defines a cell surface molecule important in the growth of lung cancer.

To study well characterized antibodies against growth factor receptors, we have tested an anti-transferrin receptor monoclonal antibody from Dr. I. Trowbridge (Salk Institute) that binds to the receptor and blocks transferring binding. We have found that all lung cancer lines bind the antibody

indicating that the transferrin receptor is expressed. When added to tumor cloning assays, dramatic growth inhibition is seen. We plan to test several more cell lines, and related monoclonal anti-transferrin antibodies that bind to the receptor but do not block transfer in binding.

5. Project 9:

This is just beginning, and there are no data to report.

6. Project 10:

In collaboration with Dr. J. Berzofsky, we have prepared several monoclonal antibodies with specificity for sperm whale myoglobin following immunization with myoglobin (4,5). Antibodies were found to produce linear Scatchard plots predicted for homogeneous antibodies with a single binding site. They also possessed high affinities for myoglobin ($K_a \ 10^9 \text{ M}^{-1}$). None of the antibodies reacted with cyanogen bromide cleavage fragments of myoglobin indicating they reacted with a secondary or tertiary protein structure. Studies of binding of the monoclonals to a panel of myoglobins from different species allowed us to pinpoint the binding site of the antibodies (5). Two of the antibodies recognized groups of residues which are far apart in the primary structure but close together in the 3-dimensional structure of the native myoglobin molecule, i.e., they were topographic determinants. The third antibody distinguished a transition at residue 140 from Lys to Asn and in addition other surface residues nearby. These determinants differed from previously reported antigenic sites on sperm whale myoglobin in that they were topographic rather than sequential and that they were outside of previously reported sites.

The monoclonal anti-myoglobin antibodies were used to raise guinea pig anti-idiotypic antibodies that were then absorbed to remove common anti-mouse immunoglobulin determinants. The anti-myoglobin antibodies all bound to different sites on the myoglobin molecule. Surprisingly, by competitive binding studies, anti-idiotypic antiserum to one monoclonal inhibited binding of myoglobin not only to itself but to other monoclonals. We conclude that despite having different combining sites, the monoclonal antibodies share idiotopes. This sharing of idiotopes within otherwise distinct antibodies may reflect the action of idiope-specific regulatory T cells in the animals from which these clones were derived.

Our future plans include preparation of anti-idiotypic monoclonal antibodies against the anti-myoglobin antibodies to specifically study with Dr. Berzofsky the idiotypic regulation.

7. Project 11:

In collaboration with Dr. S. Wilson (NCI) we have prepared a

panel of rat monoclonal antibodies against highly purified calf thymus alpha DNA polymerase. Two of these antibodies were selected for study. In binding assays, these antibodies react with purified alpha polymerase from calf EBT and monkey BSC-1 cells, and with crude human polymerase. One also reacts with purified beta polymerase.

Immunoprecipitation studies show that major targets of the antibodies include the already described alpha polymerase proteins of 157, 110, 70, and 50 kdaltons. In addition, polypeptides of 230, 195 kdaltons were also specifically immunoprecipitated. A major 81 kdalton peptide was also precipitated from monkey but not calf cells. The 157, 195, and 230 kdalton peptides contain immunobinding determinants visualized on Western blots.

After 30 min incubation, the 195 kdalton protein was heavily labeled with 35-S methionine. After a 4.5 hour chase, the label in the 195 and 67 kdalton proteins declined markedly whereas label accumulated in the other polypeptides. These results suggest precursor product relationships of these peptides.

We feel these findings using monoclonal antibodies reflect significant steps forward in the understanding of the biology and metabolism of DNA polymerases. We plan to exploit these antibodies in studies of our human tumor cells using immunohistochemical and immunoprecipitation techniques.

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O F

M O N O C L O N A L A N T I B O D Y D A T A
A G A I N S T H U M A N L U N G C A N C E RPart I - Small Cell and Variant Line Lung Cancer
Directed Monoclonal Antibodies

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T A B L E 1

HYBRIDOMA SCREENING DATA SUMMARY (N = 32 fusions)
 FOR ANTI-SMALL CELL LUNG CANCER MONOCLONALS

Immunogen	Wells seeded	# With Hybrids	Selective RIA	# Stabilized
SCLC (NCI-H69 + NCI-H128)	11,951	5,360	217	82
SCLC (Defined products) bombesin	1,536	903	12	6
NSE	4,000	1,600	32	
SC/LC Converter (NCI-N417)	3,164	--	108	9

ANTI SMALL CELL LUNG CANCER MONOCLONAL ANTIBODIES DATA SHEET

<u>Antibody Number</u>	<u>Species</u>	<u>Isotype</u>	<u>Immunogen</u>	<u>(Cell Type)</u>	<u>Antigen</u>
534F8	Mouse	IgMk	NCI-H69	SCLC	LNFP III
525A5	Mouse	IgMk	NCI-H69	SCLC	LNFP III
538F12	Mouse	IgMk	NCI-H69	SCLC	LNFP III
248E7	Mouse	IgG	NCI-H69	SCLC	(p50+p70kd)
2A11	Mouse	IgG1	Lys 3-Bombesin		Bombesin
600A6	Rat	IgMk	NCI-H69	SCLC	?
600D11	Rat	IgMk	NCI-H69	SCLC	LNFP III
602G2	Rat	IgMk	NCI-H69	SCLC	LNFP III
603F5	Rat	IgMk	NCI-H69	SCLC	Glycolipid-2
604A9	Rat	IgMk	NCI-H69	SCLC	Glycolipid-1
612D5	Rat	IgMk	NCI-H69	SCLC	Glycolipid-4
624A3	Rat	IgMk	NCI-H69	SCLC	Glycolipid-1
624A6	Rat	IgMk	NCI-H69	SCLC	LNFP III
6234A12	Rat	IgMk	NCI-H69	SCLC	LNFP III
624H12	Rat	IgMk	NCI-H69	SCLC	Glycolipid X
624E5	Rat	IgMk	NCI-H69	SCLC	Glycolipid-3
624H7	Rat	IgMk	NCI-H69	SCLC	?
11-G11	Rat	IgMk	NCI-N417	Variant	Glycolipid-120kd
2H-H11	Rat	IgMk	NCI-N417	Variant	Protein-40kd
2H-H7	Rat	IgMk	NCI-N417	Variant	Glycoprotein 120kd
2H-D6	Rat	IgMk	NCI-N417	Variant	Glycoprotein 120kd
300A8	Mouse	IgG1	NCI-N417	Variant	?
325G11	Mouse	IgMk	NCI-N417	Variant	?
325D2	Mouse	-	NCI-N417	Variant	?
325E6	Mouse	-	NCI-N417	Variant	?

ANTI-SCLC MONOCLONAL ANTIBODIES BINDING TO LUNG CANCER
 CELL LINES BY RIA

Human target cells	Ref. or source	Binding ratio* π		
		∇ 525A5	∇ 534F8	∇ 538F12
SCLC				
NCI-H390	18	99	102	105
NCI-H69	18	46	50	49
NCI-H128	18	63	76	62
NCI-H187	This laboratory	35	43	46
NCI-H209	This laboratory	75	87	77
NCI-H64	18	56	62	64
NCI-H146	18	13	16	14
NCI-H60	18	4.7	13	13
SCLC to large cell converters				
NCI-H82	This laboratory	14	20	20
NCI-N231/417	This laboratory	8.2	10	17
Adenocarcinoma of the lung				
NCI-H125 [†]	This laboratory	21	25	38
NCI-H23	This laboratory	2.7	5.8	9.0
Large cell lung cancer				
NCI-H157	This laboratory	0.1	1.3	0.9
9812 [‡]	19	0.1	0.3	<0.1
Squamous cell lung cancer				
U1752	J. Ponten, Uppsala, Sweden	5.7	1.4	16
Adenocarcinoma lung (? bronchioloalveolar)				
A549	20	0.7	0.5	0.8

Assays were performed in quadruplicate, using rabbit anti-mouse IgM, and the ¹²⁵I-protein A assay, with monoclonal antibodies from ascites fluids tested at a dilution of 1:10⁴.

* Binding ratio = (cpm test well - cpm negative control) + cpm negative control to allow comparison between assays. Results are the average of quadruplicate determinations (less than 20% variance between wells for any one test). The negative control (within the range of 100-300 cpm for all cell lines tested) was obtained by omitting the monoclonal antibody and substituting P₁/NaCl or mouse myeloma IgM λ (MOPC-104E, Litton Bionetics, Rockville, MD) in the reaction.

[†] NCI-H125 is a malignant pleuropulmonary neoplasm compatible morphologically with adenocarcinoma or mesothelioma.

[‡] The tumor type of 9812 has been variously referred to as lung cancer and melanoma. Histologically the *nude* mouse heterotransplant is a large cell undifferentiated tumor compatible with either type.

π All results of RIA in this document are expressed as Binding Ratio.

∇ All binding Lacto-N-Fucopentaose III.

REACTIVITY OF MONOCLONAL ANTIBODIES
AGAINST
SMALL CELL LUNG CANCER TISSUE CULTURE LINES

Monoclonal Antibody	60	64	69	231	128	146	187	209	249	250	390	464
600A4	3.9	25.3	24	10.9	7.6	4.4	6.9	8.5	<1	4.7	9.6	4.3
600A6	1.7	8.2	15	3.3	3.8	<1	3.3	<1	7.3	5.1	2.1	6.7
600A10	2.6	23.5	26	6.4	5.3	1.1	7.3	2.5	11	5.2	3.1	8.7
600D11	2.1	17.5	12	7.3	7.4	<1	5.9	5	<1	<1	9.3	2.2
602G2	4.5	22.8	12	11.5	11.1	4.8	8.2	8.3	<1	2.9	14.5	6.1
603A6	5.8	45.8	18	13.3	13.2	4	8.5	11.3	<1	5.1	12.2	12
603F5	4.4	42.4	17	12.8	13.3	N.T.	8.3	9.8	<1	2.9	15	6
603A11	4.0	39.2	9.1	5.5	3.9	1.8	4.4	7.8	<1	1.3	3.5	4.8
604A9	10	13.0	13	9.2	4.2	3.7	6	8.8	8.1	23	9.9	6.8
604B4	4.7	8.6	24	5.3	4.1	<1	4.9	1.89	<1	<1	14.6	<1
610A8	1.8	24.5	16	4.4	2.7	2.1	4.5	7.1	<1	<1	3.1	3.4
612D5	2.6	26.9	13	5.9	4.2	<1	1	9.3	<1	<1	4.9	3.9
624A3	4	37	22	10.5	12.5	3.2	8	10.3	<1	3.3	12.6	11
624A6	8.2	6.2	17	6.9	6.5	1.6	7.9	7.0	<1	2.1	9.4	<1
624A12	5.4	16.6	12	13.3	9.3	9.7	8.2	11.6	<1	18	12.3	4.4
625E8	1.6	24.4	15	8.1	4.2	<1	6.1	9.7	<1	3.5	9.9	4.1
624H7	4	23.9	20	7.6	4.6	2.1	6.5	7.6	<1	1.5	8.9	5.2
624H12	5.3	13.2	8.7	11.7	5.4	4.7	6.8	10.7	<1	9	11.4	<1
625B4	4.7	45.7	8.2	9.0	12.4	3.6	9.9	11.6	<1	2	13.4	11
625E3	5.8	36.4	19	10.7	11.3	2.9	8.3	10.7	<1	2.8	13	9.1
625E5	4.9	20.1	16	7.8	7.4	1.9	7.3	8.7	<1	1.8	10.6	5.8
625G10	5.3	15.2	14	11.9	9.9	1.7	9.8	8.9	<1	5.5	12.6	4.2
625G12	2.4	31.7	18	9.2	9.5	1.5	7.5	9.0	<1	3.2	12.9	5.1

REACTIVITY OF MONOCLONAL ANTIBODIES
 AGAINST
 SMALL CELL LUNG CANCER TISSUE CULTURE LINES
 THAT HAVE TRANSFORMED
 TO
 LARGE CELL VARIANTS

Monoclonal Antibodies	82	231/417	60/177
600A6	2.5	2.5	1.1
600D11	3.4	<1	<1
602G2	<1	5.3	<1
603A6	<1	6.1	<1
603F5	<1	6.5	<1
603A11	5.9	<1	<1
604A9	<1	<1	<1
604B4	<1	<1	<1
610AB	<1	<1	<1
612D5	<1	<1	<1
624A3	1.4	5.4	<1
624A6	1.6	<1	<1
624A12	1.3	<1	1.9
624E8	<1	2.9	1.7
624H7	1.7	1.1	<1
624H12	1.2	<1	<1
625B4	<1	8.1	<1
625E3	<1	4.6	<1
625E5	<1	1.3	<1
625G10	2.2	2.2	1.0
625G12	1.3	3.2	<1

REACTIVITY OF MONOCLONAL ANTIBODIES
 AGAINST
 PLEURO-PULMONARY HUMAN TISSUE CULTURE HISTOLOGIES

FETAL LUNG VA2	NONCLONAL ANTIBODY	EPI U1752	AC 125	AC 23	AC 495	AC 324	AC A549	LC 157	LC 9812	LC 28	MESOTHELIOMA 226 290
<1	600A4	2.1	4	<1	20	3.4	<1	<1	<1	4.5	<1 <1
<1	600A6	2.7	1	<1	2.2	1.2	1.9	<1	<1	<1	<1 <1
<1	600A10	9.3	2.7	<1	11	5.5	8.2	<1	7.1	1.8	<1 <1
<1	600D11	<1	<1	<1	<1	<1	<1	<1	<1	3.4	<1 <1
<1	602G2	1.1	5.8	<1	<1	<1	<1	<1	2.3	9.5	<1 <1
<1	603A6	1.3	19	<1	6.6	17	<1	<1	8.4	11	<1 <1
<1	603F5	1.8	12	<1	6.2	5.2	<1	<1	2.1	9.6	<1 <1
<1	603A11	<1	18	<1	<1	1.2	<1	<1	2.5	5.7	<1 <1
<1	604A9	<1	21	<1	8.7	41	<1	<1	<1	<1	<1 <1
<1	604B4	<1	1.7	<1	<1	1.1	<1	<1	1.2	1.2	<1 <1
<1	610A8	<1	15	<1	<1	1.5	<1	<1	<1	<1	<1 <1
<1	612D5	<1	16	<1	<1	4.1	<1	<1	<1	5.3	<1 <1
<1	624A3	1.4	22	<1	5.6	13	<1	<1	1.5	11	<1 <1
<1	624A6	<1	3.7	<1	<1	6.3	<1	<1	<1	1.2	<1 <1
<1	624A12	2.2	20	<1	14	44	<1	<1	<1	6.5	<1 <1
3.9	624E8	4.5	17	<1	<1	5.4	2.6	<1	4	5.5	<1 <1
<1	624H7	<1	19	<1	<1	2.6	<1	<1	3.4	6.8	<1 <1
<1	624H12	1.1	18	<1	0.1	26	<1	<1	<1	<1	<1 <1
<1	625B4	2.0	22	<1	1.6	23	1.6	<1	6.5	11	<1 <1
<1	625E3	1.0	23	<1	3.1	25	1.3	<1	<1	9.5	<1 <1
<1	625F5	1.2	19	<1	<1	5.1	<1	<1	1.5	5.5	<1 <1
<1	625G10	2.4	12	<1	<1	3.4	<1	<1	<1	8.4	<1 <1
<1	625G12	4.7	18	<1	2.1	8.0	<1	<1	5.8	6.6	<1 <1

EPI - Epidermoid AC - Adenocarcinoma LC - Large Cell

T A B L E 3 Z01 CM 06575-08 NMOB
 ANTI-HUMAN SMALL CELL/LARGE CELL CONVERTOR MONOCLONAL ANTIBODIES

Binding of monoclonal antibodies to human lung cancer and fetal lung cell lines in solid phase radioimmunoassay[¶]

Cell Lines*	2H-H7	2H-D6	1I-G11	2H-H11
	----- (p120)			----- (p40)
	(Binding Ratio)§			
<u>Large Cell Variants</u>				
NCI-N231/417	57	50	60	21
NCI-H82	6	4	49	4
NCI-H446	16	ND	26	5
NCI-N177	17	ND	55	7
<u>Small Cell Lung Cancer</u>				
NCI-N231	10	11	20	<1
NCI-H187	5	3	8	<1
NCI-H209	7	9	13	<1
NCI-H146	8	5	12	<1
NCI-H249	2	2	5	<1
NCI-H123	2	2	7	1
NCI-H69	2	2	7	<1
NCI-N390	<1	<1	<1	<1
<u>Large Cell Lung Cancer</u>				
NCI-H157	12	11	14	<1
NCI-N650	<1	1	<1	<1
<u>Adenocarcinoma</u>				
NCI-H125	2	<1	5	<1
NCI-H23	1	<1	2	<1
A549	<1	<1	<1	<1
NCI-H324	1	<1	4	<1
NCI-N495	2	1	7	<1
<u>Mesothelioma</u>				
NCI-H226	5	<1	3	1
NCI-H28	2	1	6	<1
<u>Fetal Lung</u>				
IMR90	7	<1	4	<1
HR6	2	<1	4	<1

¶ Results represent the mean of quadruplicate samples using ascites fluid. Variance between tests was + 10%. Dilutions of 1:3000 for 2H-H7 and 2H-D6, 1:6000 for 1I-G11, and 1:1600 for 2H-H11 was used.

* All NCI cell lines were generated in this laboratory. A549 was obtained from G. Todaro, NCI. IMR90 and HR6 were obtained from HEM Research, Rockville, MD. All cell lines used were free of mycoplasma contamination (Microbiological Associates, Bethesda, MD).

BINDING OF ANTI-SCLC MONOCLONALS TO NON-PULMONARY CELL LINES

1	Target cells	Binding ratio			Ref. or source	Target cells	Binding ratio		
		525A5	534F8	538F12			525A5	534F8	538F12
	Human target cells					Human target cells (continued)			
	B lymphoblastoid					Fetal lung			
	NCI H128BL SCLC*	<0.1	<0.1	0.1	This laboratory	WI-38	<0.1	<0.1	<0.1
	NCI H209H SCLC*	0.4	0.8	0.4	This laboratory	WI-18-VA2 (transformed by simian virus 40)	<0.1	<0.1	<0.1
	NCI H263H SCLC*	<0.1	<0.1	<0.1	This laboratory	Erythrocytes	<0.1	<0.1	<0.1
	NCI H134BL SCLC*	0.4	0.2	0.1	This laboratory	Type A1	<0.1	0.8	0.4
	NCI H151BL MF†	0.4	0.6	1.0	23	Type B†	0.4	1.3	0.9
	Burkitt lymphoma (American)				I. McGrath, NCI	Type O†	0.9	1.3	1.7
	PC119	<0.1	0.6	1.0	I. McGrath, NCI	Type B-	0.4	0.8	1.2
	EW36	1.4	2.6	1.2	32	Rodent cell lines			
	Multiple myeloma					Mouse			
	U266	<0.1	0.1	0.7		RAG (BALB/c, renal cell carcinoma)	0.1	1.2	0.3
	T-cell leukemia/lymphoma					B82 (C3H, transformed fibroblast)	0.8	1.8	0.9
	MO1.T 4 (lymphoblastic leukemia)	0.1	0.1	0.3	32	1.51784R (DBA, L cell)	0.1	0.5	<0.1
	NCI J178 (Sézary syndrome)	<0.1	<0.1	0.1	23	Rat			
	Oatogenic sarcoma				This laboratory	NRK-536/5A6 (Oaborne- Mendel rat, kidney)	<0.1	0.4	<0.1
	NCI H135	<0.1	<0.1	<0.1		BRL30E (Buffalo rat, hepatocyte)	<0.1	0.3	<0.1
	Mesothelioma					Hamster			
	NCI H1226	<0.1	<0.1	<0.1	This laboratory	E36 (Chinese hamster, transformed lung)	0.6	0.7	0.5
	Hypertrophoma					BIH1 (Golden Syrian hamster, kidney fibroblast)	0.9	1.7	0.4
	NCI H201	1.3	2.9	1.1	This laboratory				
	Malignant melanoma								
	6759; EN	0.3	<0.1	0.6	S. Rosenberg, NCI				
	624R; WE	0.1	0.2	0.1	S. Rosenberg, NCI				
	Fibroblasts (skin)								
	NCI H290SK3†	1.1	1.2	1.4	This laboratory				
	6625; EN	0.9	2.4	0.7	S. Rosenberg, NCI				
	6750; ER	1.6	3.2	0.6	S. Rosenberg, NCI				
	2628; SL	0.3	1.8	0.3	S. Rosenberg, NCI				
	6359; VE	<0.1	<0.1	<0.1	S. Rosenberg, NCI				

Assays were performed in quadruplicate as described for Table 1. NCI, National Cancer Institute.

* B-lymphoblastoid line derived from SCLC patient.

† B-lymphoblastoid line derived from mycosis fungoides patient.

‡ Skin fibroblast line from SCLC patient NCI-H390.

T A B L E 4 (Continued)

BINDING OF ANTI-SCLC MONOCLONALS TO NON-PULMONARY CELL LINES

Target cells	Ref. or source	Binding ratio		
		525A5	534F8	538F12
Human cell lines				
SCLC				
NCI-H390	18	99	102	105
NCI-H69	18	46	50	49
NCI-H60	18	4.7	13	13
Neuroblastoma				
CHP-100	D. Glaubiger, NCI; 39	14	14	12
CHP-126	D. Glaubiger, NCI; 39	18	13	16
KO	D. Glaubiger, NCI	26	29	20
Breast cancer				
MCF-7	40	24	66	7.7
ZR-75-1	41	4.0	9.9	8.0
MDA-MB231	42	1.3	2.1	1.4
Rodent cell lines				
Mouse				
NIE-115 (A/J, neuroblastoma)	43	0.3	0.3	0.3
Rat				
RIN-m, CL5 (NEDH, insulinoma)	44	0.8	0.6	0.6
PC12 (NEDH, pheochromocytoma)	45	0.3	0.5	0.3

T A B L E 4 (Continued)

BINDING OF ANTI SCLC MONOCLONAL ANTIBODIES
WITH
NEUROECTODERM-DERIVED TUMORS

Monoclonal Antibody	Neuroblastoma				Glioma		Melanoma	
	IMR 32	KOP8	CHP 100	CHP 126	N. Glioma	M. Glioma	NCI-H234	6208-WE
	(Binding Ratios)							
600A4	1.9	7.9	8.5	6.8	<1	<1	14.1	7.8
600A6	2.0	1.8	4.3	<1	1.4	2.7	1.4	<1
600A10	2.7	8.4	8.9	2.3	4.3	5.6	17.6	8.8
600D11	<1	8.7	5.2	6.1	<1	<1	<1	<1
602G2	1.7	8.5	8.3	7.0	<1	<1	<1	<1
603A6	5.5	12	9.5	2.9	<1	<1	<1	<1
603F5	2.0	8.1	9.4	9.3	<1	<1	<1	<1
603A11	<1	5.1	5.5	<1	<1	<1	<1	<1
604A9	<1	<1	<1	<1	<1	<1	<1	<1
604B4	<1	3.2	3.5	3.7	<1	<1	<1	<1
610A8	<1	9.9	1.7	<1	<1	<1	<1	<1
612D5	2.0	10	2.2	<1	<1	<1	<1	<1
624A3	5.5	9.8	11	20	<1	<1	<1	<1
624A6	<1	1.7	5.5	8.1	<1	<1	<1	<1
624A12	<1	5.7	6.2	9.5	<1	<1	1.4	<1
624E8	2.4	8.5	4.2	1.7	<1	<1	<1	<1
624H7	1.8	8.4	4.2	6.3	<1	<1	<1	<1
624H12	<1	1.7	3.3	6.1	<1	<1	<1	<1
625B4	3.3	14	7.9	9.2	<1	<1	1.0	<1
625E3	5.8	12	9.2	7.3	<1	<1	<1	<1
625E5	3.7	11	5.5	4.6	<1	<1	<1	<1
625G10	<1	9.7	7.8	12	<1	<1	<1	<1
625G12	<1	9.5	3.7	5.2	3.8	11	1.9	<1

T A B L E 4 (Continued)

CELL LINES WHICH FAILED TO GIVE SIGNIFICANT BINDING REACTIONS (BINDING RATIO < 1)
WITH THE MONOCLONAL ANTIBODY PANEL (600 SERIES ANTI-SMALL CELL ANTIBODIES)

<u>HUMAN CELL LINES</u>	<u>(SOURCE)</u>	<u>HUMAN CELL LINES</u>	<u>(SOURCE)</u>
<u>B Lymphoblastoid:</u>		<u>Me1aroma:</u>	
NCI-H128BL	(This lab)	NCI-H234	(This lab)
NCI-H209BL	(This lab)	A875	(J. Schlom, NCI)
NCI-H26BL	(This lab)	A375	(J. Schlom, NCI)
NCI-H134BL	(This lab)	A101	(J. Schlom, NCI)
		6108-WE	(S. Rosenberg, NCI)
<u>Burkitt's Lymphoma:</u>		<u>Red Blood Cells:</u>	
PC119	(I. McGrath, NCI)	Type A	(This lab)
EW36	(I. McGrath, NCI)	Type B	(J. Schlom, NCI)
<u>T-Cell Lymphoma:</u>		Type 0	(J. Schlom, NCI)
HUT78	(This lab)	<u>Fibroblasts:</u>	
HUT102	(This lab)	NCI-H390SK3	(This lab)
<u>Osteogenic Sarcoma:</u>		HR6	HEM Research
NCI-N377/T35	(This lab)	IMR 90	HEM Research
<u>Glioblastoma:</u>		<u>Rodent Cell Lines:</u>	
N glioma	(P. Kornblith, NIH)	RAG (Mouse)	ATCC
M glioma	(P. Kornblith, NIH)	L(TK-)(Mouse)	(A. Nienhuis, NIH)
		B82 (Mouse)	(J. Littlefield, Johns Hopk)
		E36 (Chinese hamster lung)	(T. Caskey, NIH)

BINDING OF ANTI-SMALL CELL/LARGE CELL CONVERTOR MONOCLONAL ANTIBODIES TO HUMAN
 CANCER CELL LINES IN SOLID PHASE RADIOIMMUNOASSAY*

Cell Lines	Source	2H-H7	2H-D6	1I-G11	2H-H11
		----- (p120)			----- (p40)
(Binding Ratio)§					
<u>Melanoma</u>					
4T43	BRL	4	16	47	< 1
NCI-H234	This Lab	5	8	14	< 1
A875	J. Schlom, NCI	3	ND	ND	< 1
6208-WE	S. Rosenberg, NCI	21	17	28	< 1
A375	J. Schlom, NCI	6	5	14	< 1
A3827	J. Schlom, NCI	4	6	3	< 1
A101 D	J. Schlom, NCI	3	4	5	< 1
<u>Neuroblastoma</u>					
IRM32	ATCC¶	2	3	6	< 1
CHP134	S. Baylin, J. Hopkins	7	5	7	2
CHP126	D. Glaubiger, NCI	7	3	2	< 1
<u>Breast Cancer</u>					
MDA-MB231	M. Lippmann, NCI	1	<1	1	< 1
MCF-7	M. Lippmann, NCI	2	3	4	< 1
<u>T Lymphoma</u>					
HUT-78	This Lab	<1	2	3	< 1

* Assays were performed in quadruplicate as described in Table 3.

§ Binding Ratio = cpm test well minus cpm negative control / cpm negative control. ND is not determined.

¶ American Type Culture Collection, Rockville, MD.

IMMUNOHISTOCHEMICAL ASSAY OF ANTI SMALL CELL MONOCLONAL ANTIBODY BINDING TO HUMAN LUNG CANCERS HETEROTRANSPLANTED IN NUDE MICE.

Monoclonal Antibody	Cell Line				
	NCI-N679 (SCLC)	NCI-H378 (SCLC)	NCI-H329 (SCLC)	NCI-H82 (Variant)	NCI-H23 (Adenoca.)
	(Immune histochemical reaction score)				
600A6	+	+	+	-	-
600D11	-	-	-	-	-
602G2	-	-	-	-	-
603F5	-	-	-	-	-
604A9	-	-	-	-	-
612D5	-	-	-	-	-
624A3	+	-	+	-	-
624A6	+	-	-	+	-
624A12	+	+	+	+	+
624H12	+	-	-	-	-
624E5	+	+	+	+	+
624H7	+	+	+	+	-
625B5	-	+	-	-	-
625E3	+	+	-	-	-

IMMUNOHISTOCHEMICAL ASSAY OF ANTI-SCLC MONOCLONAL ANTIBODY BINDING
TO NORMAL ADULT HUMAN LUNG

Monoclonal Antibody	Antigen	Structure Within Adult Human Lung				
		Bronchial Epithelium	Serous Glands	Chondrocytes	Alveolar Lining Cells	Macrophages
600A6	-	+	+	-	-	+
600D11	LNFP 3	+	+	-	-	+
602G2	LNFP 3	+	+	-	-	+
603F5	GL-2	+	+	-	-	+
612D5	GL-4	+	+	-	-	+
624A3	GL-1	+	+	+	-	+
624A6	LNFP III	+	+	+	-	+
625A12	LNFP III	+	+	+	+	+
625H12	GL-X	+	+	-	-	-
624E5	GL-3	+	+	-	-	+
625H7	-	+	+	-	-	-
625B5	LNFP III	+	+	-	-	-
625E3	GL-Y	+	+	-	-	+

All Antibodies were tested on serial sections from the same embedded blocks.

IMMUNOHISTOCHEMISTRY ASSAYS OF THE BINDING OF HUMAN TUMORS BY ANTI
SMALL CELL/LARGE CELL CONVERTOR MONOCLONAL ANTIBODIES§

Tumor	Monoclonal antibodies			Comments
	2H-H7	1I-G11	2H-H11	
	(Number positives/totals)			
Large cell variants	1/2	2/2	1/2	Diffuse
Small cell lung cancer	1/4	2/4	0/4	Focal
Lung adenocarcinoma	0/2	1/2	2/2	Focal
Squamous cell lung carcinoma	1/1	1/1	0/1	Tumor cells and extracellular keratin
Breast cancer	01/	1/1	1/1	Focal

§Immunohistochemical assay described in "Material and Methods."

ANTI SMALL CELL/LARGE CELL CONVERTER MONOCLONAL ANTIBODY BINDING USING
CELL SORTER ANALYSIS OF LUNG CANCER AND B LYMPHOBLASTOID CELL LINES*

<u>Cell Lines</u> [†]	<u>MONOCLONAL ANTIBODIES</u>		
	<u>2H-H7</u>	<u>11-G11</u>	<u>2H-H11</u>
	<u>(% Positive Cells)</u>		
<u>Large Cell Variants</u>			
NCI-N231/417	64	67	10
NCI-H82	19	65	5
<u>Small Cell Lung Cancer</u>			
NCI-H146	44	59	4
NCI-H128	57	70	0
NCI-H209	63	50	6
NCI-H60	25	30	0
NCI-H123	26	24	0
NCI-N592	41	56	0
NCI-N691	71	59	4
<u>Large Cell Lung Cancer</u>			
NCI-H157	49	87	0
<u>Adenocarcinoma</u>			
NCI-H23	10	28	0
NCI-H125	35	29	0
A549	5	9	0
<u>B-Lymphoblastoid</u>			
NCI-H180BL	0	0	0
NCI-H155BL	2	3	0

* Results represent the mean of duplicate samples using purified monoclonal antibody at 40 ug/ml. Variance between tests was 10%.

†All NCI cell lines were generated in this laboratory. A549 was provided by G. Todaro, NCI.

TABLE 7

 BINDINGS ON ANTI SMALL CELL LUNG CANCER MONOCLONAL
 ANTIBODIES TO NECROPSY TISSUE

Source of cells	Source of monoclonal antibody*	^{125}I -Labeled detector reagent†	^{125}I -SAM or ^{125}I -GAM bound, cpm		
			525A5	534F6	538F12
Cell lines					
SCLC					
NCI-H390	AF	GAM	1018	1237	1227
NCI-H390	TCS	SAM	1215	1237	1365
NCI-H69	TCS	SAM	1038	576	999
NCI-H128	TCS	SAM	986	857	871
NCI-H187	TCS	SAM	897	1236	1361
B-lymphoblastoid					
NCI-H128BL	TCS	SAM	55	0	141
NCI-H26	TCS	SAM	192	63	1
NCI-H51	TCS	SAM	165	30	95
Autopsy‡					
SCLC tumor					
PET	AF	GAM	736	1428	1577
MUL	TCS	SAM	699	550	578
GRA	AF	GAM	601	949	1261
PVR	AF	GAM	356	619	586
Normal tissue					
Lung-1 (SND)	AF	GAM	90	103	121
Lung-2 (HAR)	TCS	SAM	0	0	0
Lung-3 (MUL)	TCS	SAM	23	17	42
Liver-1 (HAR)	TCS	SAM	33	0	13
Liver-2 (SND)	AF	GAM	42	150	164
Skeletal					
muscle (SND)	AF	GAM	10	46	0
Kidney (SND)	AF	GAM	152	182	168
Spleen (HAR)	TCS	SAM	29	13	29

Tissues were obtained at autopsy (courtesy of L. Ortega, Washington Veterans Administration Medical Center Laboratory Service), minced with fine scissors, passed through a 300- μm grid mesh screen, taken up in P_i/NaCl , and dispersed through a 25-gauge needle. The cell suspension was washed three times in P_i/NaCl , fixed with 0.25% glutaraldehyde/ P_i/NaCl for 5 min, washed twice again, and then adjusted to a 1% vol/vol suspension, and 0.05 ml of this suspension was added to each well of a polyvinyl microtiter plate and fixed as for the cell lines. ^{125}I -GAM and ^{125}I -SAM were used to test the binding of monoclonal antibodies to autopsy specimens because of the large background binding of ^{125}I -protein A to these human tissue samples. Results in cpm are the average of quadruplicate determinations with the backgrounds (200–400 cpm) subtracted.

* AF, ascites fluids; TCS, tissue culture supernatant fluids.

† To each well, 50,000–70,000 cpm of ^{125}I -labeled GAM or SAM was added.

‡ Different patients are identified by initials. Primary lung tumors and liver metastases were tested.

T A B L E 7 (Continued)
 REACTIVITY OF MONOCLONAL ANTIBODIES

Z01 CM 06575-08 NMOB

AGAINST

SMALL CELL LUNG CANCER, NECROPSY SPECIMENS, NORMAL

LUNG, NORMAL LIVER AND NORMAL KIDNEY

MONOCLONAL ANTIBODY	NL LUNG	NL LIVER	NL KIDNEY	1		2		3		4		5		6		7		8		9		10	
				SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER	SCIG LIVER
600A4	<1	<1	13.6	2.2	9.1	2.8	1.0	3.7	4.2	1.4	2.5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
600A6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
600A10	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
600D11	<1	<1	5.6	2.6	<1	7.8	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
602G2	<1	<1	8.6	4.0	13.7	3.4	1.1	<1	1.7	<1	6.0	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
603A6	1.8	7.8	0.0	6.0	39	<1	1.5	4.0	3.3	1.7	0.5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
603F5	3.3	3.4	8.4	4.5	27	5.3	<1	1.0	3.1	1.8	0.7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
603A11	<1	<1	4.2	3.4	14.0	<1	<1	1.0	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
604A9	<1	<1	<1	<1	32	2.7	<1	<1	1.3	<1	11.3	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
604B4	<1	<1	4.7	2.3	11.4	<1	<1	7.8	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
610A8	<1	<1	2.0	2.5	10	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
612D5	<1	<1	4.3	2.6	25	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
624A3	2.5	0.5	7.0	5.5	40.3	<1	<1	2.0	2.5	6.0	6.7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
624A6	<1	<1	3.7	1.9	19	<1	<1	3.9	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
624A12	1.6	<1	4.3	3.1	41	5.2	<1	22	14	1.0	4.1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
624E0	<1	<1	4.7	2.6	24	<1	<1	<1	<1	<1	1.4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
624H7	1.3	<1	4.5	2.6	35	<1	<1	<1	<1	<1	1.5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
624H12	<1	<1	3.7	0.3	43	<1	1.0	5.7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
625H4	3.3	0.0	5.4	3.5	30	<1	3.1	2.0	7.0	4.5	7.1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
625E3	3.3	3.7	5.2	3.5	31	<1	<1	1.7	3.3	3.4	6.5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
625E5	1.3	<1	6.6	2.3	25	<1	<1	<1	<1	<1	2.2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
625G10	1.0	<1	5.7	2.7	15	<1	<1	<1	<1	2.0	1.6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	
625G12	2.4	0.7	5.7	4.5	6.8	4.6	<1	2.2	2.8	2.7	3.0	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	

INHIBITION OF SOFT AGAR COLONY FORMATION BY MONOCLONAL ANTIBODIES

Antibodies	Cell Lines		
	NCI-N231/417	NCI-H82	NCI-H146
	(% Growth inhibition)		
None	0¶*	0	0
TEPC 183 IgM k§	-10	-13	-3
2H-H7	42	15	31
1I-G11	59	70	38
2H-H11	-9	7	4

¶Colony forming efficiency with no antibody added was: 20% for NCI-N231/417, 10% for NCI-H82 and 2.5 % for NCI-H146.

*All samples were done in triplicate at antibody concentrations of 10 µg/ml. The results represent the mean of 3 experiments. Variance between experiments was $\pm 10\%$.

§Myeloma protein. Litton Bionetics, Kensington, MD.

T A B L E 9

DOSE-RESPONSE EFFECT OF MONOCLONAL ANTIBODY 1I-G11
IN THE SOFT AGAR COLONY FORMATION ASSAY*

Concentration of Monoclonal Antibody 1I-G11	Cell Lines	
	NCI-N231/417	NCI-H82
(ug/ml)	(% Growth inhibition)	
None	0 [¶]	0
0.1	12	3
0.5	11	19
1	14	36
2	59	ND
10	59	60
50	57	ND

* Assay was performed as described for Table VII.

[¶] Colony forming efficiency with no antibody added was: 17% for NCI-N231/417 and 9% for NCI-H82. ND is not determined.

SCREENING HYBRIDOMA FOR NON SMALL CELL LUNG CANCER MONOCLONAL (N=6 FUSIONS)

	<u>Wells Seeded</u>	<u>No. With Hybrids</u>	<u>Selective RIA</u>	<u>No. Stabilized</u>
Immunogen				
NCI-H157	2688	578	38	5
NCI-H23	1152	968	102	12

ANTI NON SMALL CELL LUNG CANCER MONOCLONAL ANTIBODY DATA SHEETS

<u>Antibody Number</u>	<u>Species</u>	<u>Isotype</u>	<u>Immunogen (Cell Type)</u>	<u>Antigen</u>
701B2	Mouse	IgG2AK	NCI-H157 (Large Cell)	Protein 31kd
702A6	"	"	"	"
703D4	"	"	"	"
704B4	"	"	"	"
704A1	"	"	"	"
316F5	"	IgG1	NCI-H23 (Adeno)	(p65+85kd)
315F6	"	IgG1	"	(p70+200kd)
315G7	"	IgG2A	"	-
315G2	"	-	"	(p100kd)
313B2	"	IgG2A	"	(p80kd)
312B1	"	-	"	(p65+70kd)
317G7	"	IgG	"	(p80+150kd)
315A5	"	IgG	"	-
315C2	"	Igm	"	(p65+85kd)
330C9	"	IgG3	"	(p80+200kd)
334D9	"	"	"	(p65+85kd)
315A3	"	IgM	"	-

BINDING OF MONOCLONAL ANTIBODIES 703D4 and 704A1 TO HUMAN
LUNG CANCER LINES IN SOLID PHASE RADIOIMMUNOASSAY^a

<u>HUMAN LUNG CANCER LINES</u>		<u>SOURCE^d</u>	<u>BINDING RATIO^b</u>	
			<u>703D4</u>	<u>704A1</u>
Large Cell	NCI-H157	This Laboratory	170	140
	9812 ^c	G. Todaro, NIH	6	<1
Adenocarcinoma	NCI-H125	This Laboratory	1	1
	NCI-H23	This Laboratory	20	3
	A549	ATCC	18	18
	SK-LU-1	J. Fogh, <u>MSKI</u>	32	2
	NCI-H324	This Laboratory	2	3
	Calu-6	J. Fogh, <u>MSKI</u>	6	1
Mucopidermoid	NCI-H292	This Laboratory	7	<1
Mesothelioma	NCI-H28	This Laboratory	17	9
	NCI-H226	This Laboratory	6	1
Small Cell	NCI-H69	This Laboratory	2	<1
	NCI-H187	This Laboratory	1	<1
	NCI-H146	This Laboratory	1	2
	NCI-H60	This Laboratory	1	1
	NCI-H209	This Laboratory	1	1
	NCI-H249	This Laboratory	2	2
	NCI-N231	This Laboratory	<1	<1
	NCI-H128	This Laboratory	<1	<1
	NCI-H123	This Laboratory	1	2

T A B L E 12 (Continued)

HUMAN LUNG CANCER LINES	SOURCE	BINDING RATIO ^b		
		703D4	704AT	
SC/LC Converters ^e	NCI-N231/417	This Laboratory	1	1
	NCI-H82	This Laboratory	1	2

^aAssays were performed in quadruplicate using ¹²⁵I-protein A assay with monoclonal antibodies from ascites purified with 40% ammonium sulfate and used at concentration of 1 ug/ml.

^bBinding ratio = (cpm test well - cpm background) / cpm background to allow comparison between assays. Results are the average of quadruplicate determinations (less than 20% variance between wells for any one test). The background (mean 108 cpm, with a range of 50-300 cpm for all cell lines tested) was obtained by omitting the monoclonal antibody and substituting PBS in the reaction. We scored significant Binding Ratio as greater than two. Positive reactions thus had a range of total cpm bound of 430-8670 (mean 2460). In contrast, the total counts bound for the SCLC lines (all scored as negative) had a range of 80-190 (mean 130 cpm).

^cTumor type of 9812 has been referred to as lung cancer and melanoma. Histologically, the nude mouse heterotransplant is a large cell undifferentiated tumor compatible with either type.

^dABBREVIATIONS: ATCC - American Type Culture Collection, Rockville, Maryland; MSKI - Memorial Sloan Kettering Institute; NIH - National Institutes of Health.

^eCell lines which in culture have undergone histologic conversion from small cell to large cell with loss of typical small cell APUD characteristics.

T A B L E 13

NONPULMONARY HUMAN TUMOR CELL LINES WITH SIGNIFICANT BINDING RATIO IN
SOLID PHASE RADIOIMMUNOASSAYS WITH MONOCLONAL ANTIBODIES
703D4 and 704A1^a

<u>CELL LINE</u>	<u>TYPE</u>	<u>SOURCE</u>	<u>BINDING RATIO</u>	
			<u>703D4</u>	<u>704A1</u>
Renal Cell Carcinoma	NCI-H201	This Laboratory	23	30
Osteogenic Sarcoma	NCI-H135	This Laboratory	9	3
Melanoma	6208-WE	S.A. Rosenberg, NIH	17	20
	A375	J. Schlom, NIH	9	5
	NCI-H234	This Laboratory	4	4
	A875	J. Schlom, NIH	8	6
	SKMEL-28	K. Foon, NIH	20	3
	A3827	J. Schlom, NIH	20	3
	A101	J. Schlom, NIH	14	2

^aAssays were performed in quadruplicate as described for Table I.

T A B L E 13 (Continued)

CELL LINES WITH LOW OR INSIGNIFICANT BINDING OF ANTI-HUMAN LARGE CELL
LUNG CANCER MONOCLONAL ANTIBODIES 703D4 and 704A1 IN SOLID PHASE
RADIOIMMUNOASSAY^a

TARGET CELL		SOURCE	BINDING 703D4	RATIO 704A1
B-Lymphoblastoid	NCI-H128BL ^b	This Laboratory	<1	<1
	NCI-H209BL ^b	This Laboratory	<1	<1
Macrophage	U937	K. Foon, NIH	2	1
Multiple Myeloma	U266	J. Bergh, Uppsala U.	<1	<1
T-cell leukemia/ lymphoma	Hut78	This Laboratory	1	3
	Hut102	This Laboratory	<1	<1
Neuroblastoma	CHP100	D. Glaubiger, NIH	<1	2
	IMR-32	ATCC	2	<1
Breast Cancer	MCF-7	M. Lippman, NIH	1	1
	MDA-MB231	M. Lippman, NIH	2	1
Melanoma	SKMEL-31	K. Foon, NIH	2	1
Colon	SWI-222	K. Foon, NIH	3	3
Fibroblasts, human	IMR-90	HEM Research, Rockville, Maryland	3	2
	HR-6	HEM Research, Rockville, Maryland	2	2

^aAssays were performed in quadruplicate as described for Table I.

^bB-lymphoid line derived from SCLC patient.

T A B L E 13 (Continued)

	<u>TARGET CELL</u>	<u>SOURCE</u>	<u>BINDING RATIO</u>	
			<u>703D4</u>	<u>704A1</u>
Rodent Cell Lines				
Mouse	RAG (BALB/c, renal cell carcinoma)	ATCC	1	1
	B82 (C3H, transformed fibroblasts)	J. Littlefield, Johns Hopkins	<1	<1
	L51784R (DBA, L Cell)	J. Bertino, Yale	<1	2
	L cell (TK-) (mouse fibroblasts)	A. Niehuis, NIH	<1	<1
Rat	GH ₃ (pituitary tumor, (rat))	ATCC	<1	<1
	PC12 (Pheochromocytoma)	L. Greene, Harvard	1	1
	Chinese Hamster E36 (transformed lung)	T. Caskey, NIH	2	2

^aAssays were performed in quadruplicate as described for Table I.

T A B L E 14

BINDING OF NUDE MOUSE TUMOR OF HUMAN LUNG CANCER CELL LINES
 HETEROTRANSPLANTS IN IMMUNOHISTOCHEMICAL ASSAY WITH MONOCLONAL
 ANTIBODIES 703D4 and 704A1.

	<u>STAINING</u>	
	<u>703D4</u>	<u>704A1</u>
Non-Small Cell Carcinoma of Lung		
NCI-H23 (Adeocarcinoma)	+	+
NCI-207 (Adenocarcinoma)	+	+
NCI-H292 (Mucoepidermoid)	+	+
A549 (Adenocarcinoma)	-	+
NCI-H348 (Squamous Cell)	+	-
NCI-125 (Adenocarinoma)	-	-
Small Cell Lung Cancer		
NCI-N179	-	-
NCI-H69	-	-
NCI-H128	-	-
NCI-328	-	-
NCI-329	-	-
Small Cell to Large Cell Converters		
NCI-N231/417	-	-
NCI-H82	-	-

^aImmunohistochemical assay described in "Material and Methods"

T A B L E 15

BINDING OF ANTI LARGE CELL MONOCLONAL ANTIBODIES 703D4 and 704A1 WITH
 NORMAL HUMAN TISSUES IN IMMUNOHISTOCHEMICAL ASSAY^a

<u>NORMAL TISSUE</u>	<u>703D4 #POSITIVE/TOTAL</u>	<u>704A1 #POSITIVE/TOTAL</u>
Lung	0/5	0/5
Liver	0/3	0/3
Kidney	0/3	0/3
Brain	0/4	0/4
Pancreas	0/2	0/2
Prostate	0/2	0/2
Bladder	0/2	0/2
Skeletal Muscle	0/1	0/1
Colon	0/1	0/1

^aImmunohistochemical assay described in "Materials and Methods"

Title: EXPRESSION OF HLA AND HISTOCOMPATIBILITY ANTIGENS IN LUNG CANCER CELLS

I. Personnel

- A. Permanent Senior Staff: John D. Minna, M.D., Paul A. Bunn, M.D.
- B. Medical Staff Fellows: L. Austin Doyle, M.D., James Mulshine, M.D.
- C. Other Professional Staff: None
- D. Technical Staff: Marion Nau, Chemist

II. Introduction

A. Objectives

1. To determine the extent of the deficiency of class I histocompatibility antigens (HLA framework and beta 2 microglobulin) on the surface of small cell lung cancer using radiobinding and fluorescent cell sorter assays.
2. To test for related defects in class II histocompatibility antigens on small cell lung cancer lines using anti-HLA-DR monoclonal antibodies.
3. To determine whether immunoperoxidase staining with anti-HLA framework monoclonal antibodies could distinguish between small cell and non-small cell lung cancers.
4. To use immunoprecipitation, protein gel electrophoresis, and Southern and Northern blotting techniques to evaluate the level of the defect in class I histocompatibility antigens.
5. To examine whether expression of Lacto N Fucopentaose III (LNFP III), the dominant glycolipid antigen on small cell lung cancer lines, identical to Stage Specific Embryonic Antigen (SSEA), is related to class I antigen expression or development.

B. Rationale and Background .

HLA, B2M, and HLA-DR antigens are of major biologic importance (1). We observed in preliminary experiments that W6/32, a commercially available anti-HLA framework monoclonal antibody reactive with almost all cells, did not bind to human small cell lung cancer in radiobinding assays. Also, work from 2-D gel membrane protein analysis did not reveal spots corresponding to HLA antigens. In addition, we noted as described in the general report on monoclonal antibodies, that the glycolipid antigen lacto-N-fucopentaose III was immunodominant on small cell lung cancers (2). While we were making these observations, other groups reported deficiencies in HLA and beta 2 microglobulin (B2M) expression on choriocarcinoma cells and neuroblastoma cells (3,4). In addition, work in 59 mouse teratocarcinoma cells revealed deficient

expression in H2 and B2 microglobulin antigens and the expression of the stage specific embryonic antigen (SSEA-1) of the mouse which subsequently was shown to be Lacto-N-fucopentaose (LNFP III) (5). LNFP III (SSEA-1) is related to T locus antigens in the mouse (6). All of these facts made us decide to pursue, and evaluate the expression of class I and II antigens (HLA, B2M, and HLA-DR) in lung cancer cells.

C. Specific Aims and Outline of Methodology

1. Plate binding assays using glutaraldehyde-fixed tumor cells and monoclonal antibodies to HLA framework, B-2 microglobulin and HLA-DR with a 125-I Protein A detector.
2. Fluorescent cell sorter assays using live cells and the same monoclonal antibodies.
3. Immunoperoxidase staining, using monoclonal antibodies to class I determinants and an avidin-biotin complex technique on slides of small cell and non-small cell lung tumors.
4. Immunoprecipitation of 35-S methionine labelled cell lysates of small cell and non-small cell lines with subsequent SDS protein electrophoresis to determine if HLA-frame and B-2 microglobulin are deficient at an intracellular protein level.

IV. Progress Report and Future Plans

1. Radiobinding and cell sorter assays have shown a marked reduction or total absence of both HLA, B2M, and HLA-DR antigens on the surface of 8 small cell lung cancer lines, and 2 "variant" small cell lung cancer lines. In contrast, all 7 non-small cell lung cancer lines, and 3 non-lung cancer lines expressed the class I antigens. All of the cell lines other than small cell had HLA-DR antigens by radiobinding assay, but only some reacted with anti-HLA-DR in the live cell, cell sorter assays.

We plan to systematically test all of our lung cancer lines with the anti-class I and anti-class II monoclonal antibodies. In addition, we plan to obtain lung cancer tissue from autopsy and surgical specimens to test fresh tumor specimens for the expression of these antigens. If this situation found in cell lines is mirrored in the patient the phenotype will be of clinical utility.

2. Immunoprecipitation and gel electrophoresis shows absence of B2M bands in all 4 small cell lung cancer lines tested. All 4 non-small cell lines tested had distinct B2 microglobulin bands. Immunoprecipitation with anti-HLA framework monoclonals revealed that 4 of 5 small cell lines were missing HLA framework bands altogether while 4 of 5 non-small cell lung cancer lines had distinct HLA bands of normal molecular weight. We plan to continue to improve technically the immunoprecipitation results and also use heteroantisera.

3. To determine whether absence of these histocompatibility antigens lies at the DNA, RNA, or protein level we have performed Southern blot analysis of the lung cancer lines with a human cDNA HLA probe (a gift of Dr. S. Weisman). To date there appears to be no difference between the lines whether or not they express the antigens. We are currently attempting to perform the same experiments with a mouse beta 2 microglobulin probe (a gift of Dr. J. Siedman). We will use the same probes to perform "Northern" blot analysis of RNA expression of these genes.
4. Because of the findings in mouse teratocarcinoma cells that histocompatibility antigens can be expressed following induction of differentiation with retinoic acid, with a concurrent decrease in SSEA-1 expression, we will try similar experiments with the small cell lung cancers.

V. References and Publications

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TITLE: IMMUNOHISTOCHEMICAL AND CELL SORTER ANALYSIS OF MONOCLONAL ANTIBODIES (MoAb) FOR USE IN DIAGNOSTIC AND THERAPEUTIC CLINICAL TRIALS.

I. Personnel

- A. Permanent Senior Staff: Gazdar, A.F., Matthews, M.J., Bunn, P.A., Minna, J.
- B. Clinical Associates/Medical Staff Fellows: Mulshine, J., Doyle, A.
- C. Other Professional Staff: Cuttitta, F. (Staff Fellow), Fargion, S. (Visiting Fellow)
- D. Technical Staff: Fedorko, J., Jewett, P.

II. Collaborators

Worsham, F., Bibro, M. (Anatomic Pathology, Naval Hospital, Bethesda)
 Polack, J (Histochemistry Department, Royal Postgraduate Medical School, Hammersmith Hospital, London, England)

III. Introduction

A. Objective

The major objectives of this report ask:

1. Can we use the antibodies to type lung cancer into clinically relevant categories?
2. Do the antibodies have enough specificity to warrant their introduction into diagnostic and therapeutic trials?
3. Can they teach us more about the biology of lung cancer?

B. Rationale and Background

We have developed many monoclonal anti-lung cancer antibodies that we would like to introduce into clinical trials. However, before monoclonal antibodies can be introduced into clinical trials of either a diagnostic or therapeutic nature, it is crucial to know the following: What processing and fixation techniques will be tolerated by the antigen detected by a specific monoclonal antibody (MoAb) and is the antigen localized on the cell surface or elsewhere? Is the antigen expressed on uncultured tumor cells? What fraction of lung cancers will react with a MoAb? Does the antibody distinguish lung cancer from other types of cancer and how much heterogeneity exists within a tumor? In addition, which normal adult and fetal cells express the antigen? The latter is particularly important before giving antibody to patients. The best way to approach these questions is with immunohistochemical and in some cases cell sorter techniques. In addition, heterologous anti-sera are available that bind to certain well defined SCLC markers such as

neuron specific enolase, the BB isozyme of creatine kinase, and the peptide hormone calcitonin. While we are developing monoclonal antibodies to some of these markers (e.g., neuron specific enolase) we will use the heteroantisera in the immunohistochemistry work. We have established these techniques and collected a panel of tumors and normal tissue to begin study of these questions. The development and initial characterization of these antibodies has been discussed in detail in the general report on monoclonal antibodies. Tabular data summarizing many of the characteristics of these antibodies was also presented.

C. Specific Aims and Outline of Methodology

Using immunohistochemical and cell sorter assays to test a panel of monoclonal antibodies against lung cancer cells and their products to determine their reactivity with:

1. A panel of normal tissues.
2. A panel of lung tumors of various histologic types.
3. A panel of tumors arising outside the lung.

As part of this also:

4. To determine the degree of heterogeneity of antigen expression within any one tumor.
5. To see whether the expression of an antigen profile is associated with a specific histologic type of lung cancer.
6. To see if antigen expression has prognostic value.

Methodology

We have adapted and slightly modified the methodology of Su-Ming (1), who was responsible for the development of the avidin-biotin-complex immunohistochemistry assay system (2). For many lymphoma studies as described elsewhere we are collaborating with him. The current method employs nickel chloride impregnation of diaminobenzidine which forms a black precipitate when exposed to horseradish peroxidase.^(3,4) The peroxidase is part of the connecting antibody tagged avidin-biotin-complex. The deposition of this precipitate contrasts with the methyl green counter stain allowing for very sharp resolution of detail.

Cell sorter assays are performed in Dr. Bunn's laboratory using a TPS II Coulter cell sorter with attachments to allow dual parameter analysis, e.g., for DNA content and bound antibody).⁽⁵⁾

Using both paraffin embedded and frozen tissue samples, four pathologists score the material independently for conventional H and E light microscopic criteria and antibody deposition. The four pathologists are:

Drs. Adi Gazdar and Mary Matthews, (NCI-Navy), and Drs. Fred Worsham and Mary Bibro (Naval Hospital Anatomic Pathology). To facilitate this we are developing a standardized scoring format and recording system which ultimately will be computerized.

IV. Progress Report

We have done work over the last 18 months, to standardize the immunohistochemistry assay including establishing antigen fixative requirements for 25 of the antibodies. We have done titration studies of the individual antibodies to determine optimal antibody concentration and incubation conditions. However, we nearly all always use high concentrations (10 ug/ml or greater) of monoclonal antibody to facilitate detection of small amounts of antigen.

Of primary importance is a source of tissues to test. We have thus developed a tissue bank including formalin fixed, paraffin embedded blocks of nude mice heterotransplants of 25 different cell lines of various human lung cancers, and 70 blocks of different human lung cancers from patients obtained from the hospital archives. For each of these blocks the four pathologists have agreed upon a diagnosis by routine light microscopy after independent review. In addition, we have made arrangements to obtain 100 more blocks of various non-pulmonary neoplasms. For antibodies which recognize determinants which are not stable to routine fixation we have a bank of 25 frozen nude mouse tumors of various lung cancer cell lines and 6 different human lung cancer specimens. The tissue bank is being further expanded with clinical material from current patients as specimens become available. In addition, various cooperative cancer study groups (CALGB) have provided lung cancer blocks of patients entered onto their protocols.

For initial characterization with immunohistochemistry we first determine with nude mouse tumors or normal tissues whether or not the antigen is stable after fixation and embedding or whether we have to use frozen sections. Most of the antigens tolerate the fixation. We then screen the candidate antibody for binding to normal lung and kidney, and then the panel of lung cancer tissues. Interesting antibodies identified by these tests have a more extensive survey of binding to normal and malignant tissues. Currently, we are screening 10 MoAb with the large panel of lung cancers and normal tissues.

Tables 1, 2, and 3 summarize immunohistochemical work with six different antibodies on normal tissues, and human lung cancer specimens taken directly from patients. These antibodies include two that react with the glycolipid lacto-*n*-fucotetraose III (also known as the stage specific embryonic antigen of the mouse) but see different determinants on this molecule, two that react with two other distinct glycolipids which we currently are characterizing chemically with Dr. Ginsberg's group, and two that are against proteins of 120 and 31 kdaltons respectively. (Table 1). Most do not react with any cells in adult liver, several react with proximal tubules in the kidney, and some with distal tubules in the kidney. Many react with normal cells in adult lung but this reactivity is very restricted. It includes bronchial epithelium and

serous glands, but not mucous glands, chondrocytes, alveolar lining cells, or macrophages. Just with normal lung alone the six antibodies give 4 different patterns. When the lung and kidney staining patterns are combined, all 6 MoAb are distinct.

In staining of tumors taken directly from patients (Table 3), we see that within any histologic type most antibodies do not stain every tumor. However, the tumors that are negative for one antibody are positive for another, so that together combinations of antibodies can stain nearly all tumor samples. In addition, immunohistochemistry has demonstrated heterogeneity of antigen expression on tumor cells within individual tumors. We are planning to also study tumor biopsy samples from different metastatic sites within the same patient. So far none of the tumor MoAb generated against SCLC are absolutely specific for this cell type. Staining of at least some of the non-small cell cancers is also seen. These results are early and we are planning to extend them to our entire panel of lung tumors.

Studies of immunohistochemistry with anti-bombesin monoclonal antibodies:

As described under the general monoclonal antibody report, we have generated a monoclonal antibody with specificity for the peptide hormone bombesin and defined the antibody binding site, and shown the antibody can inhibit the clonal growth of SCLC in defined medium. We have started using this antibody in immunohistochemistry studies and can show staining of SCLC nude mouse heterotransplants, patient tumor samples, and bombesinergic cells in human fetal bronchus and the chick foregut (proventriculus). We have started collaborating with the immunohistochemistry Department of the Hammersmith Hospital (England) who are expert in the immunohistochemistry of neuropeptides and the APUD system.^(6,7) With this group we are pursuing these three projects: First the light microscopic localization of bombesin containing cells in the CNS, gut, and lung in fetal and mature tissues. The second involves giving them the antibody to screen lung cancer specimens in their department (Total of the two groups is 150 specimens). The final project is an ultrastructural study by the Hammersmith to determine the intracellular localization of bombesin in SCLC cells producing abundant amounts of the neuropeptide using the Hammersmith's strep-avidin gold techniques. To begin these projects, Drs. Mulshine and Cuttitta visited the Hammersmith for 2 weeks to learn techniques and perform preliminary experiments.

Flow cytometric analysis of lung cancer specimens:

Dr. Bunn's laboratory has analyzed a large number of tumor specimens taken directly from patients using single parameter DNA content measurements. We have shown that the majority of lung cancers (85%) have aneuploid DNA content. We have also shown that tumor cell DNA content correlates highly with modal chromosome numbers and rarely changes after in vitro cultures. In fact, the DNA index serves as a convenient signature of the cell lines. Because of the precious nature of fresh lung cancer specimens for tissue culture studies we are perfecting the cell sorter techniques with antibodies on the established cell lines.

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T A B L E 1

SPECIFICITIES OF MONOCLONAL ANTIBODIES GENERATED AGAINST HUMAN LUNG CANCER
 IN A IMMUNOHISTOCHEMICAL ASSAYS WITH NORMAL HUMAN TISSUE

<u>Monoclonal</u>	<u>Immunogen</u>	<u>Immunostaining or Normal</u>			<u>Antigen</u>
		<u>Lung</u>	<u>Kidney</u>	<u>Liver</u>	
534F8	SCLC	+	P.T*	-	LNFP III
624A12	SCLC	+	-	-	LNFP III
600A6	SCLC	+	P.T.	+	-
624H12	SCLC	+	P.T.	-	Glycolipid X
11-G11	SC/LC	+	D.T.	-	Glycoprotein 120kd
703D4	NSCLC	-	-	-	Protein 31k

*Abbreviations: P.T. - proximal tubules, D.T. - distal tubules, LNFP - Lacto-N-Fucopentaose III

T A B L E 2

ANTI HUMAN LUNG CANCER MONOCLONAL ANTIBODY BINDING TO NORMAL HUMAN LUNG
IN IMMUNOHISTOCHEMICAL ASSAYS

Monoclonal Antibody	STRUCTURAL WITHIN ADULT LUNG						
	Bronchial Epithelium	Serous Glands	Mucous Glands	Chondrocytes	Alveolar Lining Cells	Macrophages	
534F8	+	+	-	-	-	-	
624A12	+	+	-	+	+	+	
600A6	+	+	-	-	-	+	
624H12	+	+	-	-	-	-	
11-G11	+	+	-	+	+	+	
703D4	-	-	-	-	-	-	

T A B L E 3

ANTI LUNG CANCER MONOCLONAL ANTIBODIES BINDING TO HUMAN
LUNG CANCER SPECIMENS IN IMMUNOHISTOCHEMICAL ASSAYS

<u>Monoclonal Antibody</u>	<u>Tumor Histology (Positive/Total)</u>			
	<u>SCLC</u>	<u>Squamous</u>	<u>Adeno</u>	<u>Large Cell</u>
534F8	15/22	8/10	17/31	3/5
624A12	3/7	0/2	1/6	1/1
600A6	3/7	0/2	4/6	1/1
624H12	2/7	2/2	0/6	0/1
11G11	7/7	2/2	5/6	1/1
703D4	1/7	1/2	3/6	0/1

T A B L E 4

PATTERNS OF BINDING OF A MONOCLONAL ANTIBODY TO A PANEL OF SMALL
CELL LUNG CANCER SPECIMENS IN IMMUNOHISTOCHEMICAL ASSAYS (N=7)

<u>Monoclonals</u>	<u>SCLC</u>	<u>Specimens</u>						
		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
624A12	-/0	-/0	+/1	+/2	+/3	+/2	-/0	
600A6	-/0	+/3	-/0	+/3	+/1	-/0	-/0	
624H12	+/2	-/0	-/0	-/0	+/3	-/0	-/0	
1IG11	+/2	+/3	+/2	+/2	+/3	++/2	++/2	

Grading Intensity: (-) negative, (+, to +++) positive

Percent Staining: (0) No staining, (1) <25% of cells staining,
(2) <50% of cells staining, (3) <75% of cells staining
(4) \leq 100% of cells staining

Title: A MOLECULAR CYTOGENETIC APPROACH TO QUESTIONS ON THE MECHANISM OF CHROMOSOMAL TRANSLOCATIONS AND CANCER

I. Personnel

- A. Senior Staff Fellow: Dr. Ilan Kirsch (Principal Investigator), NCI-NMOB
- B. Other Professional Staff: To be arranged
- C. Technical Staff: None

II. Collaborators/Collaborating Branches

Dr. P. Leder, Department of Genetics, Harvard Medical School;
Dr. S. Korsmeyer, Metabolism Branch, NCI; Dr. C. Morton, Harvard Medical School; Dr. J. Strominger and Dr. James Skare, The Dana Farber Cancer Center; Dr. Ian Magrath, Pediatric Branch, NCI.

III. Introduction

A. Objectives

To use molecular genetic and molecular cytogenetic approaches to study chromosomal changes in malignant cells. Specifically to: Molecularly clone and map translocation breakpoints in malignant cells; using in situ chromosomal hybridization with cloned cellular oncogene probes to study malignant cells with interesting cytogenetic abnormalities.

B. Rationale and Background

With advances in the cytogenetics of tumor cells it has become apparent there are many specific cytogenetic abnormalities associated with tumor cells. These include among others: del 13q retinoblastoma; del 11p Wilms' tumor; translocations of chromosomes 2, 14, and 22 with 8 in Burkitt's lymphoma, del 3p in small cell lung cancer; t3;8 in renal cancer; del 1p in neuroblastoma; many examples of double minute chromosomes (DM's) and homogeneous staining regions (HSR's) indicating gene amplification; a variety of changes in copies, deletions and translocations of specific chromosomes in acute leukemias, and the 22; 9 translocation of chronic myelogenous leukemia. In addition, the development of cloned DNA probes for oncogenes and for genes coding for differentiated cell products, coupled with the development of chromosomal in situ hybridization technology, permits us to localize genes to very precise regions on chromosomes.

The molecular cytogenetic experiments currently in progress and projected for the future are based on work in which I participated while in the laboratory of Dr. Philip Leder. We had reached a point in our cloning and study of the mouse and human immunoglobulin genes where we wished to know more precisely their position in the genome. Initially, through the use of somatic cell hybrids, we and others were able to map the human kappa, lambda, and heavy chain genes to chromosomes 2, 22, and 14, respectively. We turned to the technique of chromosome in situ hybridization for more detailed mapping studies.

This technique involves hybridizing slides of metaphase spreads of chromosomes to specific tritium or iodine labelled DNA probes, autoradiographing the slides under a nuclear track emulsion, developing the emulsion, and then quinacrine banding the chromosomes. When reviewed under an incident light fluorescent microscope, a banding pattern is seen which allows for individual chromosome identification. When the visible light is added to the field, silver grains stand out where the hybridized probe has exposed the emulsion.

Using this technique, we are able to localize the immunoglobulin heavy chain locus to a particular band of chromosome 14, 14q32, and the lambda light chain locus to 22q11. The kappa light chain has been mapped by Malcolm et al. to the region 2c-2p13.

What is striking about these immunoglobulin gene localizations is that they are the precise bands seen in the reciprocal translocations characteristic of Burkitt's lymphoma. In Burkitt's lymphoma 90% of cases are associated with a translocation between chromosome 8q24 and 14q32, while 10% of cases are associated with t2;8 (p12;q24), or t8;22 (q24;q11). We wished to know how closely linked immunoglobulin genes were to the translocated portion of chromosome 8, and what was on band 8q24.

We already had in our laboratory another tumor system, an IgA producing mouse plasmacytoma, which also had a specific chromosomal translocation in which one of the partners was an immunoglobulin gene-bearing chromosome. We had previously cloned the actual translocated segment of DNA in this analogous tumor system. Our analysis demonstrated that the translocation in this case occurred within the heavy chain constant region locus at the heavy chain "switch" target site, the normally recombinationally active area that is necessary for the switch of the immune response from the production of primarily an IgM antibody to the production of a different heavy chain class IgD, IgG, IgE, or IgA. Furthermore, the piece of DNA brought into this locus was demonstrated by us and others to encode the mouse c-myc oncogene, the oncogene already implicated in the transformation of avian B cells by avian leukosis virus.

We demonstrated that precisely the same event often occurred in Burkitt's lymphoma cells. Through chromosome in situ hybridization, Southern blot analysis, and cloning, it was demonstrated that the human c-myc oncogene resided on chromosome 8q24, and could be translocated in a way completely analogous to the mouse plasmacytoma into the immunoglobulin loci. These findings are leading to intense investigations concerning (1) the relationship of this translocation to the process of malignant transformation and (2) the lessons to be learned about the mechanism of chromosomal translocation by this phenomenon. The work in progress and prospective projects are designed to explore issues of the mechanism of chromosomal translocation as well as approach the question of carcinogenesis in Burkitt's lymphoma from a related, but different aspect.

C. Methodology

This work will involve the standard methodology of molecular biology and cytogenetics (see references for details).

IV. & V. Progress Report and Future Plans

1. Studies on the mechanism of chromosomal translocation: It was striking that in a cell committed to the production of antibody molecules (a B cell), the characteristic c-myc translocations were occurring to precisely those regions encoding immunoglobulin molecules. Analysis by Gilbert Lenoir of numerous individual tumors from patients with Epstein-Barr virus positive and negative Burkitt's lymphoma have demonstrated an almost 100% positive correlation between the light chain produced by the malignant lymphocytes and whether the translocation involved chromosome 2 (in kappa producers) or 22 (in lambda producers) when these less common translocations occurred.

We therefore asked whether genes with high levels of expression were involved in the process of chromosomal translocation. We looked for another cell type, e.g. non-lymphocytic, where we could identify a primary product made by the cell, and ask if a chromosomal translocation occurred in this cell, would it involve the region to which that primary gene mapped. The globin gene appeared to be a good choice because of its potential expression in erythroleukemia. A patient with erythroleukemia presented to the Medical College of Virginia. We analyzed the karyotype of this patient's malignant cells on a direct bone marrow preparation and found a consistent reciprocal translocation between chromosomes 7 and 11. (Chromosome 11 is known to be the site of beta globin.) In collaboration with Cynthia Morton, we have analyzed these metaphase spreads using a beta-globin probe and found the breakpoint on chromosome 11 to be precisely that region where the beta globin gene cluster is encoded. This is not a unique finding. The K562 cell line derived from a patient with chronic myelogenous leukemia in which globin production could be induced shows an identical 7;11 translocation. We are also pursuing a recent report of a patient with erythroleukemia whose malignant cells carry a small t16;17. (The alpha-globin genes are encoded on chromosome 16).

The point could be made that translocation actually occurs randomly, but is selected for by transcriptional activity or growth advantages brought about by oncogene activation. This is possible, but we think we have an example which supports a more etiologic role to genomic activity in the genesis of chromosomal translocation. In collaboration with Stan Korsmeyer and Ken Nakahara, we have analyzed phytohemagglutinin stimulated T cells from a patient with ataxia-telangiectasia. We observed the characteristic t14;14 translocation already reported to be associated with this disease. However, when we analyze an Epstein-Barr virus transformed B-lymphocyte line from the same patient, we observed instead t2;14, the breakpoints on chromosomes 2 and 14 being identical to those seen in the t2;8, t2;14 Burkitt's lymphoma mode. Epstein-Barr virus, itself, has never been implicated as causing these translocations. Thus, the

translocation that we observed was correlated more with the cell type in which it occurred (B cell) than with the malignant state.

A key feature in the analysis of the c-myc translocation, as well as immunoglobulin gene switching, is the cloning of the breakpoint or switch region. Therefore, we are planning to: (1) clone and analyze the breakpoint of the t2;14 ataxia-telangiectasia B-cell line using kappa and heavy chain genes as probes; (2) clone and analyze the breakpoint in the erythroleukemia cell line K562 and in fresh malignant cells from erythroleukemia patient using the beta-globin and Ha-ras probes (Ha-ras has been shown to map to a contiguous region of chromosome 11 in the t7;11 examples, and using an alpha-globin probe in the cases involving chromosome 16. This will also involve a more general screening of patients with erythroleukemia (several of which are in our clinic) for translocations involving chromosome 11 or 16 since these cytogenetic abnormalities have not been previously reported. In these cases, DNA libraries obtained from cells with the translocations will be screened using the relevant immunoglobulin or globin gene probes. The resultant clones will then be tested to see if they have material allowing in situ hybridization with the translocated chromosome other than the one the screening probe is assigned to. For example, in the case of the t7;11 translocation we will test to see if a clone selected with a beta globin probe normally on 11p2 contains material that now hybridizes to chromosome 7.

The analysis of these tumor/disease systems using already defined probes is exactly reminiscent of where the Burkitt's lymphoma work was approximately 1-1/2 years ago. From the above mentioned studies we hope to gain insight into the mechanism of chromosomal translocation. Currently our theory is that transcriptionally active sites become involved in chromosomal aberration.

2. Chromosome in situ hybridization: We expect to continue to utilize the tool of chromosome in situ hybridization as an aid in a number of investigations currently underway, in collaboration with other researchers.

A) For example, we will be analyzing the large cell transformants of the small cell lung cancer lines which have shown amplification and/or rearrangements of the myc sequences. Three of these 4 c-myc amplified lines have HSR regions on chromosomes other than 8 (the normal site of the c-myc gene). We will use the tritium or iodine labelled c-myc gene as a probe in these studies.

B) We are also involved in a collaboration with Jack Strominger and James Skare to analyze the nature and specificity of Epstein-Barr virus integration in four cell lines that carry a low copy number (1-2) of the Epstein-Barr virus genome as well as in a cell line of Dr. Ian Magrath which is Epstein-Barr virus negative, but Epstein-Barr nuclear antigen positive.

Title: THE REARRANGEMENT, DISPERSION AND TRANSLOCATION OF IMMUNOGLOBULIN GENES

I. Personnel

A. Senior Staff Fellows: Gregory F. Hollis, Ph. D. (Principal Investigator)
Ilan I. Kirsch, M.D., Ph.D.

B. Other Professional Staff: William Cox, Ph. D.

II. Collaborators

Dr. P. Leder, Department of Genetics, Harvard Medical School

III. Introduction

A. Objectives

To use the immunoglobulin gene system as a model to study changes that occur in DNA structure in normal and malignant cells. Specifically, to study what mechanisms are involved in duplication and dispersion of genetic information from one chromosomal location to another. Further, we are interested in examining, at the molecular level, the mechanisms involved in chromosomal translocations often associated with malignancy. We intend to approach this by identifying genes that have carried with them sequences that are normally involved in DNA recombination events.

B. Rationale, Background and Progress Report

We have used the immunoglobulin gene as a model system to study the fluidity of the human genome. In addition to rearrangements that occur normally in the formation of functional immunoglobulin genes, we have shown that these genes can be dispersed to other chromosomes or be involved in reciprocal translocations. The results of these findings are discussed below. From 1980-1982 Dr. Hollis was a Staff Fellow in the Laboratory of Molecular Genetics, NICHD, NIH. In July, 1982 he became a Staff Fellow of the NCI-Navy Medical Oncology Branch and because of the transition of the two laboratories (establishing the NCI-Navy molecular genetics group and the movement of Dr. Leder's lab to Harvard) he was assigned to the Department of Genetics, Harvard Medical School. During this period he has pursued the work given in this program report. While this arrangement is not routine, it has provided a unique opportunity for Dr. Hollis to continue his work and to develop skills to apply to NCI-Navy Branch projects. He begins full-time work at the NCI-Navy unit June, 1983.

Immunoglobulin light chain genes are encoded by three discrete DNA segments in the germline, the C or constant region gene, the V or variable region gene segment and the J or joining segment. All light chains of a particular class, kappa or lambda, share a common carboxyterminus, amino acids 1-97, and the J which encodes amino acids 98-109.

For a functional light chain to be produced, a somatic recombination event at the level of DNA must take place which brings one of many V regions next to a J segment. This recombination completes the formation of the variable part of an immunoglobulin light chain gene. By encoding the variable part of the light chain protein in two pieces, V and J, the number of different proteins that can be made is a product of the number of V and J segments in the germline. For light chains there are five tandemly duplicated J segments upstream from a single constant region.

We were interested in the structure of the human lambda light chain genes and whether a similiar expansion of J segments had occurred. We began by cloning the lambda constant region locus from a human DNA phage library. The lambda locus consists of a complex array of six lambda constant like genes. These six genes are evenly spaced along the chromosome, approximately 5000 base pairs apart. The genes are very similiar to each other, some differing by only a single amino acid. The structures of these genes suggest that they arose by duplication.

How many lambda J segments are there and where are they located?

We began our studies on the J segments by examining where rearrangements occurred in lambda producing B cells. The location of the rearrangements suggested that each lambda constant region gene had a J segment associated with it. We confirmed this by sequence analysis of the DNA upstream from lambda constant region 2. The lambda system, like the kappa, has expanded its V region repertoire by increasing the number of J's available for recombination, but the duplication unit in lambda includes both lambda J and constant region.

While work was progressing on the lambda locus, we discovered several other lambda-like genes that were not directly linked to the functional locus. We were able to demonstrate that one of these unlinked genes had moved from chromosome 22, the location of the active locus, to chromosome 19. We examined this unlinked gene, first by cloning and later by DNA sequence analysis. This dispersed gene showed a great deal of homology to a functional lambda light chain polypeptide. When we examined this dispersed gene further we found that the homology to lambda C ended abruptly ten nucleotides 3' to the polyA addition signal where the dispersed gene broke into a string of adenine residues. This run of adenines positioned approximately ten bases away from a polyA addition signal is characteristic of mRNA structure. That is, this dispersed gene appeared to have undergone one of the hallmark reactions of mRNA maturation. Was it possible that when this gene moved from chromosome 22 to 19 it had done so through an RNA intermediate? If so, we would predict that another reaction of mRNA maturation, RNA splicing, would have removed the intervening sequence that separates the J segment from the C region in the DNA. Sequence analysis of the 5' end of this dispersed gene demonstrated that the J had been joined to the C precisely in accord with the rules of RNA splicing. This dispersed pseudogene had undergone two of the processing reactions of mRNA maturation. Therefore, we suggested that when

it moved it had passed through an RNA intermediate, and thus referred to it as a dispersed "processed" gene.

The movement of genetic information from DNA to RNA and then back to DNA is not novel to this cellular gene, but rather is known to be a part of the life cycle of retroviruses. Retroviruses are RNA containing particles which replicate through a DNA intermediate. Because their life cycle involves an RNA phase, the genes that they code for do not have intervening sequences.

Retroviruses have been the subject of intense study because particular types are efficient agents of transformation. These viruses carry a transforming oncogene as part of their genome. These virally carried oncogenes have normal cellular counterparts which differ in structure from the viral gene because they have intervening sequences. It has been proposed that the transforming retroviruses have incorporated a cellular oncogene into their genome, and as the virus moved through its RNA phase the intervening sequences were lost. The similarity of the structure of the oncogene carried by the retrovirus and the dispersed processed gene is striking. We are interested in determining whether dispersed processed genes are a product of a virally mediated event or are created by a normal cellular process in which genetic information can flow "backwards" from RNA to DNA.

This can be approached in two ways. First, if processed gene dispersion is virally mediated, we would expect the DNA sequences flanking the processed gene to contain virus sequences. We can test this directly by cloning the flanking DNA and examining its structure. Second, if processed gene dispersion is mediated by cellular systems, we would expect cells to be able to carry out the reactions that would allow RNA to be converted to DNA and inserted into the genome. By introducing intermediates along this pathway into the cell, we can determine the cell's ability to carry out the subsequent steps.

Recently, an important relationship between the transforming retrovirus avian myelocytomatosis virus and its oncogene *v-myc* and the human disease, Burkitt's lymphoma, has been established. Burkitt's lymphoma cells have characteristic reciprocal chromosomal translocations that may be critical to their malignant transformation. These translocations involve the chromosomal segments that encode the immunoglobulin genes and chromosome 8 band q24. This band of chromosome 8 contains the cellular homologue, *c-myc*, of the avian transforming gene *v-myc*. From studies done with cloned fragments from Burkitt's lymphomas, it has been demonstrated that in these cells the 8;14 translocation has joined the Ig heavy chain locus to *c-myc*. An analogous situation has been described in mouse plasmacytomas which contain a 12;15 translocation.

In all translocations described to date at the molecular level, the translocations involve a head-to-head joining of the heavy chain locus with *c-myc*. The exact location of the crossover varies, but an area 5' to IgH normally involved in DNA recombination events, called the heavy chains switch, has joined to an area 5' to *c-myc*.

The two other characteristic translocations in Burkitt's lymphoma, 2;8 and 8;22, occur at the chromosomal segment containing the kappa and lambda genes, respectively. While the 8;14 translocation involves the heavy chain class switch, it is unlikely that a switch sequence would be involved in the 2;8 and 8;22 translocations. This is because no switch sequence has been described that is directly associated with these genes. In fact, we have just cloned a 8;22 translocation from a Burkitt's lymphoma cell line and preliminary evidence suggests that the translocation did not occur in a switch-like sequence. The nature of the recombination event is being pursued at the DNA sequence level.

If the heavy chains translocate in their switch-like sequences, what type of sequence from the light chain might be involved in translocation? Light chains normally undergo DNA recombination events in cells of the B-cell lineage. These recombinations form functional variable regions by joining V to J. It is possible that the DNA sequences that direct V-J joining may mediate chromosomal translocations. If so, we would expect that these sequences would be present at the point of translocation. This can be directly tested by cloning the DNA containing the translocations and determining its nucleotide sequence along with its normal cellular counterpart. What sequence would be an efficient partner for a translocation involving the light chain? The normal partner for the DNA recombination event involving J segments is a germline

V. These DNA rearrangements are intrachromosomal events. However, we have shown that in the case of lambda genes, at least one joining segment and constant region have moved to another chromosome and, at least in theory, could function as a target for an interchromosomal rearrangement between a normal lambda V on chromosome 22 and this dispersed J on chromosome 19. If we extend this theme one step further, it is possible that lambda and kappa V regions are also dispersed to other chromosomes. If this were true, they could also serve as partners in DNA recombination events that would produce translocations. We would like to explore this possibility by examining the chromosomal locations of kappa and lambda variable region genes. Further, if we find these genes are dispersed, we intend to clone these genes and determine their structure. With the cloned genes available we can ask whether these genes are involved in specific chromosomal translocations.

IV. Specific Aims and Future Plans

We have cloned a lambda light chain gene that has been dispersed from the active locus on chromosome 22 to chromosome 19. DNA sequence studies of this gene have revealed that the extent of homology to a functional gene is restricted to a DNA fragment approximately 750 basepairs in length, which includes all of the coding region. We plan to examine by restriction mapping, Southern blotting, and DNA sequence analysis the DNA that flanks this dispersed gene. We wish to determine if this flanking DNA is important in the dispersal

of this gene and whether similar sequences may be involved in the movement of other genes. To this end, we have now cloned four other lambda-like genes that are not directly linked to the function locus. We plan to assign them to chromosomal locations, and determine their fine structure to compare their features to the dispersed processed lambda gene. By comparing these five lambda-like genes and their flanking sequence, we hope to identify areas that are involved in their movement.

These studies involve one gene family, the human lambda constant region. We wanted to determine whether other multi-gene families might have also undergone dispersion. To determine this we have cloned a human variable region gene, and we intend to use it as a probe to look for dispersed lambda variable region genes.

After we have established the chromosomal location of the cloned lambda-like genes we plan to determine whether they are targets for chromosomal translocations. This can be done by correlating the position of these genes with already described translocations. Direct participation in the chromosomal translocation can be assessed by probing genomic blots of cells containing translocations with specific dispersed gene probes. If translocations do involve these dispersed genes, the translocation breakpoints will be cloned and their nucleotide sequence determined.

In addition to describing the structural features of genes that have moved, we intend to investigate the mechanisms involved in their movement. We have proposed a model for gene dispersion that involves priming an RNA, converting this RNA to a DNA copy by reverse transcription, and introducing this copy into a new chromosomal location. We intend to study the model by introducing specific intermediates of the pathway into the cell and examining the cell's ability to carry out subsequent steps in the pathway.

In summary, we wish to examine the structure of genes that have moved, the mechanism by which they have moved, and determine whether dispersed genes play a role in chromosomal translocations.

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Title: ORGANIZATION AND EXPRESSION OF CELLULAR ONCOGENES IN HUMAN
LUNG CANCER CELLS

I. Personnel

- A. Permanent Senior Staff: J.D. Minna, A.F. Gazdar, D.N. Carney
- B. Medical Staff Fellow: C. Little
- C. Other Professional Staff: I. Kirsh, G. Hollis
- D. Technical Staff: M. Nau

II. Collaborators/Collaborating Branches

- J. Biedler, Memorial Sloan-Kettering; J. Trent, University of Arizona;
- U. Rapp, NCI LVC, M. Perucho, SUNY; E. Scolnick, Merck; E. Chang, USUHS;
- G. Stark, Stanford.

III. Introduction

A. Objectives

To study the organization and expression of cellular oncogenes in human lung cancer including lung cancer cell lines and direct patient tumor samples.

B. Rationale and Background

Recently there has been an explosion of information concerning the nature, status, and expression of cellular genes related to the retroviral transforming genes (cellular oncogenes, or c-onc). While nearly 20 such genes have been identified and shown to be highly conserved in evolution, three have been strongly implicated in human tumors. These include the c-Harvey-ras gene isolated from human bladder cancer (1), c-Kirsten-ras (ras-2) isolated from human lung and colon cancers (2), and c-myc translocated and often rearranged in Burkitt's lymphoma (3) and other B cell lymphomas and amplified in a promyelocytic leukemia and colon tumor (4) (5).

The information that these oncogenes play a role in the malignant process comes from two sources: first, their viral counterparts, the v-onc genes are able to cause tumors; and second, they exist sometimes in an altered fashion in human tumors or tumor cell lines as detected by transfection studies, DNA blot analyses, or direct sequencing (7). Their analyses in human tumors was made possible by the molecular genetic approaches and the development of mammalian cell transfection technology. Since they exist in normal as well as malignant cells, there are two basic possibilities for their role in malignancy: either a mutation of the structural gene leading to a mutant gene product, or a change in the expression of the product in terms of amount of or timing from the normal status. In fact, both of these mechanisms probably occur.

There are currently four general methodologies for approaching the role of c-oncogenes in human lung cancer. The first involves transfer of DNA or chromosomes from a lung cancer cell to a non-malignant cell (such as mouse NIH3T3 cells in the DNA transfection assay) and selection for a malignant mouse cell (i.e. transformed focus) caused by the presence of specific human genes. Such an approach has led to the identification of the k-ras-2 gene as a lung/colon oncogene. The second involves using cloned molecular c-oncogene probes to look at lung cancer DNA and chromosomes for rearrangements and/or amplification of c-oncogenes. No such changes have yet been reported in lung cancer. The third involves using cloned molecular c-oncogenes probes to look at mRNA for expression of these genes and changes in either the amount or size of the message. No such changes have been reported as yet for lung cancer. The fourth involves using antibody precipitation and similar techniques to look at the c-oncogene protein product for change in amount or type. Altered types of ras-related protein products (p21 protein detected by a monoclonal antibody) have been reported in lung cancer cells able to donate transforming DNA in the NIH3T3 transfection assay (6). A fifth but related approach is to directly isolate c-oncogenes from lung cancer cells or nonmalignant cells transfected by lung cancer DNA and determine any sequence differences in the lung cancer genes. No such changes have yet been reported.

C. Specific Aims and Outline of Methodology

1. Using already cloned c-oncogene and v-oncogene probes to test the status of the c-Har-ras, c-Ki-ras, and c-myc oncogenes in lung cancer cell lines, i.e., to look for rearrangements and amplification using restriction enzyme digestions and Southern blot hybridization analyses.
2. Using the same probes to look for expression and status of c-oncogene messenger RNA in lung cancer cell lines using "Northern" blot hybridization.
3. Using monoclonal antibodies against the oncogene product(s) to look for changes in level or type of oncogene product.
4. Using calcium phosphate DNA transfection and somatic cell fusion technology to isolate NIH3T3 transformants following exposure to lung cancer DNA and subsequent study of the transformants using Southern blot analyses to determine the presence of human genes and c-oncogenes.
5. To relate any changes found in the above studies to the histologic type or biologic behavior of lung cancer, and to see if they occur in normal tissue from the same patient as well. While the first studies will be done with cell lines, studies will be confirmed with direct samples from patients.

D. Methodology

1. DNA organization: Preparation of DNA; digestion of DNA using restriction endonucleases; Southern gel blotting; labelling of oncogene probes and other DNA probes with ^{32}P by nick translation; hybridization of digested and blot transferred DNAs.
2. RNA expression: Preparation of RNA; "Northern" gel blotting; nick translation and hybridization as described above.
3. Preparation of oncogene probes: Growth of specific oncogene-containing plasmid; preparation of DNA from the plasmid; isolation of specific oncogene DNA fragment using restriction endonuclease digestion, agarose gel electrophoresis and removal of fragment from the gel.
4. Protein expression: Immune histochemical studies using biotin avidin peroxidase technology with anti-oncogene product antibodies of lung cancer cell lines with ^{35}S methionine and then SDS PAGE analysis with and without immunoprecipitation using monoclonal antibodies.
5. Transfection analysis: Isolation of high molecular weight DNA; preparation of a calcium phosphate DNA precipitate; transfection into NIH3T3 cells and selection for transformed foci. Propagation into NIH3T3 cells and testing of such foci for growth in soft agarose, tumorigenicity in nude mice, presence of human repetitive sequences using a "Alu" probe (BLUR8) or nick translated human DNA, and then cloned c-oncogene probes.
6. Chromosomal analysis: Using nick translated cloned c-oncogene probes in situ chromosomal hybridization (see section of Dr. I. Kirsch).

IV. Progress Report

A. DNA Studies

Using the cloned probes we have found no obvious amplification or rearrangements of c-Har-ras and c-K-ras oncogenes from 16 different lung cancer DNAs. In contrast, we found a 4 to 32-fold amplification of the c-myc oncogene in six lung cancer cell lines (from four different tumors). The amplification involves the authentic, non-rearranged c-myc gene (i.e., 12.5 kb Eco R1 fragment). We found a 2 to 4-fold amplification of the authentic c-myc genes. These include a new 14 kb and a new 16 kb fragment both amplified for c-myc and novel, unamplified 2 kb c-myc bands in three other lung cancers (all Eco R1 gene fragments).

B. RNA Expression

The cell lines greatly amplified for the c-myc oncogene express increased (10-fold or greater) amounts of c-myc RNA compared to controls. Another tumor lacking c-myc oncogene amplification expresses elevated amounts of c-myc RNA intermediate between control cell lines and cell lines with c-myc amplification. All of the cell lines, both unamplified and

amplified for the c-myc oncogene, express the same size mRNA (i.e., 2.7 kb) corresponding to previous reports.

C. Protein Expression

In collaborative studies with Dr. Edward Scolnick (formerly NCI, now Merck Research) lung cancer cell lines have been studied for immunoprecipitable ras p21 related proteins using a broadly reactive rat monoclonal anti-ras antibody. At least some of these lines (predominantly non-small cell) have been found to have abnormal p21 proteins. These in turn have been studied for mRNA expression by Dr. Chang.

D. Transfection Studies

Nine different lung cancer DNAs have been studied by us and six different DNAs (part of the same group) have been tested by Dr. Manuel Perucho (SUNY) for their ability to transform NIH3T3 cells to malignant behavior, as well as to donate active TK genes to LTK-Cells. In addition, we tested control DNAs including NIH3T3 DNA, B lymphoblastoid DNA from a small cell lung cancer patient, T lymphoma DNAs, cloned v-mos DNA (pMI from Dr. G. Vande Woude, transfected v-Ha-ras cellular DNA (from Dr. Perucho), and cloned PTK DNA.

We have found the following: five different lung cancer DNAs (including 3 SCLC lines, 1 variant SCLC line, and 1 adenocarcinoma) have been able to transform NIH3T3 cells. These transformed NIH3T3 lines form colonies in soft agarose and tumors in nude mice. The efficiency of transfection range from 1-20 colonies per 100 ug of DNA for both NIH3T3 cells, and LTK-Cells (transformed foci and TK+ clones, respectively). When tested for the presence of human sequences with the BLUR8 probe, the TK transformants contained abundant human DNA sequences. In contrast DNA from only one set of NIH3T3 transformed foci contains ALU sequence, and in this case the ALU sequences were contained in a 5-6 kb fragment. The transformed clones were then probed with a v-Ki-ras clone (pKBE-2 from Dr. E. Chang). With Eco RI digestion, three bands corresponding to the authentic mouse Ki-ras bands were seen for NIH3T3 cells and the transformants. In addition, nearly all of the transformants had new ras bands appearing. These bands appeared to be of similar size to those previously reported in human DNA, or Ki-ras transfectants. One of these included the ALU.

E. Relation to Biologic Behavior

The findings of high levels of c-myc amplification was correlated with variant types of SCLC. In all cases, the cell lines harboring greatly amplified c-myc genes were: more clonogenic, more tumorigenic in nude mice, grew rapidly, and had lost expression of L-dopa decarboxylase, peptide hormones and neurosecretory granules characteristic of "classic" SCLC. All of these lines are also more radioresistant (by virtue of an increased "shoulder" on the radiation survival curve) than the "classic" SCLC lines. Three of the four variant lines have been studied

karyotypically and all have either double minute chromosomes (DMs), or homogeneous chromosomal staining regions (HSRs). The three cell lines with minimal c-myc amplification (2 to 4-fold) are all adenocarcinomas (NSCLC).

V. Future Plans

1. Continue to obtain various c-oncogene probes and test the lung cancer cell lines for amplification, rearrangement, and expression of the c-oncogenes in the lung cancer cell lines. Genes of particular interest would be those residing on chromosome arm 3p, the site of a deletion in SCLC. One current candidate is the c-raf oncogene, recently assigned to chromosome 3 (U. Rapp, unpublished). Currently we are collaborating with Dr. Rapp and his group in studying the c-raf oncogene status in lung cancer cell lines.
2. The amplification of the c-myc gene is striking. We plan to look for this amplification in fresh tumor specimens, particularly of variant SCLC types and in normal tissue specimens. The finding of this amplification in a direct patient tumor specimen would allow important questions to be asked at the DNA, RNA, and protein levels of these amplified lung cancer cell lines. In collaboration with Drs. J. Biedler (MSKCC), and J. Trent (Univ. of Arizona), we (Dr. I. Kirsch) are performing in situ chromosomal hybridization studies to determine the chromosomal placement of the amplified gene (including its relationship to the HSR or DM bodies) and whether a translocation has occurred (quite likely in the HSR containing lines). We are studying the size of the amplification unit with various restriction enzymes and using subcloned probes of the human c-myc gene to determine how much of the c-myc gene is involved in the amplification event and with what fidelity the amplification occurred. It appears that a very large fragment has been amplified. However, we already know that several types of c-myc rearrangements can take place in both the amplified and unamplified cell lines. These same c-myc subprobes can identify the portions of the genes involved in these events as well. We are particularly interested in the novel, small 2kb Eco RI fragment found now in the three independent tumors. Rearranged fragments that are found to have interesting changes will be cloned for further study.
3. At the RNA level we wish to know if the increased expression of the message in both amplified and unamplified cell lines is a normal transcript or not. We will approach this again using specifically defined c-myc probes, Northern blots, and S1 nuclease mapping. We note the possibility that the lines expressing large amounts of c-myc amplification may have a novel promoter region or may have a different structural gene.
4. At the protein level we would like to demonstrate an amplified c-myc protein. While this can be approached with metabolic labelling, clear answers await the use of specific antisera/monoclonal antibodies against

c-myc. If we can obtain these from other groups we will. If not, we may have to try to make our own anti-myc reagents. The amplified lines are one source of c-myc protein for immunization as it is reported anecdotally that many c-myc derived synthetic peptides are not efficient in generating antibodies. Biologic reasons for our group being interested in these protein studies is the nuclear localization of c-myc product and the radioresistance and aggressive biologic nature of the c-myc amplified lung tumors. Such antibodies could be used to type tumors directly in patients. In addition, they could be used to ask if a mutant c-myc product is present by immunoprecipitation studies.

5. Cloning of c-myc from amplified lung cancer lines.

In collaboration with C. Clayton and G. Stark (Stanford University) c-DNA clones from an amplified c-myc cell line have been prepared. A c-DNA library of line NCI-N417 (greatly amplified for c-myc) was prepared by Dr. Clayton and screened using a v-myc probe. Approximately 1/1000 of the phage contained c-myc. These c-myc containing phage are being sent to us for characterization. They provide another tool for studying the amplification event.

6. The study of other variant cell lines and early passages of the c-myc amplified lines will answer questions concerning the timing and generality of the c-myc amplification event.

7. The transformation studies will continue to determine if transformation is a rare or a common event with lung cancer DNA from different kinds of tumors (i.e., SCLC, NSCLC, etc.). In addition, we will use DNAs from normal tissue from the same patients.

Transformants already in hand that contain novel Ki-ras reactive fragments will be studied. The major question is whether the new ras fragments represent human or mouse Ki-ras genes. We are approaching this using cloned human c-Ki-ras gene subprobes (provided by Dr. E. Chang) to distinguish Ki-ras-1 from Ki-ras-2, and human from mouse ras sequences. In addition, it is possible the fragments are related to the recently described N-ras-gene from human neuroblastoma (8), or an as yet undescribed ras-related gene. We note recent work by Dr. Chaganti (MSKCC) demonstrating in situ hybridization ras-related sequences on human chromosome 3p (personal communication).

8. The ras protein studies will be continued to define whether or not abnormal p21 is found in lung cancer cells and whether this is related to the ability to transform NIH3T3 cells and express high levels of Ki-ras RNA.

VI. Publications

1. Amplification and Expression of the c-myc Oncogene in Human Lung Cancer Cell Lines. Little, C.D., Nau, M.M., Carney, D.N., Gazdar, A.F., and Minna, J.D.: Nature, submitted.

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Title: STUDIES ON THE GENETICS OF LUNG CANCER

I. Personnel

- A. Permanent Senior Staff: J. Minna, A. Gazdar, D. Carney
- B. Clinical Associates/Medical Staff Fellows: None
- C. Other Professional Staff: I. Kirsch, G. Hollis
- D. Technical Staff: M. Nau, S. Stephenson

II. Collaborators

J. Whang-Peng, Medicine Branch; J. Mulvilhill, Clinical Genetics, NCI;
J. Biedler, Sloan Kettering Cancer Center.

III. Introduction

A. Objectives

To define the genes in lung cancer cells responsible for their malignant behavior.

B. Rationale and Background

An understanding of lung cancer will ultimately require identification and characterization of the genes in lung cancer cells responsible for their malignant behavior. The changes found in such genes will provide insight into the results of mutagenic agents such as cigarette smoking and environmental pollutants on the bronchial epithelium. They should also indicate whether or not there are predisposing genetic changes in patients which allow them to develop lung cancer.

Despite the common occurrence of lung cancer there is little evidence that lung cancer occurs in families. In fact, very few families with lung cancer have been reported (1). This could be explained by both a recessive nature for lung cancer and the requirement for multi-step events in carcinogenesis. For example, one reasonable hypothesis is that there is an inherited recessive defect carried predominantly in the heterozygous form in the population. When people who have this defect smoke, some of them will develop mutations or deletions in the corresponding normal allele. This second event exposes the mutant gene in a homozygous fashion so that its mutant phenotype can be expressed. Possible models for this are retinoblastoma and Wilm's tumor (2). Other events are also likely to be required either for mutant gene expression or tumor progression. Candidates for these other genes include: the cellular oncogenes; peptide hormones which could act as autocrine growth factors; or genes switching on or off various programs of development and differentiation. With development of clinical disease comes the need to control tumor growth and the manifestations of tumors in primary, metastatic sites, and paraneoplastic syndromes. Here genes for drug and radiation resistance

and genes controlling the production of peptide hormones produced by the tumors that have biologic activity become important.

Previous work by our group has approached the question of the genetics of carcinogen metabolism. Using standard somatic cell hybrid techniques, we were able to assign a gene for aryl hydrocarbonhydroxylase (AHH) to human chromosome 2 (3). This mixed function oxidase plays an important role in metabolism of benzopyrenes. However, the metabolism of carcinogens could be carried out by cells other than the final target of the carcinogen. Other work by our group had looked at the expression of human genes regulating retrovirus DNA polymerase (reverse transcriptase), and primate (M7 baboon) retrovirus replication insomatic cell hybrids. (31,32). We stopped these earlier studies because of the lack until recently of "clean" assays to pursue their molecular genetics. With the new information on cellular oncogenes, our finding a chromosomal deletion (3p) in small cell lung cancer, and the new information on cellular oncogenes, we have turned to study the genetics of lung cancer cells directly.

The cell biologic studies reported in other sections of this report by Drs. Gazdar and Carney provide a rationale and background for many of the genetic studies. Namely, it is now possible to grow lung cancer cells in vitro and study their biology, patterns of differentiation, production of peptide hormones, biochemistry, cytogenetics, and response to therapy. We now know there are a series of biochemical markers (e.g., creatine kinase BB isozyme, neuron specific enolase, neurosecretory granules, L-dopa decarboxylase, the entire array of membrane and cytoskeletal proteins and glycolipids defined by 2-D gel and monoclonal antibody analysis), peptide hormones (e.g., bombesin, ACTH, calcitonin, AVP), autocrine growth factors (e.g., bombesin, AVP, and other uncharacterized peptides) which distinguish lung cancer types. In addition, we know that there are "variant" cell lines which either do not express the full pattern of differentiated features characteristic of a lung cancer cell type (e.g., best studied in small cell lung cancer and its variants), or which appear to be able to differentiate along two separate pathways (e.g., small cell and epidermoid carcinoma).

One direct approach is to be able to identify chromosomal abnormalities. Cytogenetic studies by our group in collaboration with Dr. Whang-Peng have revealed a chromosomal defect (deletion 3p) associated with many, if not all, small cell lung cancers (4,5). This deletion was not found in normal cells from the same individuals and no translocation of this region has yet been identified. This acquired deletion suggests a recessive defect. In addition, these same studies show double minute chromosomes (DMs) and homogeneously staining chromosomal regions (HSRC's) in some lung cancer cells suggesting the presence of gene amplification (5,6). These amplifications could be related to therapy resistance, growth advantage, malignant behavior, or unrelated to the biology of the lung cancer cells.

While the cytogenetic results showing a 3p deletion need confirmation, there are other cytogenetic data suggesting that this region of chromosome 3 plays a role in malignancy. These are summarized in Table 1. Some familial tumors have translocations into 3p including areas with known oncogenes. In addition, the transferrin gene and its receptor are located on chromosome 3. Because of the recent evidence of a transferrin-like sequence and its relationship to a transforming gene (29), the transferrin locus is a candidate for change in small cell lung cancer. All of these findings taken together indicate we should proceed with the study of the 3p chromosome region.

Studies of DNA transfection from lung cancer cells have implicated the Ki-ras-2 gene as a dominant cellular oncogene in human lung cancer (7-14). The Ki-ras-2 gene has been assigned to chromosome 12 (15). However, studies of cell hybridization of lung cancer cells by our group have, in at least one case, revealed suppression of malignancy despite the presence of chromosome 12 (16). Finally, cloning and immunohistochemical studies have revealed the great degree of heterogeneity that exists within individual lung cancers (9). This, and the host of other data from hybrid cell studies, suggests that malignancy may commonly be a recessive rather than dominant defect (23 for summary). In addition, the del 3p of small cell carcinoma and the difficulty in transfecting transforming genes from lung cancer cells (only successful in 20% of cases or less in the literature)(7,10, our lab, and Perucho, personal communication) suggests that the majority of lung cancer cells may carry a recessive rather than dominant genetic defect. All of these results demonstrate the potential complexity of the genetics of lung cancer.

The recent expansion of knowledge about cellular oncogenes, including their structure, rearrangement, amplification, chromosomal location, and involvement in translocations provides a direct approach to the lung cancer gene problem (11,16,18). This is covered in detail in the preceding project report. This report focuses on approaches to the study of the 3p deletion.

C. Specific Aims and Outline of Methodology

1. Organization, expression, structure, amplification and chromosomal location of cellular oncogenes in lung cancer cells is a specific aim and is covered in detail in the preceding report and the report by Dr. Kirsch on molecular cytogenetics. Here we would add the following specific aims:
 - a. Using lung cancer cell lines with the del 3p to determine if the cytogenetic changes are associated with changes in the structure or expression of cellular oncogenes assigned to the chromosomal region involved.

We are particularly interested in finding cellular oncogenes which are located in the smallest detected region of 3p (14-23) found in small cell carcinoma. The most direct approach will be with the c-ras gene (assigned to chromosome 3) and the newly described c-ras associated genes detected on chromosome 3p by in situ hybridization (20, and personal communications Rapp, Chaganti).

- b. To study the expression of c-oncogenes in hybrid cells made between lung cancer cells expressing particular c-oncogenes and mouse cells and relate this to the malignancy and pathway of differentiation of the hybrids.

The methodology used for these studies is given in the preceding reports.

2. Cytogenetic studies of lung cancer cells. To identify new lesions and confirm the already described specific cytogenetic changes (del 3p SCLC) in lung cancer cells using chromosome banding techniques. These include continuing studies of small cell lung cancer, and analyzing our non-small cell lung cancer material. The methodology used is trypsin Giemsa banding as carried out by cytogenetic labs expert in this area such as Dr. J. Whang-Peng (NCI) and Dr. J. Biedler (MSKCC).
3. To develop molecular genetic markers for the chromosome 3p deletion using cloned DNA restriction fragment polymorphism probes, and other probes for genes assigned to chromosome region 3p.

The methodology here involves obtaining cloned DNA restriction fragments which have been shown to be polymorphic (vary among individuals) and assigned to chromosome 3 (17,18,19). Normal cells from a patient are studied with the probe and those demonstrating both alleles (i.e., heterozygous) are selected for tumor cell study. This patient by definition is heterozygous at the locus defined by the restriction fragment probe. The tumor cells of this patient are then studied with the same probe. If one of the two alleles is absent, there is then proof at the molecular level of a deletion involving this chromosomal region. We note that this approach is similar in concept to the use of esterase D analysis for defining genetic linkage in human retinoblastoma (21,22). The technology for these studies is the same as in the preceding section. We are carrying these out in collaboration with Dr. S. Naylor (Roswell Park Memorial Institute) who has assembled a large number of polymorphic restriction fragment probes assigned to chromosome 3 (17,18, 19).

4. Attempt to correct the defect in small cell carcinoma (del 3p) with genes from non-malignant human, mouse or rat cells using somatic cell hybrid and DNA transfection technology. In addition, to study the expression of malignancy in hybrid cells formed between non-malignant cells and lung cancer cells. Somatic cell hybrid and DNA transfection methodology will be used.

IV. Progress Report and Future Plans:

1. Project 1 - Cellular Oncogenes in Lung Cancer Cells

These studies are reviewed in detail in the previous report. Dr. Rapp has so far found no obvious change in the structure or amplification of the c-raf gene in our lung cancer cells, but these studies are early and we are just starting to prepare the c-raf probe for our own use for in situ hybridization and DNA/RNA blotting studies. In addition, Dr. Kirsch and Dr. Biedler are just starting in situ hybridization studies with various ras probes to see if any activity can be detected on 3p.

2. Project 2 - Cytogenetics of Lung Cancer Cells

In collaboration with Dr. J. Whang-Peng we have been studying the cytogenetics of lung cancer cells with the chromosome banding studies (4,5,6). Until we began this work there was only very limited information on the banded karyology of lung cancer cells. This was primarily because of the difficulty in obtaining good metaphase preparations with which to work with. Thus, our laboratory's ability to grow lung cancer cells both in short- and long-term culture provided material previously not available for chromosome banding studies. So far, we have found a consistent defect in human small cell lung cancer including short term cultures, established cell lines, and direct tumor preparations (from tumor contaminated bone marrow). Namely, a deletion involving a greater or lesser part of chromosome 3p (4,5). The defect is found in all aneuploid (tumor cell) metaphases but not in diploid cells. However, we would stress the following features: the size of the defect can vary from a small interstitial deletion to loss of an entire chromosome; shortest region of overlap analysis shows the common deleted region to be 3p (14-23)(5); all of the metaphases contain at least one intact chromosome 3; some have been noted. However, since this is an interband region, such a translocation could be missed; finally, no confirmatory reports of this lesion have been published. Because such changes are complex, we feel the first order of business is to have the results confirmed by another cytogenetics laboratory. In this regard, Dr. Whang-Peng has met with Dr. Wurster-Hill of Dartmouth (another major center studying the biology of small cell lung cancer) and they have compared cytogenetic notes. Suffice it to say, that at present there is a difference of no translocations of this part of chromosome 3 to another chromosome between the two cytogeneticists. However, a 3p deletion can be found in some small cell lung

cancer lines from Dartmouth, but the number of such lines and the possibility that other samples may lack the 3p deletion is a matter of controversy. To help resolve this, we have turned to another cytogeneticist, Dr. J. Biedler (MSKCC) who will study several of our small cell lung cancer lines for their chromosome banding status. We are trying to give Dr. Biedler tumors with as near diploid chromosome number and DNA content as possible to facilitate this examination.

In addition, we have had the opportunity to study an interesting patient who developed erythroleukemia after treatment for small cell lung cancer (30). In this patient, the erythroleukemia cells had a 3p deletion (30). If other examples such as this can be obtained, it would suggest the exciting possibility of several tissue types becoming malignant with deletion of 3p.

Dr. Whang-Peng has done a considerable number of banding studies on our non-small cell lung cancer lines (which appear not to have the 3p deletion found in small cell lung cancer), and the results of these studies are being analyzed.

3. Project 3 - Development of Molecular Genetic Markers for the 3p Deletion

In collaboration with Dr. S. Naylor (RPMI) we have studied two pairs of small cell lung cancers and B lymphoblastoid cell lines from the same patients for one chromosome 3 assigned polymorphic restriction fragment probe D3S1 (17,18,19). One of the patients was not heterozygous for the gene and, thus, was not informative. However, the other patient was. The B lymphoblastoid cells showed two bands on the Southern Blot after appropriate restriction endonuclease digestion. Of great interest, the small cell lung cancer showed only one band. Thus, one of the two alleles had been deleted in this tumor. This is of importance because this tumor (NCI-H128) has a karyotype with three normal appearing chromosome 3's, as well as the deleted 3's. Thus, this analysis shows that the normal appearing chromosome 3's all came from the parent. We are highly encouraged by this preliminary result and are continuing this work in collaboration with Dr. Naylor.

4. Project 4 - Correct the defect in human small cell carcinoma (del 3p) with normal mouse, rat, or human chromosomes and study the expression of malignancy in lung cancer and normal rodent and human cell hybrids. Previously, we have shown that one lung cancer line (A549, an adenocarcinoma) when fused to 3T3 cells was suppressed for malignancy (16). We have plated these hybrids in soft agarose and some of them yield colonies at low efficiency. These colonies were picked and tested for tumorigenicity in nude mice. However, only a few were tumorigenic. We have started to approach this problem again by fusing lung cancer cells to non-malignant mouse and rat cells. The selection assay for dominant

expression of malignancy is either focus formation or growth in soft agarose, and ultimately tumorigenicity in nude mice. Unfused human cells can be removed with ouabain. The impressive problem is that it has been almost impossible to make viable, continuously replicating hybrid cells in these crosses. Of interest, the same lung cancer cells fused to malignant mouse, rat or Chinese hamster cells readily form viable, replicating hybrids. Thus, there seems to be factors in normal cells which are lethal for the replication of lung cancer cells.

We are continuing this project in the following ways: first, to continue the attempts to make viable hybrids using other nonmalignant rodent and human cell lines and freshly established rodent embryonic cells; second, to isolate mouse chromosomes in microcells that have only one, or at most a few, mouse chromosomes using standard techniques (28). We will then fuse these to the human lung cancer cells. Hybrids formed in this way may have a better chance of being viable. Specifically, we would like to test if a specific mouse or human chromosome could suppress malignancy in a human lung cancer cell, particularly small cell lung cancer. In particular, we would like to know if human chromosomes or the mouse homologue of human 3p could correct the malignant defect. Currently, mouse chromosome 9 is the leading candidate for such a homologue (Table 1). Because of the conservation of linkage groups that occurred in evolution, it is predicted that the mouse homologue of human 3p should contain the gene(s) deleted from 3p in small cell carcinoma. Besides cytogenetic analysis, there are several biochemical markers that will allow detection of mouse chromosome 9 in the hybrid cells. DNA restriction fragment probes will allow distinction of different human chromosomes (Table 1). Thus, we can test if a normal copy of these genes (donated by a mouse or human) can correct this defect. Previously, our lab had played a major role in the mapping of mouse genes using somatic cell hybrids, and so we are very familiar with the comparative gene mapping area and its technology.

If any mouse chromosome contains genes that would suppress malignancy of lung cancer, then subsequent rounds of transfection studies could be used to isolate the responsible mouse genes. Because the hybrid cell and microcell mediated chromosome transfer studies would require testing of only a small number of cell lines for suppression of malignancy, they could be screened using standard techniques. However, if transfection technology is required, then some form of selection or the use of a shuttle vector would be required. However, the first thing to discover is if such suppression can take place. Once this is shown, the isolation of the normal gene can be contemplated, picked, grown up and tested for malignancy in nude mice.

TABLE 1

GENES ASSIGNED TO HUMAN CHROMOSOME 3

<u>Genes Including Enzymes and Others</u>	<u>Region on Human Chromosome 3</u>	<u>Reference #</u>
Aminoacylase-1 (ACY1)	p21	Mouse 9 (17, 18, 19)
Beta-galactosidase-1 (GLB1)	p21 to q21	Mouse 9 "
Glutathione peroxidase-1 (GPX1)	p13 to q12	"
Transferrin		Mouse 9 "
Transferrin receptor		"
Somatostatin (SST)	q28	"
AF8T Temperature Sensitivity Complementing (AF8T) Restriction		
<u>Fragment Polymorphisms</u>		
D3S1 (D3S1 DNA segment)	q12	(17, 18, 19)
D3S2 (p12-32 DNA segment)	q21 to qter	"
D3S3 (1-37 DNA segment)	pter to cen.	"
<u>Cytogenetic Abnormalities</u>		
Human small cell lung cancer (del 3p)	p14-23	(4, 5)
Hereditary renal cell carcinoma t(3;8) (p21; q24)	p21	(24)
Familial retinoblastoma with insertional translocation (t3p; 13q)	p12	(25)
Familial renal cell with 3p; 11p translocation in tumor cells	3p	(26)
Retinoblastoma HSR insertion (1p to 3p)	3p	(27)

VI. Publications (*) and References
 (* Indicates publications of our group)

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2. Knudson, A.G.: Contribution and mechanisms of genetic predisposition to cancer: hereditary cancers and anti-oncogenes. Proceedings of the 13th International Cancer Congress, September 1982, Seattle, Washington (in press).
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- 23b. Stanbridge, E.J., Der, C.J., Doersen, C.J., Nishimi, R.Y., Peehl, D.M., Weissman, B.E., Wilkinson, J.E.: Human cell hybrids: analysis of transformation and tumorigenicity. *Science* 215:252-259, 1982.
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06811-01

P

PERIOD COVERED

October 17, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Controlled Trial of Adjuvant Chemotherapy in the Treatment of Osteosarcoma

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Angela Miser, Visiting Fellow, Pediatric Branch, NCI

COOPERATING UNITS (if any)

Surgery Branch, NCI; Pediatric Oncology Group, Gainesville, FL

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.2

PROFESSIONAL:

1.0

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Osteosarcoma, a malignant bone tumor, occurs most commonly in the second and third decades of life.

Treatment of localized disease with amputation alone has historically resulted in a long-term survival of approximately 20%, although over the past ten years there appears to have been an increase in survival rates such that figures of 40 to 50% five year survival are now being projected. Although several chemotherapy agents have been found to cause tumor stabilization or regression in patients with metastatic disease, the benefit of these drugs in the adjuvant setting, ie., after surgical removal of a localized tumor, is much debated.

The purpose of this study, which is being conducted in a multiinstitutional setting, is to evaluate the efficacy of adjuvant chemotherapy, using all the currently known "first line" drugs with activity against osteosarcoma, in children with localized tumor of the extremity who have been rendered disease-free by amputation or a limb-salvage procedure. The patients are randomly assigned to receive either immediate adjuvant chemotherapy using bleomycin/actinomycin D/cyclophosphamide, high dose methotrexate, adriamycin and cisplatinum for 43 weeks (regimen 1) or to receive no immediate chemotherapy (regimen 2). Those patients on regimen 2 who subsequently experienced tumor relapse then receive the chemotherapy schema given to regimen 1 patients after surgical resection of as much recurrent tumor as possible.

The study was opened in May 1982 and results thus far are too early for detailed evaluation, although to date there is no significant difference between the relapse rate in the two arms. Although morbidity has been significant, no treatment-related mortality has thus far been reported.

Professional Personnel:

P. Pizzo	Chief	P C
S. Rosenberg	Chief	SB C
A. Baker	Senior Investigator	SB C
M. Link	Assistant Professor	Stanford, POG

Objectives:

To assess the role of adjuvant chemotherapy in patients with localized, high-grade osteosarcoma of the extremity following total surgical ablation of the primary tumor.

Methods Employed:

Patients less than 30 years of age who have a localized, biopsy proven, high-grade osteosarcoma of an extremity undergo amputation or limb-salvage procedure to secure complete surgical ablation of the primary tumor. Patients are then randomized to receive either adjuvant postoperative chemotherapy using high-dose methotrexate 12 gms per m^2 ; bleomycin 15 $u/m^2/day$ x 2/cytosar 600 $mg/m^2/day$ x 2/actinomycin D 0.6 $mg/m^2/day$ x 2, adriamycin 30 mg/m^2 x 3, and adriamycin 50 $mg/m^2/cis-platinum$ 100 mg/m^2 in cycles for 43 weeks, or to receive no immediate chemotherapy.

All patients are followed closely for development of metastases. Relapses in bone are treated surgically where possible. Patients relapsing in lung undergo thoracotomy (usually median stenotomy) with resection of all palpable tumor if possible. Following optimal surgical management of relapse, patients who have received no prior chemotherapy will receive the drugs as detailed above in an identical sequence to those patients receiving immediate adjuvant chemotherapy. Those patients relapsing after exposure to all the chemotherapy agents outlined will either receive no further chemotherapy (where all metastatic disease has been resected) or a phase I or II agent (where active tumor remains).

The study was opened in May 1982. To date the Pediatric Oncology Group has accrued approximately 16 randomized patients of whom 8 are NCI patients. Approximately 26 other localized osteosarcoma patients have been registered by POG but have refused randomization of whom 14 elected to receive immediate adjuvant chemotherapy, and 12 elected no immediate chemotherapy. Data is being collected on these patients but their evaluability is dubious. Results of the study are too preliminary to draw definitive conclusions, but thus far there is no significant difference between the number of relapses in each arm of the study. Of the eight patients randomized from NCI, 2 have been non-compliant and 6 continue on study of whom 4 were randomized to receive chemotherapy, and 2 received no immediate chemotherapy. These 6 have been followed from 1-10 months, and one patient receiving chemotherapy has relapsed.

In addition, during the study period, three patients refused randomization and refused chemotherapy, of whom 2 have relapsed and one is lost to follow-up. Ten patients with known metastatic disease have been enrolled in the chemotherapy arm, of whom 5 are evaluable for chemotherapy response (5 were rendered disease-

free by surgery). Three of these five have shown objective tumor regression following chemotherapy.

Toxicity has been as anticipated, and no life threatening toxicities have been encountered.

Significance to Biomedical Research and the Program of the Institute:

As the role of adjuvant chemotherapy in osteosarcoma is at present unknown and much debated, this study is intended to address the issue of efficacy of the most active presently available agents given in the adjuvant setting.

Proposed Course:

Patient accrual has been slower than the anticipated 100 patients per year from all institutions, but the study is being frequently reviewed and will continue according to protocol. Ways to improve the patient accrual are being explored.

Publications:

None to date.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-CM-06813-01 P
PERIOD COVERED April 1, 1983 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Pediatric Tumors		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Mark A. Israel, Senior Investigator, Pediatric Branch		
COOPERATING UNITS (if any) Department of Pathology, Harvard Medical School; Department of Microbiology, Columbia University;		
LAB/BRANCH Pediatric Branch		
SECTION -----		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> During the past year, our laboratory efforts have been directed towards two goals: (1) elucidating the molecular mechanisms underlying the biologic activity of polyoma virus (Py) and (2) developing a line of research which applies state of the art molecular biological technology to the evaluation of pediatric tumors. </p> <p> Experiments focused on studies of oncogenic transformation induced by polyoma virus include: (1) characterization of the physiologic activity of the viral oncogene product using monoclonal antibodies; (2) genetic characterization of the polyoma viral oncogene by <u>in vitro</u> site-specific mutagenesis; (3) characterization and genetic evaluation of regions of the viral genome important for the determination of tissue specificity of virus-induced tumor formation. </p> <p> Studies directed towards an understanding of the molecular events which are important for the malignant characteristics of pediatric tumors have focused on the development of tissue culture models for neuroblastoma. Our finding that some human neuroblastoma tissue culture cell lines can be differentiated to morphologically and biochemically resemble differentiated sympathetic ganglion cells should provide a basis for identifying and characterizing the molecular mechanisms which mediate both the normal cellular differentiation of this tissue and the pathologic alterations which may be important during oncogenesis. </p>		

Professional Personnel:

J. Bolen	Senior Staff Fellow	P C
C. Dawe	Staff Pathologist	Harvard, M.S.
C. Prives	Associate Professor	Columbia Univ.

Objectives:

- A. Characterization of the molecular mechanisms which mediate polyoma virus induced oncogenesis.
1. To determine precisely the regions of the polyoma viral genome required for polyoma virus induced tumorigenicity.
 2. To determine the molecular basis of tissue specificity of tumor induction by different strains of polyoma virus.
 3. To determine the physiologic role of the polyoma virus oncogene, the middle T-antigen, in the induction of cellular transformation.
- B. Characterization of the molecular mechanisms which mediate the growth pattern and phenotype of cell lines from pediatric tumors.
1. To develop well characterized tissue culture models for several pediatric tumors including neuroblastoma, Ewing's sarcoma, and lymphoma.
 2. To identify and evaluate the genetic basis by which the growth rate and phenotype of these tumor cell lines is regulated.
 3. To identify and characterize antigens which are characteristic of specific stages of cellular differentiation in neuroblastoma cells.

Methods Employed:

- A. Characterization of the molecular mechanisms which mediate polyoma virus induced oncogenesis

While a cDNA clone encoding only the polyoma viral oncogene product, middle T antigen is known to transform flat, continuous rodent cell lines in culture, it will not transform primary cells or cells maintained in low serum. We have determined that the cDNA clone encoding middle T antigen is not tumorigenic in animals.

To evaluate what additional virus encoded information is required for tumorigenicity, we constructed a polyoma viral mutant which encodes a functional middle T antigen, a full-sized small T antigen, but only 20 amino acids of the unique region of the polyoma large T antigen. DNA prepared from this mutant viral genome is tumorigenic in newborn hamsters. This mutant was prepared by inserting a 6 base pair synthetic oligonucleotide at a predetermined site in the early region of the polyoma viral genome. This oligonucleotide contains a termination codon in the reading frame which encodes the polyoma large T antigen and 2 amino acids, serine and threonine, in the

reading frame which encodes the polyoma middle T antigen. Small T antigen does not complement middle T antigen to mediate the transformation of primary cells. Furthermore, Py small T antigen differs from the amino terminal end of large T antigen by only a few amino acids.

Our finding that this mutant genome is tumorigenic in animals suggests that a very short region of the large T antigen (20 amino acids) is critical for virus-induced tumorigenicity.

We have begun an evaluation of several strains of wild type polyoma virus which induce a different spectrum of tumors following their inoculation into newborn mice. Preliminary evidence suggests that a non-coding region of the viral genome close to the origin of DNA replication mediates tissue specific expression of viral proteins critical for both viral DNA replication and oncogenic transformation and may thereby determine the tissue specificity of host cell-viral interactions.

The primary focus of our laboratory effort has been to characterize the physiologic activity of the polyoma virus oncogene product, the polyoma middle T antigen. In vitro studies have identified a polyoma middle T antigen associated tyrosyl kinase activity, and we have directed our attentions towards characterizing this enzymatic activity. Towards this end, we have constructed and characterized a hybridoma cell line which produces a monoclonal antibody that immunoprecipitates the polyoma middle T antigen and inhibits the associated tyrosyl kinase activity. Also, we have identified group-specific reagents which inhibit this kinase activity. Preliminary results using an in vitro assay we recently developed for Py middle T Ag associated kinase activity suggest that the polyoma middle T antigen can function only as a receptor of the transferred phosphate group and that the detected tyrosyl kinase activity results from a closely associated cellular tyrosyl kinase.

B. Characterization of the molecular mechanisms which mediate the growth pattern and phenotype of cell lines from pediatric tumors

We have studied the ability of a variety of biologic modifiers to induce the differentiation of neuroblastoma cell lines in culture. Amongst these, retinoic acid, under certain growth conditions, may induce the terminal differentiation of neuroblastoma tissue culture cell lines. This altered phenotype is characterized by growth arrest, complete loss of detectable clonogenicity in soft agar, the induction of physiologic levels of neurotransmitters, and the outgrowth of characteristic neurites. Once the characterization of this in vitro system is complete, we will use a variety of molecular biological techniques to identify the alterations in gene expression associated with the neoplastic and differentiated cellular phenotypes.

Significance to Biomedical Research and the Program of the Institute:

While the development of powerful molecular biological techniques remains an explosive area of current research, several new techniques including recombinant DNA technology and monoclonal antibody production have provided important new lines of investigation for scientists interested in understanding the molecular basis of neoplastic transformation. We are planning to use these technologies

to study a variety of pediatric tumors. We believe that some pediatric tumors may represent the escape of certain tissues from normally functioning developmental controls. Hopefully, an understanding of the mechanisms of cellular regulation of growth and differentiation in these tissues will provide a rational basis for designing new therapeutic approaches for the management of patients with these tumors.

Proposed Course:

We are planning to taper our current efforts to characterize the molecular mechanisms underlying the biologic activity of polyoma virus and focus greater energies on molecular biological studies of Pediatric tumors. I anticipate that during the next year we will complete our characterization of the virally encoded information required for tumorigenesis. The various mutant viral genomes and recombinant genomes for these studies have been constructed, characterized, and their biologic activity *in vivo* examined. Experiments remaining will focus on the *in vitro* characterization of these altered viruses. On the other hand, we are only beginning our molecular studies of Pediatric tumors. There is little doubt that initially we will focus on neuroblastoma as we have been most successful in obtaining and manipulating cell lines from this particular tumor in culture. We anticipate that the altered growth characteristics of neuroblastoma cells in culture treated with retinoic acid will reflect similar alterations occurring during the differentiation of neurocrest tissue. To characterize this transition as well as the molecular events which regulate it, we plan to use a variety of molecular biological techniques. These include evaluation of hybridization kinetics to identify differences in gene expression of cells maintained under different culture conditions, cDNA cloning of messenger RNA molecules characteristic of a particular phenotype, and isolation of monoclonal antibodies which recognize antigens characteristic of a specific phenotype.

Publications:

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2. Tanka, K., Chowdhury, K., Chang, K., Israel, M., Ito, Y.: Isolation and characterization of polyoma virus mutants which grow in murine embryonal carcinoma and trophoblast cells. The EMBO J. 1: 1521-1527, 1982.
3. Kenichi, T., Chowdhury, K., Liang, L., Chang, K., Israel, M. and Ito, Y.: Relationship between growth restriction to polyoma virus and stages of differentiation in embryonic cell lines. Cold Spring Harbor Symposium, in press.
4. Chowdhury, K., Garon, C.F., and Israel, M.A.: Structural analysis of integrated polyoma viral DNA in a polyoma-induced hamster tumor cell line. J. Virol., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06814-01 P

PERIOD COVERED

October 17, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology and Treatment of Pediatric Soft Tissue and Ewing's Sarcomas

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James S. Miser, Expert, Pediatric Branch

COOPERATING UNITS (if any)

Experimental Immunology Section, MB; Radiation Branch, NCI; Surgery Branch, NCI; Laboratory of Pathology, NCI; Genetics Branch, NCI; Rehabilitation Medicine, CC

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study of Ewing's Sarcoma, rhabdomyosarcoma, and undifferentiated sarcoma is being undertaken in two areas: biological studies and therapeutic trials. The biological studies address: 1) in vitro tissue culture evaluation and characterization of the cell lines from tumors of patients with these sarcomas; 2) in vitro differentiation of cell lines derived from tumors of patients with these sarcomas; 3) development of monoclonal antibodies to pediatric sarcomas; 4) definition of the cytogenetics of pediatric sarcomas; and 5) in vitro radiation and chemosensitivity of cell lines derived from tumors of patients with these sarcomas.

The therapeutic studies address: 1) improvement in therapy for patients with high risk pediatric sarcomas a) by improving the initial induction rate using an intensive induction and b) by utilizing intensive consolidation including high dose chemotherapy, total body radiotherapy, and autologous bone marrow reinfusion; 2) improvement in therapy for patients with low risk pediatric sarcomas; 3) improvement in the detection, evaluation, and treatment of pulmonary metastasis in patients with pediatric sarcomas; 4) careful evaluation of the short and long term effects of chemotherapy and total body radiotherapy on cardiac and pulmonary function, as well as other major organ systems; 5) evaluation of the efficacy and toxicity of autologous bone marrow transplantation in the treatment of pediatric sarcomas using a new chemotherapeutic and radiotherapeutic regimen.

This protocol, begun in early 1983, has already demonstrated that high-dose induction chemotherapy is successful and safe in the initial treatment of pediatric sarcomas. Further, the induction rate on this program has shown significant improvement over previous regimens. The induction regimen consists of high-dose cytoxan, high-dose adriamycin, and standard dose vincristine and actinomycin D on a dosing schedule which requires frequent administration.

Professional Personnel

P. Pizzo	Chief	P C
E. Glatstein	Chief	RO C
T. Kinsella	Senior Investigator	RO C
T. Triche,	Head	LP C
J. Mulvihill	Chief	CEB C

Objectives:A. Therapeutic:

1. To devise an effective therapeutic regimen for patients with Ewing's Sarcoma of "high risk" (rib, pelvis, sacral primaries, proximal extremity primaries and any primary with metastatic disease).
2. To devise an effective and relatively safe therapeutic regimen for patients with Ewing's Sarcoma of "low risk" (primaries from non-rib, pelvis, sacrum, proximal extremity, without metastatic disease).
3. To devise an effective therapeutic regimen for patients with Stage III and IV rhabdomyosarcoma and undifferentiated sarcoma.
4. To devise an effective and relatively safe therapeutic regimen for patients with Stage I and II rhabdomyosarcoma and undifferentiated sarcoma.
5. To study the short and long term effects of therapy on cardiac, pulmonary, and other major organ systems.
6. To study the short and long term effects of high-dose chemotherapy and total body radiotherapy followed by autologous bone marrow transplantation.
7. To study the evaluation of the detection methods of pulmonary metastases in patients with pediatric sarcomas.
8. To study the interaction of vincristine with other factors (e.g., radiotherapy, tumor induced nerve damage).
9. To study the effect of the rapidity of response to long-term outcome in patients with pediatric sarcomas.

B. Biologic

1. To establish and characterize cell lines from tumors of patients with sarcomas of childhood in tissue culture.
2. To study the pattern of differentiation and the effect of differentiating agents on these cell lines in tissue culture.

3. To develop monoclonal antibodies to pediatric sarcomas.
4. To define the cytogenetics of pediatric sarcomas.
5. To evaluate the *in vitro* radiation and chemosensitivity of cell lines derived from patients with pediatric sarcomas.
6. To study the epidemiology of Ewing's Sarcoma.

Methods Employed:

Clinical Studies

1. Current Treatment Protocol (PB 83-C-73) for high risk pediatric sarcomas.
The current treatment protocol for patients with high risk pediatric sarcomas addresses two major therapeutic problems:
 - a. The resistance to initial induction therapy, and
 - b. The relapse following initially successful induction therapy.

This protocol investigates a high-dose chemotherapy induction schedule emphasizing high-dose adriamycin in combination with cyclophosphamide, vincristine, and actinomycin D. The hypothesis being tested is that chemotherapy emphasizing adriamycin given at maximal doses and at frequent intervals will result in a higher initial induction rate and subsequent long term survival. This study also assesses the utility of an intensified consolidation in place of maintenance therapy to prevent relapse. This consolidation consists of high-dose chemotherapy in combination with total body radiation therapy followed by autologous bone marrow infusion. Experience in approximately 25 patients enrolled to date has shown that this intensive therapy is well tolerated and, thus far, effective in the initial induction therapy of newly diagnosed patients with high risk pediatric sarcomas.

Patients with relapsed sarcomas are also being evaluated on this protocol with the primary additional question under evaluation being whether high-dose chemotherapy is effective in a patient who has relapsed. Further, the characteristics of these patients are being related to the outcome of therapy. Other Phase II and Phase III studies of therapy for patients with recurrent pediatric sarcomas are under development.

2. The study of late effects of therapy:

The study of the late effects of intensive therapy for patients with sarcomas is presently in preparation and will address:

- a. Pulmonary function.
- b. Cardiac function.
- c. Endocrine function including growth, pubertal development, and hormone function.
- d. Gonadal function.

- e. Intellectual function.
- f. Other major organ systems function.

3. The study of the methods of detection of pulmonary metastases in patients with pediatric sarcomas.

The study of the methods of detection of pulmonary metastases in patients with pediatric sarcomas is presently in preparation and will address the effectiveness and accuracy of chest radiographs, whole lung tomograms, and chest CT scanning when compared to the results of thoracotomy in the evaluation of pulmonary masses.

Biological Studies:

1. Tissue culture

The primary goals of the work in this are:

- a. The establishment and characterization of cell lines from tumors of patients with Ewing's Sarcoma.
- b. The study of *in vitro* differentiation and the effect of differentiating agents on these cell lines in tissue culture.
- c. The development of monoclonal antibodies to Ewing's Sarcoma and other pediatric sarcomas.
- d. To evaluate the *in vitro* radiation and chemosensitivity of cell lines derived from patients with pediatric sarcomas.

These studies have just recently commenced.

2. Cytogenetics

Limited information is available about the cytogenetics of pediatric sarcomas. This study is in development.

3. Epidemiology

The epidemiology of pediatric sarcomas will be investigated for clues to the pathogenesis.

Proposed Course:

The studies described are all in their development. The major clinical study will be developed initially as a pilot study and subsequently as a randomized study to evaluate the efficacy and necessity of autologous bone marrow transplantations in the treatment of these patients.

The biological studies will be developed and expanded over the next 12 months with a greater emphasis on *in vitro* work with the goal of better characterization of the tumor cells and cell lines of patients with high risk sarcoma.

Publications:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06815-01 P

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Investigation and Treatment of Patients with Non-Hodgkin's Lymphoma

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ian T. Magrath, Senior Investigator, Pediatric Branch

COOPERATING UNITS (if any)

Clinical Chemistry, Clinical Center

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland 20205

TOTAL MANYEARS:

10

PROFESSIONAL:

8

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

78 patients have now been admitted to the primary protocol for the treatment of non-Hodgkin's lymphoma, and the goals of this protocol, namely, to define different prognostic groups within this broad category of patients have largely been achieved. Utilizing a CHOP - high dose methotrexate regimen, the results in lymphoblastic lymphoma without marrow involvement and patients with entirely resected intraabdominal undifferentiated lymphoma or localized disease have been excellent (currently 100% and 92% disease-free survival). Among the remaining patients the most important prognostic feature is bone marrow involvement. With the exception of this category, even patients with extensive undifferentiated lymphomas have a disease-free survival rate of about 50%. These findings will be incorporated into protocols now under development in which treatment will be tailored to prognostic groups.

Overall, the results of the present protocol show a 15% improvement in terms of disease-free survival over the two previous protocols used in the Pediatric Branch, when the previous results are combined (justified on the basis of a previous multi-institutional study which showed no difference in outcome between these protocols).

A detailed analysis of staging studies in undifferentiated lymphoma in children has been completed. This indicates the value of gallium scanning which gave no false negative results in the abdomen. Gallium is inferior to ultrasound or CAT scan in detecting hepatic lesions, and to a chest x-ray in detecting pleural effusions. A negative scan of the abdomen, however, is sufficient to exclude intraabdominal disease, obviating the need for further imaging studies except for helping to determine resectability and in determining whether hydronephrosis is present. No skeletal lesions were detected on bone scan which were not also seen on gallium scan.

Personnel

Philip A. Pizzo	Chief, Pediatric Branch	P	C
David G. Poplack	Head, Leukemia Biology Section	P	C
Mark A. Israel	Senior Investigator	P	C
James A. Miser	Expert	P	C
Nicholas Papadopoulos	Chemist	CP	CC

Objectives

- 1) To better define prognostic groups in young people with non-Hodgkin's lymphoma by treating all patients with an identical regimen.
- 2) To compare the relative value of various diagnostic imaging procedures in determining the extent of tumor.
- 3) To provide material for study of the biology of non-Hodgkin's lymphoma.

Methods and Major FindingsA. Definition of Prognostic Groups

An analysis of the results of the first 65 patients entered into protocol 77-04 carried out in January 1983 has led to the following conclusions:

1. Overall, approximately 60% of patients achieved long term survival.
2. One of the most important prognostic factors was the presence of bone marrow infiltration. Eleven of 12 patients with bone marrow involvement relapsed. All of the patients with bone marrow involvement in protocol 77-04 had at least 50% of the bone marrow replaced by malignant cells. These patients in other institutions would normally have been treated on a protocol designed for acute lymphoblastic leukemia. The disease-free survival of patients without bone marrow involvement treated according to protocol 77-04 was approximately 70%.
3. Among patients without bone marrow involvement the best prognosis was enjoyed by patients in one of the following categories:
 - a. lymphoblastic lymphoma (100% relapse-free survival in 10 patients)
 - b. localized (stage A) or completely resected intraabdominal tumor (stage AR) in patients with undifferentiation lymphomas (92% relapse-free survival in 14 patients)
4. Overall, age was not a significant prognostic factor, although partial responses were confined to patients over the age of sixteen with undifferentiated lymphoma.

5. No difference in outcome (or clinical features) was observed between patients classified as Burkitt's lymphoma, and those classified as having undifferentiated, non-Burkitt's lymphoma.
6. There was no difference in outcome among patients with stages B, C, or D undifferentiated lymphomas. Patients with stage B were few in number, and little weight can therefore be attached to the finding for this group of patients.
7. Since patients with undifferentiated lymphomas other than stages A or AR, achieved a predicted survival of only approximately 40%, this group of patients is worthy of the major focus in the next protocol.

B. Imaging Studies

A rapid staging work-up was applied to 51 patients with diffuse undifferentiated non-Hodgkin's lymphoma. The objective of this study was to determine the relative value of specific diagnostic imaging procedures in effort to establish the most appropriate means of rapidly defining the extent of disease. Gallium citrate scanning proved an excellent screening test for abdomino/pelvic disease. There were no false positive or false negative results. When the gallium scan is negative, there is no need for further imaging of the abdomen except in stage AR patients. However, in the presence of an abnormal abdominal scan, further, more detailed anatomical delineation of tumor is necessary and can be achieved using ultrasound (US) or computed tomography (CT). Overall, ultrasound was more sensitive than CT in assessing abdomino/pelvic disease. Extra-abdominal sites of involvement are usually obvious clinically and are frequently detected by the imaging procedures. In particular, the chest x-ray is useful in showing pleural effusions which might not be clinically apparent. Similarly, gallium scanning is extremely sensitive to bone and nasopharyngeal involvement by tumor.

C. Other Investigations

Two other observations have been made recently.

- 1) In patients with extensive Burkitt's or non-Burkitt's undifferentiated lymphoma, monoclonal immunoglobulin bands are frequently (60%) detected in serum.
- 2) At presentation, at least 20% of microscopically normal bone marrows in patients with undifferentiated lymphomas contain occult tumor cells. This has been demonstrated by the development of tumor cell lines from such marrows, or the demonstration of cells containing an 8;14 translocation in the marrow.

Significance to Biomedical Research and the Program of the Institute:

The Pediatric Branch is committed to improving the diagnostic and therapeutic approaches to children with malignant diseases. The present work has provided a number of insights into prognostic variables in non-Hodgkin's lymphoma and

upon this basis protocols are currently being developed which will be more intensive for high risk patients, and will be slightly less intensive for low risk patients. Data obtained from the imaging studies will enable patients to be worked up more expeditiously and unnecessary studies will be avoided. The demonstration of monoclonal bands in undifferentiated lymphomas is of relevance to the biology of these tumors, but may also be of diagnostic value in some circumstances. This initial step towards the development of a tumor marker will be followed by attempts to develop a free light chain (immunoglobulin) assay which may prove to be more sensitive.

The detection of occult marrow involvement confirms fears that programs utilizing autologous bone marrow reinfusion must be approached cautiously, and the use of methods aimed at removing occult neoplastic cells prior to reinfusion may need to be developed.

Thus, progress has been made which should result in improvement in current approaches to diagnosis and management.

Proposed Course:

New protocols are currently being developed which will focus upon further improving results of therapy by separating out different prognostic groups and treating them differently.

One approach currently underway is to develop a "non-crossreactive regimen" which will be piloted initially in relapsing patients.

Publications:

1. Cossman, J., Chused, J.M., Fisher, R.I., Magrath, I., Bollman, F., and Jaffe, E.S.: Diversity of immunologic phenotypes of lymphoblastic lymphoma. Cancer Research, in press.
2. Csako, G., Magrath, I.T., and Elin, R.: Serum total and isoenzyme lactate dehydrogenase activity in American Burkitt's lymphoma patients. Amer. J. Clin. Path. 78: 712-717, 1982.
3. DeCristoforo, R., Corden, B.J., Hood, J.C., Narang, P.K., Magrath, I.T.: High dose morphine therapy complicated by chlorobutanol induced somnolence. Annals Int. Med. 98: 335-336, 1983.
4. Janus, C., Sariban, E., and Magrath, I.T.: Surgical resection and limited chemotherapy for abdominal undifferentiated lymphomas. Cancer Treat. Rep., in press.
5. Kemeny, M.M., Magrath, I.T. and Brennan, M.F.: The role of surgery in the management of American Burkitt's lymphoma and its treatment. Annals of Surgery 196: 82-86, 1982.
6. Levine, P.H., Kamarajn, L.S., Conelly, R.R., Berard, C.W., Dorfman, R.F., Magrath, I.T., and Easton, J.M.: The American Burkitt's lymphoma registry: Eight years experience. Cancer 49 (5): 1016-1022, 1982.

7. Magrath, I.T.: Burkitt's Lymphoma. In Mollander, D. (Ed.): Diseases of the Lymphatic System: Diagnosis and Therapy. New York, Springer Verlag, in press.
8. Magrath, I.T.: Infectious Mononucleosis - Its Relationship to Malignant Neoplasia. In Schlossberg, D. (Ed.): Infectious Mononucleosis. New York, Springer Verlag, in press.
9. Magrath, I., Benjamin, D. and Papadopoulos, N.: Serum monoclonal immunoglobulin bands in undifferentiated lymphomas of Burkitt's and non-Burkitt's types. Blood 61: 726-731, 1983.
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12. Sariban, E., Donahue, A., and Magrath, I.T.: Jaw involvement in American Burkitt's lymphoma. Cancer, in press.
13. Sariban, E., Janus, C., Edwards, B., Magrath, I.T.: Central Nervous System in American Burkitt's Lymphoma. J. Clin. Oncol., in press.
14. Sariban, E., and Magrath, I.T.: Dysphagia -- an unusual presentation of Burkitt's lymphoma. Am. J. Dis. Child. 136: 172-173, 1982.
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT		Z01-CM-06830-13 P
PERIOD COVERED October 1, 1982 - September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Infectious Complications of Malignancy: Diagnosis, Management and Prevention		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Philip A. Pizzo, Head, Infectious Disease Section; Chief, Pediatric Branch		
COOPERATING UNITS (if any) Medicine Branch, NCI; Surgery Branch, NCI; Diagnostic Microbiology, CC; Clinical Mycology Section, LCI, NIAID; USUHS; WRAIR; U. Penn; Johns Hopkins; Clinical Section, NIDR		
LAB/BRANCH Pediatric Branch		
SECTION Infectious Disease		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 5.5	PROFESSIONAL: 4.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Infection is a major cause of morbidity and mortality in cancer patients. Our studies are devoted to developing methods to define patients at high risk for infection, improving the ability to diagnose infections early, treat them effectively and ultimately prevent them.</p> <p>We are seeking to differentiate granulocytopenic patients at heightened risk for infection by measuring tissue-bound phagocytes, opsonizing antibody and local defense factors. We are seeking to improve rapid diagnosis of fungal infections by measuring circulating antigens and to define the role of viruses in infection in immunocompromised patients.</p> <p>Effective management of the granulocytopenic patient requires empiric broad-spectrum antibiotic therapy. We are developing new therapeutic approaches based on new antibiotic developments, particularly the third generation cephalosporins. Our results show that a new cephalosporin, ceftazidime, is as effective as a triple drug combination. Our studies are also defining the appropriate antibiotic therapy for documented bacterial infections, the necessary duration of empiric therapy for patients with unexplained fever, the choice of empiric antifungal therapy, and the merits of empiric therapy vs invasive procedures in patients with pulmonary infiltrates.</p> <p>Prevention of infection has largely resided in decreasing acquisition of new organisms and in reducing the endogenous burden with prophylactic antibiotics. We are continuing our study of total protected isolation for high risk patients but are developing new prevention strategies to improve host defenses. These include passive immunization with post-vaccine antisera to the core glycolipid of enterobacteriaceae as well as pooled immunoglobulins. Our pre-clinical studies are further focused on attaching (arming) leukocytes with polyclonal or monoclonal antibodies to improve their bactericidal activities and ultimately leukocyte transfusion therapy. We are also developing methods to accelerate granulopoiesis using chemically-defined immunoregulatory agents, and ultimately to study their mechanisms of action.</p>		

Professional Personnel:

D. Cotton	Senior Staff Fellow	P C
J. Hiemenz	Investigator	P C
J. Hathorn	Clinical Associate	P C
I. Ioannou	Visiting Fellow	P C
D. Marshall	Research Nurse	P C
J. Gress	Research Nurse (Guest Worker)	P C
D. Longo	Head, Experimental Immunology Section	MB C
S. Brower	Investigator,	SB C
J. Roth	Senior Investigator,	SB C
A. Macher, V. Gill	Staff	LCI I
J. Bennett	Head, Clinical Mycology Section	LCI I
B. Lange	Assistant Professor	U. Pennsylvania
M. Pollack	Senior Investigator	USUHS
J. Sadoff	Senior Investigator	WRAIR
R. Yolken	Associate Professor	Johns Hopkins
P. Fox	Investigator	NIDR
B. Baum	Clinical Director	NIDR

Objectives:

1. To assess the current etiologies of febrile episodes in patients with malignancy, and the relationship of these episodes to cancer treatment, prior infection, degree of host compromise, underlying disease, and granulocytopenia.
2. To determine the most appropriate means for the evaluation of fever in the compromised host by assessing invasive and noninvasive diagnostic techniques.
3. To improve methods by which occult infections may be detected and treated.
4. To assess the possibility of reducing the incidence of infectious complications in granulocytopenic cancer patients by the use of prophylactic antibiotics.
5. To assess the efficacy and toxicity of empiric and specific antibiotic regimens for patients who become febrile and/or infected during periods of granulocytopenia. The role of antifungal therapy in high-risk patients will also be determined.
6. To improve the utility of the "total protected environment" in reducing the incidence of severe infection in patients with various lymphomas and solid tumors who are undergoing very intensive chemotherapy (with or without autologous marrow rescue), and to learn whether the intensive treatment possible in this circumstance results in improved tumor response and prolongation of survival.
7. To evaluate methods for bolstering host defenses and stimulating granulocyte recovery following chemotherapy-induced immunosuppression as an adjunct to the treatment and prevention of infections.

METHODS AND MAJOR FINDINGSCLINICAL STUDIES:

Prospective study of the diagnosis, management and prevention of infectious complications in cancer patients have been ongoing since 1976. During the first 5 years of study only pediatric patients were evaluated; since July, 1981 adults from the Medicine Branch have been available for study. To date, more than 1500 episodes of fever and granulocytopenia have been entered into clinical studies:

A. Studies Related to the Diagnosis of Infection

1. The role of viruses as co-factors or antecedent agents causing fever and/or secondary bacterial infection is being prospectively evaluated with R. Yolken at Johns Hopkins using ELISA assays on throat washings and stool samples from patients who become febrile. Since this study began in December, 1982, 140 samples have been collected and the study is still in progress.
2. The prevalence of C. difficile as a cause of diarrhea in patients receiving chemotherapy or antibiotics is being prospectively investigated. The study began in March 1982, and 134 samples have been analyzed so far, 15 containing a toxinogenic C. difficile. Correlation of these findings with the patient's chemotherapy is underway.
3. The ability to detect antigens to Candida and Aspergillus in either serum or urine is being analyzed with Dr. J. Bennett using antibody-coated latex beads or double sandwich ELISA samples from patients with documented fungal infections.
4. The association of the indwelling right arterial silastic catheters of the Hickman-Broviac type with bacteremia has been evaluated in 111 catheter episodes. The incidence of bacteremia is 39% in both granulocytopenic and nongranulocytopenic patients, predominately with gram-positive organisms. Infectious morbidity related to the catheters is negligible and the majority can be treated with parenteral antibiotics without the need for catheter removal.
5. Approach to the management of pulmonary infiltrates appearing in patients already receiving broad spectrum antibiotics was retrospectively evaluated in the 34 patients (11.7%) who developed such infiltrates over a 4-year period. The most common etiology was fungal and outcome depended significantly on whether the infiltrate occurred in association with granulocyte recovery. If infiltrates occurred and progressed without granulocyte recovery, the possibility of a fungal pneumonia was highest and such patients required early antifungal therapy with amphotericin B.

6. The initial management of patients with diffuse pulmonary infiltrates with empiric antibiotics vs an attempt to make a diagnosis using open lung biopsy represents an area of current controversy. In March of 1982 we initiated a prospective study in which patients are randomly assigned to initial empiric antibiotic therapy (without biopsy) vs initial biopsy and specific therapy. The empiric therapy regimen depends on whether or not the patient is granulocytopenic or is receiving antibiotics. To date 17 patients have been randomized, 10 to empiric therapy, 7 to an initial open lung biopsy. It is anticipated that this study will take another 12-18 months to complete.
7. Oral granulocytes are measurable in salivary mouth rinses and have recently been shown to correlate with peripheral granulocytes. The possibility that oral granulocytes (which are easy to measure) might serve as a marker of tissue phagocyte defenses and thus correlate with the risk for infection is intriguing. The fact that only 30% of comparably granulocytopenic patients actually become febrile suggests that a method for differentiating patients at higher risk for infection is highly desirable. This study measures oral granulocyte counts in patients receiving chemotherapy and follows the counts sequentially. The results are being correlated with the risk for infection.
8. Predictors of oral mucositis. To assess the role of salivary gland function in mucositis, we are serially monitoring patients receiving cytotoxic agents which have a high propensity to cause oral mucositis. Salivary secretions and crevicular fluids are collected before and after stimulation and their cellular contents, electrolytes, anionic proline-risk protein, mucin and lysozyme levels are measured and correlated with the incidence and severity of mucositis. To date, five patients have been studied, each showing a significant abnormality in the composition but not the amount of saliva secreted. Oral mucositis developed in parallel with these changes.

B. Studies Related to the Management of Infections

1. To assess whether synergistic combinations of antibiotics are necessary for febrile granulocytopenic (F+G+) patients if a single antibiotic has a very broad spectrum (particularly against gram-negative) and achieves serum levels 10-100 fold above the MIC of predominate isolates, we randomized patients to either our conventional combination (KGC: cephalothin, gentamicin, carbenicillin) vs a new third generation cephalosporin (CTZ: cef-tazidime). 204 G+ (<500 PMN/mm³) pts were randomized when F+ (>38°C x 3/24 hr or >38.5°C once) to KGC (102 pts) or CTZ (102 pts). Initial diagnosis of 186 evaluable episodes included sepsis (33 pts), pneumonia (5 pts), cellulitis (13 pts), peritonitis (1 pt), mucositis (4 pts), UTI (4 pts) and undefined fever (130 pts). Initial response (first 72 hrs) was 99% for KGC vs 100% for CTZ. Overall results were rated as: Success

Alone (SA: successful overall outcome without change or addition to study drugs(s)); Success with Modification (SM: successful overall outcome but with addition of antibiotics, antifungals or change of study drug(s) after initial response); Failure (F: death due to a proven infection on study).

Regimen	FEVER OF UNDEFINED ORIGIN				DOCUMENTED INFECTION			
	Number	SA	SM	F	Number	SA	SM	F
KGC	69	54	12	3	25	7	15	3
CTZ	61	48	12	1	31	9	17	5

While these results suggest that ceftazidime as a single drug has similar efficacy as KGC for F+G+ cancer pts with undefined fever, further study is required to confirm these observations in F+G+ pts with documented infections.

2. If patients have a microbiologically proven infection, it remains unclear as to whether appropriate treatment should be a specific antibiotic vs a broad-spectrum combination, especially if they remain granulocytopenic. Our prior retrospective study showed that broad spectrum antibiotics were optimal if the period of granulocytopenia exceeded one week. In a prospective study we are randomizing patients to specific therapy vs broad-spectrum. To date 35 patients have been randomized 18 to specific therapy and 17 to broad-spectrum.
3. For patients with unexplained fever (FUO), our previous studies have shown that it is optimal to continue antibiotics if patients remain granulocytopenic for more than one week. In present studies, patients with FUO who defervesce after the initiation of antibiotics are treated for a full course of therapy as if they had an undiagnosed infection and are then randomized on day 14 to either discontinue antibiotics or to continue until resolution of granulocytopenia. To date, 12 patients have been randomized to discontinue therapy and 12 to continue. The study is still in progress.
4. Our prior study has shown that patients with persistent fever and granulocytopenia benefit from the continuation of antibiotics and the addition of empiric antifungal therapy after one week of fever and granulocytopenia. We are presently comparing the efficacy and toxicity of a new antifungal drug, ketoconazole, with our standard, amphotericin-B. Since initiating this trial in July, 1981, 22 patients have been randomized, 10 to receive empiric amphotericin-B, 12 to receive empiric ketoconazole.

C. Studies Related to Prevention of Infection

1. Patients undergoing intensive chemotherapy may be treated in protected isolation; if the patient is receiving an autologous bone marrow infusion, the patient may be randomized to receive intensive therapy either in or out of a protected isolation. Since 1975, 90 patients have received intensive therapy in a protected environment. Results show a significant reduction

in infections and infection-related mortality, although the efficacy of this program is adversely influenced by patient compliance with the regimen. At best, only 48% of patients are fully compliant.

2. We recently completed a double blind randomized trial comparing trimethoprim-sulfamethoxazole plus erythromycin to placebo for preventing fever and infection in granulocytopenic patients. In this study, 150 patients were randomized to the antibiotics or placebos. A significant reduction in the incidence of fever and/or infection was observed for the antibiotic treated patients, but only if they totally complied in taking the medication. Compliance with the prophylactic regimen also turned out to be an independent variable.
3. In order to further extend our observations related to antibiotic prophylaxis and assess whether erythromycin might have adversely affected the prophylaxis by impeding "colonization-resistance" we began a prospective study in December, 1981 in both adult and pediatric patients. Patients are randomized to receive trimethoprim-sulfa alone, T-S plus erythromycin or to serve as a control. To date, 173 pediatric episodes and 80 adults have been randomized: 81 to B+E, 92 to Bactrim alone and 80 to control. Analysis is in progress.
4. It has recently been demonstrated that passive infusion of antibody against the core glycolipid of enterobacteriaceae (e.g., "J5 antisera") may reduce the mortality of gram-negative sepsis. We are beginning two clinical trials which will test the value of passive immunization in neutropenic pediatric and adult cancer patients. The first trial will be done in pediatric patients and randomizes patients to passive immunization with post-immune J5 antisera or to pre-immune sera (control) for both the prevention as well as treatment of fever and infection in neutropenic cancer patients. A second, concurrent study, will be done in adults using a pooled immunoglobulin preparation which has high titres against a broad range of microorganisms (including viruses). Again, patients will be randomized to either the immunoglobulin or to albumen with the objective of testing the efficacy of passive immunization in reducing the incidence of fever and infection in neutropenic patients.

PRECLINICAL STUDIES

1. Nutrition and host defense - Cancer cachexia and malnutrition can impair physical defense barriers and may also serve to impede granulocyte recovery. We studied the influence of protein carbohydrate variations on cyclophosphamide (CP)-induced myelosuppression (MS) in 6-8 week old CDF₁ mice. Three isocaloric diets differing only in their N:Ca ratio (1:22 vs 1:62 vs 1:120) were fed ad lib throughout the study. CP was administered as either a single 225 mg/kg ip dose of CP or as three daily 75 mg/kg doses; control animals were injected with ip normal saline (NS).

Retro-orbital WBC's and bone marrow CFUc's were measured every other day. There was no difference in the growth curves with the different diets and control mice receiving NS had no difference in WBC or CFUc's. Mice challenged with CP had a significant difference in their WBC nadir according to their diet:

N:Cal Ratio -	WBC nadir x 10 ⁻³		
	1:22	1:62	1:120
Single dose CP	3.5±0.4	1.65±0.5	0.3±.04
Multiple dose CP	1.8±0.3	1.0±0.2	0.1±.05

The time to WBC recovery was significantly faster for the group fed N:Cal 1:22 vs 1:62 vs 1:120; however, there was no significant difference in CFUc's in the 3 groups. Mice fed the same three diets were also challenged with 10⁵ P388 lymphoma cells per mouse and again received either NS or CP. While there was a significant difference in survival in CP-treated mice fed N:Cal 1:22 vs 1:62 vs 1:120. We conclude that high protein diet can decrease the WBC nadir and accelerate granulocyte recovery in CP treated mice but without compromising CP antitumor activity.

2. Arming Granulocytes: To improve the utility of granulocyte transfusions in clinical practice we examined whether attachment of antibody (Ab) to human PMNs enhances bactericidal activity. Dextran sedimented/Ficoll-Hypaque purified PMNs were "armed" (A) by incubation (6% PEG at 4°C for 15 min) with monoclonal Ab to LPS of *P. aeruginosa* (Ps) or polyclonal Ab (Gamimune) and centrifugation through phthalate oils (PMNs >95% viable). Attachment of Ab was confirmed by indirect fluorescence. Bactericidal assays consisted of Ab, complement (C'), Ps and PMNs. Timed samples were collected in triplicate and PMNs lysed with d H₂O. Aliquots were spread on agar plates, incubated at 37°C for 12 hrs, and the colonies counted. Data is expressed as % kill (K) or % growth (G).

TIME MIN	C', Ab, PMN, ΔC', Ab, PMN, Ab, C'		A-PMN, C', Ps		
	Ps	Ps	Ps	Monoclonal	Polyclonal
20	1 (G)	16 (G)	34 (K)	42 (K)	*50 (K)
45	35 (G)	21 (G)	49 (K)	*81 (K)	*88 (K)
90	63 (G)	42 (G)	64 (K)	*97 (K)	*96 (K)

(* % K significant at p < 0.05 compared to unarmed PMNs)

A-PMNs with mono or polyclonal Ab also enhanced bactericidal ability at various bacteria: PMN ratios (1:1 to 200:1). Preliminary data suggests that the degree of enhanced killing correlates with the specificity of the Ab used to "arm" the PMNs. "Armed" PMNs may have application in improving the efficacy of leukocyte transfusions.

3. The role of chemically-defined immunoregulatory agents on granulopoiesis has been measured in *in vitro* long-term bone marrow (Dexter) cultures of both murine and human derivation. The assays used have been cell count, CFUc and CFUs. In vivo murine studies have also been performed.

4. The cyanoaziridine, azimexon, produces a dramatic dose-response increment in WBC, granulocyte count and CFuc. The utility of this as an adjunct to infection modulation and prevention is being explored.
5. Lithium carbonate has also been explored in both in vivo and in vitro experiments of the enhancement of granulopoiesis. While effective in vitro in increasing Dexter culture cell counts, CFuc and CFUs, it fails to have this effect when administered in vivo to the same mouse strain. The mechanism of action for this is being explored.
6. The mechanism of action of the cyanoaziridines and lithium is being explored in vitro by assessing its effect on the generation of IL-2. Lithium and azimexon produce a significant increase in IL-2 production in both murine and human systems.
7. Studies to explore the role of committed granulocytes grown from in vitro bone marrow cultures as a source for therapeutic and prophylactic leukocyte transfusions in a murine model are in progress. The effect of immunoregulatory agents on the generation and function of the leukocytes for transfusion are part of this study.

Significance of Biomedical Research and the Program of the Institute:

Infection remains the leading cause of morbidity and mortality in the cancer patient. The majority of infections occur as a consequence of disease and/or treatment-induced alteration of host defenses (especially granulocytopenia), and they are the major impediment to the delivery of cancer chemotherapy. Consequently, effective supportive management of the patient is essential if the potential benefits of chemotherapy are to be achieved. This includes an understanding of the natural history of infectious complications in the compromised host, especially their early recognition and diagnosis. Our studies to date have helped to define the appropriate evaluation of the febrile, neutropenic cancer patient, as well as the specific management of particular infections and fevers of undetermined origin. These changes in management have resulted in a significant reduction in the morbidity and mortality related to infection.

Moreover, our studies of infection prevention (both the protected environment and empirically administered antibiotics) suggest that the frequency and morbidity of infectious complications can be significantly reduced, thus permitting the optimal delivery of cancer chemotherapy.

Proposed Course:

We shall continue our studies of the natural history of infectious complications in cancer patients as outlined in the progress report, since this evaluation will help further to define high-risk patients and assist in their diagnosis and management. Rapid diagnostic assays which do not depend on culture of the organisms will be studied in order to provide the most rational basis for immediate antibiotic and anti-fungal management. Our studies on the optimal empiric use of antibiotics in febrile, neutropenic patients will be continued, as will our clinical trials related to the specific management of septicemia, local bacterial infections, and viral, protozoan, and fungal complications. Our stud-

ies on antibiotic prophylaxis will be continued, and the use of chemical or immunological adjuvants which might shorten the period of granulocytopenia will be assessed. Similarly, our studies of the protected environment will be continued, with emphasis on the early intensive treatment of high-risk tumors prior to the emergence of chemotherapy resistance.

The ultimate challenge is the development of effective cancer treatment methods which are tumor-specific and which do not produce the significant compromise of host defenses which result in infectious complications. However, until this goal is realized, we will continue to investigate more effective and less toxic methods for treating and preventing infection in immunosuppressed patients. Our major emphasis will be directed at prevention. We will seek more effective methods for suppressing and/or eliminating the host's endogenous microbial flora with absorbable and nonabsorbable antimicrobial agents. Our major research target will be to develop methods for immunostimulation of the host's defenses and during chemotherapy-induced immunosuppression. Methods to activate cellular and humoral immunity, the macrophage-monocyte system, and mechanisms which expand and/or protect the neutrophil mass following chemotherapy will be sought through the use of chemically-defined immunoregulatory agents. While combining these hosts bolstering techniques with prophylactic antibiotics, we will also explore chemotherapeutic schedules which may have a more selective effect on tumor cells.

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3. Commers, J.R., Robichaud, K.J., and Pizzo, P.A.: Empiric antifungal therapy in the management of the febrile granulocytopenic cancer patient. Ped. Infect. Dis., in press.
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16. Pizzo, P.A.: The Neutropenic Patient. In Pennington, J. (Ed.): The Patient at Risk for Infection. In press.
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06840-08 P

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology and Treatment of Acute Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

David G. Poplack, Head, Leukemia Biology Section, Pediatric Branch, NCI

COOPERATING UNITS (if any)

Metabolism Branch, Immunology Branch, Biostatistics and Data Management Section (NCI); Dept. of Ped., Univ. of Pittsburgh; Dept. of Ped., Univ. of Wisconsin; Catholic Univ. of Rome.

LAB/BRANCH

Pediatric Branch

SECTION

Leukemia Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5.0

PROFESSIONAL:

3.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The biology of acute leukemia is studied and innovative approaches to its treatment explored with particular emphasis on investigation of acute lymphocytic leukemia (ALL). Biological studies are aimed at elucidating characteristics of the biology of the acute leukemic cells which will provide avenues for new therapeutic approaches. Biochemical studies have defined the crucial role of the purine pathway enzymes in lymphoid malignancies. Immunological studies have 1) confirmed the potential of in vitro monoclonal antibody immunotherapy of acute leukemia in a unique murine model; 2) examined the role of ALL cells in suppression of the immune response; and 3) evaluated the status of immunoglobulin gene rearrangement in leukemic lymphoblasts. Study of sex steroid receptors has demonstrated estrogen receptors on the leukemic cells of a subset of ALL patients, and in a murine model, has confirmed their role in leukemogenesis.

Therapeutic studies in ALL have addressed 1) improvement in therapy for patients in the high risk category; 2) assessment of mechanisms of treatment failure; and 3) characterization of adverse sequelae of antileukemic therapy and design of treatment regimens which avoid them. The major ALL treatment protocol has successfully demonstrated that high-dose, protracted systemic methotrexate infusions can substitute for cranial radiation as central nervous system CNS preventive therapy for the majority of patients with ALL. Studies on the bioavailability of orally administered maintenance chemotherapy reveal that many patients do not achieve adequate drug levels in the blood, indicating a probable mechanism of treatment failure. A clinical trial of 2'-deoxycoformycin and Ara-A seeks to take selective advantage of the specific biochemical abnormalities uncovered in our studies of leukemic lymphoblasts. Demonstration of Leydig cell dysfunction in patients treated for testicular relapse confirms the need for hormonal replacement therapy. Studies on late effects have demonstrated CT brain scan, neuroendocrine, and psychometric test abnormalities in long-term survivors, stressing the need for alternative methods of CNS preventive therapy.

Other Professional Personnel:

J. Blatt	Assistant Professor	Univ. of Pittsburgh
S. Zimm	Investigator	P C
U. Srinivasan	Investigator	P C
R. Wesley	Investigator	BDMS C
M. Trigg	Assistant Professor	Univ. of Wisconsin
R. Riccardi	Assistant Professor	Catholic Univ. of Rome
T. Waldmann	Head,, Cell Immunol Sec.	MET C
S. Korsmeyer	Senior Investigator	RR CH
R. Sherins	Senior Investigator	RR CH
D. Mann	Senior Investigator	I C

Objectives:Biological Studies

1. To study the biochemical status of the purine enzyme pathways in malignant lymphoid conditions to assess their role in lymphoid differentiation and as possible diagnostic markers and avenues of new therapeutic approaches.
2. To study the role of sex hormone receptors in lymphoblastic leukemia.
3. To evaluate the status of immunoglobulin genes in acute leukemic lymphoblasts.
4. To evaluate the potential of monoclonal antibodies as in vitro immunotherapy for acute lymphoblastic leukemia.

Therapeutic

1. To study effective therapeutic regimens in acute lymphoblastic leukemia which provide maximum tumor cell kill, while minimizing the possibility of adverse therapeutic sequelae.
2. To study the short and long term effects of antileukemic therapy on growth development and organ function.
3. To study the pharmacology of orally administered antileukemic agents and evaluate its relationship to treatment failure.

Methods and Major Findings:Biological StudiesA. Biochemical Studies of Malignant Lymphoid Cells

We have undertaken a comprehensive evaluation of the purine pathway enzymes in acute lymphoblastic leukemia and in other lymphoid malignancies. Our results have demonstrated a specific biochemical phenotype which correlates

with the major immunologic subsets of ALL, which offers an avenue for new diagnostic and therapeutic approaches. In addition, extension of these studies to other lymphoid malignancies has confirmed the ability of characterization of the purine pathway enzymes to characterize the differentiation status of lymphoid malignancies such as the Sezary syndrome.

B. The Role of Sex Hormones Receptors in Acute Lymphoblastic Leukemia

Earlier studies by this laboratory, utilizing a murine model, had demonstrated a role for steroidal sex hormones in leukemogenesis. To evaluate the status of sex hormone receptors in human ALL, estrogen (estradiol, E₂) and androgen (dihydrotestosterone, DHT) receptors were studied in human leukemic lymphoblasts. Specific hormone binding was characterized by incubation of the protamine sulfate precipitable cytosolic fraction from $0.25-1.0 \times 10^8$ cells with increasing concentrations of radiolabelled steroid in the presence or absence of 100-fold excess unlabelled steroid. Scatchard analysis of binding data revealed saturable, high affinity E₂ binding in a subset of approximately 20% of patients evaluated, including both females and males. In contrast, no DHT receptors were detected. The finding of estrogen receptors in the lymphoblasts of a subset of patients with ALL raises the possibility of a role for anti-estrogen therapy in such patients. This is being explored in current studies.

C. Studies of Immunoglobulin Genes in Acute Leukemic Lymphoblasts

In collaboration with Dr. Stanley Korsmeyer and Thomas Waldmann, studies designed to examine the status of immunoglobulin genes and ALL lymphoblasts have demonstrated that lymphoblasts from patients with "non-T, non-B" ALL have evidence of immunoglobulin gene rearrangement which indicates that they are of B-cell lineage. These studies have profound implications for evaluation of the differentiation status of malignant leukemia cells.

D. Immunotherapy of ALL

A unique murine model was developed in which the feasibility of in vitro immunotherapy using antileukemic antibodies was assessed. These studies confirmed the potential of this approach and serve as a model for future clinical studies of this strategy in patients.

Therapeutic Studies

A. Treatment studies of Acute Lymphoblastic Leukemia

1. Current treatment protocol (PB 77-02)

The current ALL treatment protocol addresses the two major therapeutic problems in this disease: a) improvement in the method of delivering CNS preventive therapy with less long-term morbidity, b) the need for improved treatment for patients possessing poor prognostic factors. This protocol investigates the efficacy of high-dose, protracted intravenous

methotrexate infusions as CNS preventive therapy. Patients are randomized to receive CNS prophylactic therapy with cranial radiation plus intrathecal methotrexate, or with high-dose, 24 hour systemic methotrexate infusions. The hypothesis being tested is that CNS preventive therapy using the methotrexate infusions alone is equally effective and less toxic than the current standard form of CNS prophylaxis (cranial radiation and intrathecal methotrexate). This study also assesses the utility of an intensified systemic maintenance schedule which alternates periodic "induction-type" chemotherapy with standard maintenance treatment. Experience to date has shown that this intensified approach can be approached without a significant increase in drug-related morbidity. To date, 177 patients have been randomized on this study; 59 to cranial radiation plus intrathecal methotrexate; 118 patients to receive high-dose, protracted intravenous methotrexate infusions (randomizations weighted on 2-1 basis). The overall remission induction rate is 98%, with a continuous remission rate at 2 years of approximately 80% for the entire study group. The overall bone marrow relapse rate is 5%; the CNS relapse rate is 6.5%. With a median duration on study of 18 months, there is no significant difference in the CNS relapse rate for both treatment arms. Long-term evaluation of neurotoxicity (by CT scan evaluation and psychometric testing) is underway. When the results are broken down in terms of patient risk groups, the CNS relapse rate for the average risk patients is approximately 3%. Thus, information to date would suggest that, for patients in this risk group, cranial radiation and intrathecal chemotherapy can be avoided and substituted by high-dose intravenous methotrexate infusions. Should these results hold up, it indicates that the use of cranial radiation can be avoided in approximately 60% of all children with ALL. The number of poor prognosis patients on this protocol is small. It is too early to assess the impact of this therapy on this subset of patients. In summary, the results to date appear to be very encouraging. Accrual on this collaborative study (4 CCSG institutions are participating) has been good and entry of new patients on this study will cease in the summer of 1983. A subsequent study is in the planning stages.

B. Relapsed ALL: Phase I Study of 2'-Deoxycoformycin and Ara-A.

Interest in 2'-deoxycoformycin (2'-DCF) was stimulated by our observations that the leukemic blast cells of ALL patients with T-cell disease have higher ADA levels than non-B, non-T lymphoblasts. An initial Phase I study with DCF alone in relapsed patients with ALL indicated that this drug had activity in ALL and that a safe dose could be identified. A subsequent study has been performed using 2'-DCF in combination with Ara-A. This study demonstrated modest antileukemic activity and confirmed that a DCF dose of 10 mg/m² and an Ara-A dose of 250 mg/m² could be given safely on an intravenous daily x 3 schedule. As an extension of this study, a larger, Phase II study comparing the efficacy of DCF alone, to that of DCF plus Ara-A has been proposed and will be initiated in mid 1983.

C. Study of the Late Effects of Antileukemic Therapy

In a previous study, we first reported CT brain scan abnormalities in asymptomatic children with ALL who have received prophylactic cranial radiation and maintenance intrathecal chemotherapy (NEJM 298:815, 1978). In that study, one or more of four types of CT scan abnormalities were observed: 1) ventricular dilatation, 2) subarachnoid space dilatation, 3) areas of decreased attenuation coefficient, and 4) pathological intracerebral calcifications. To study the natural history of these findings, repeat CT scans have been obtained on a periodic long-term follow-up basis. CT scanning was repeated between 1978-79, and again in 1981-82. Review of these scans has revealed that intracerebral calcifications have developed in approximately 20% of this patient group many years after the cessation of CNS preventive therapy. These results indicate the necessity for long-term follow-up by CT scan of patients who have received CNS preventive therapy. A detailed neuropsychological study has demonstrated for the first time a correlation between CT brain scan abnormalities and neuropsychological sequelae in long term survivors of ALL. Studies examining the growth of long-term survivors have demonstrated the value of measurement of spontaneous pulsatile growth hormone secretion in assessing the status of the hypothalamic pituitary axis in these individuals. In this study, basal growth hormone levels were measured every 20 minutes over a 24 hour period in a group of long-term ALL survivors and in a control group of age and Tanner stage matched normal children. The results indicate that perturbations of spontaneous pulsatile growth hormonal secretion are common following standard therapy for ALL, with marked blunting of the spontaneous pulsatile growth hormone secretion, indicating that this means of evaluation may be a sensitive way of detecting therapy related neuroendocrine damage. This study suggests that blunting of spontaneous pulsatile growth hormone secretion may contribute to the abnormalities in growth which are seen in children with ALL. Leydig cell function was assessed in boys treated with radiation therapy for testicular relapse. It had previously been assumed that this therapy spared testicular endocrine function. However, study of seven boys with this complication revealed that four had evidence of delayed sexual maturation with testosterone levels which were low for age and luteinizing hormone levels which were elevated. These data indicate that radiation treatment of testicular relapse compromises Leydig cell function and that hormone supplementation must be considered for these individuals.

D. Pharmacology of Orally Administered Antileukemic Agents

Study of the pharmacology of orally administered 6-MP, the backbone of maintenance therapy in ALL, has revealed that the bioavailability of oral 6-MP is extremely poor (approximately 16%), and that plasma 6-MP concentrations following uniform oral dosing are highly variable. That study demonstrated a 5-fold variation in the area under the plasma concentration-time curve (a measure of exposure of tissues to drug), following oral 6-MP administration of greater than 6-fold variation of peak plasma concentration, and an 8-fold variation in the time to achieving peak plasma concentrations following 6-MP administration. Notably, the peak level of 6-MP achieved by most

patients following oral 6-MP was approximately 1 log lower than that level shown to be optimal for cytotoxicity in in vitro systems. This study raises the question as to whether oral maintenance chemotherapy is being optimally delivered. This study has stimulated the generation of a new protocol evaluating prospectively the clinical pharmacology of p.o. methotrexate and 6-MP in patients with ALL undergoing maintenance chemotherapy. In addition to determining the inter- and intra-patient variability in plasma concentration of these drugs following oral administration, this study will attempt to correlate the results of prospective periodic pharmacokinetic bioavailability studies of these agents with relapse rate and remission duration. In addition, this study will be examining intra-patients variability in red blood cell methotrexate and 6-MP metabolites (see Project No. Z01-CM-06880-06 P).

Significance to Biomedical Research and the Program of the Institute:

Our studies on purine pathway enzymes reveal the presence of distinct biochemical differences among leukemic lymphoblasts of different immunologic subclasses and has revealed that development of a biochemical profile is a useful mechanism for classifying lymphoid malignancies in terms of their state of lymphoid differentiation. Most importantly, they have provided a rationale for the development of clinical strategies for treatment of lymphoid malignancy based on intervention in this biochemical process. The observation of estrogen receptors on lymphoid leukemic cells raises the possibility of a role for anti-estrogen treatment in a subset of patients with this disease. Study of immunoglobulin gene rearrangement has provided unique information as to the differentiation status of non-T, non-B leukemic lymphoblasts. Development of a murine model of in vitro immunotherapy for acute leukemia has provided a basis for further clinical study of this approach.

Our current ALL treatment protocol appears to be of major importance. Results to date suggest that the use of high-dose intravenous, protracted methotrexate infusions can substitute for cranial radiation and intrathecal methotrexate as CNS preventive therapy for the majority of patients with ALL. This study, which was stimulated by the results of our late effects studies which demonstrated CT scan neuroendocrine and intellectual compromise in some patients treated with cranial radiation, also appears to be the best available therapy for patients with average risk ALL.

Studies of the bioavailability of oral 6-MP have raised a crucial question regarding our method of delivering maintenance chemotherapy to patients with ALL. In addition, they have clearly demonstrated the value of pharmacologic monitoring in patients with cancer.

Proposed Course:

We are planning to expand our efforts evaluating the role of sex steroid receptors in acute leukemic lymphoblasts. In vitro studies are under way to assess the potential efficacy of anti-estrogen therapy. A trial of tamoxifen therapy for relapsed patients with ALL is a planned. Studies of immunoglobulin gene

formation in ALL patients are being pursued in an attempt to determine whether the abnormal clone is present in normal lymphocytes of patients in remission. This study may provide significant insight into the clonal origins of the leukemic process.

Entry of patients into the current clinical study for newly diagnosed patients with ALL will be completed by the summer of 1983. A subsequent study is planned which will extend the concepts set forth in this regimen. For average risk patients, the high-dose intravenous methotrexate approach utilized in the present protocol will be compared to the therapy currently being used in the BFM Study Group protocol. Poor prognosis patients will be treated both with intravenous high-dose methotrexate and with intravenous high-dose Ara-C in an effort to enhance our ability to prevent CNS disease via the systemic route of drug administration. Our studies of the bioavailability of orally administered anti-leukemic therapy is being expanded into a prospective study in which patients receiving standard maintenance chemotherapy with oral 6-MP and methotrexate will be periodically studied to determine the bioavailability of these two agents, as well as the accumulation of intra-erythrocyte metabolites of both drugs. The intention of this study is to correlate the results of these pharmacologic studies with remission duration and relapse rate in an effort to confirm the hypothesis that decreased bioavailability of orally administered drugs is a significant cause of treatment failure in patients with ALL.

Publications:

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7. Zimm, S., Collins, J.M., Riccardi, R., O'Neill, D., Narang, P.K., Chabner, B., and Poplack, D.G.: Variable bioavailability of oral 6-mercaptopurine: Is maintenance chemotherapy in acute lymphoblastic leukemia being optimally delivered? N. Engl. J. Med. 308: 1005-1009, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06880-06 P

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology;

Experimental Approaches to the Treatment of CNS Malignancy.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

David G. Poplack, Head, Leukemia Biology Section, NCI

COOPERATING UNITS (if any)

Clin. Pharm. Br. (NCI); Pharm (CC); Lab. of Chem. Pharm. (NCI); Boston Univ. Sch. of Med.; Children's Hosp. of Los Angeles; Catholic Univ. of Rome.

LAB/BRANCH

Pediatric Branch

SECTION

Leukemia Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.0

PROFESSIONAL:

4.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical pharmacology of antineoplastic agents used in the treatment of pediatric malignancies is studied. Emphasis is placed on the role of clinical pharmacologic monitoring and on both pre-clinical and clinical pharmacologic studies of Phase I agents. The clinical pharmacology of orally administered antileukemic agents has been evaluated and the limited bioavailability and variable drug levels of 6-MP achieved following oral administration has been documented. Studies are underway to determine the extent to which this phenomenon is the cause of treatment failure. The interaction of 6-MP and allopurinol, a unique example of hepatic first-pass metabolism in cancer chemotherapy has been examined in both subhuman primates and in man. Additional efforts to optimize 6-MP administration have been based on in vitro studies which have demonstrated a need for prolonged exposure to cytotoxic concentrations of drug to maximize leukemic cell kill. A clinical protocol evaluating prolonged intravenous 6-MP infusions in a Phase I setting is underway. Pre-clinical and clinical pharmacokinetic studies of the new agent, Tiazofurin, have been pursued and a pediatric Phase I study of this agent is in progress.

A major effort of this project is to study experimental approaches to the treatment of both meningeal and non-meningeal CNS malignancy. A unique sub-human primate model which allows sterile, repetitive access to cerebrospinal fluid, is utilized to study the CNS pharmacokinetics of various intrathecal and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities attendant upon various CNS treatments; and to evaluate and screen in a pre-clinical setting newer CNS treatment modalities and drug schedules. Information gained from the studies with this model is then applied to the design of clinical protocols used to treat patients with meningeal and non-meningeal malignancies. Current clinical studies of intrathecal AZQ and Concentration-x-Time intraventricular Ara-C are in progress. Pre-clinical studies evaluating intra-CSF drug administration via indwelling drug delivery devices is under way.

Other Professional Personnel:

S. Zimm	Investigator	P C
J. Collins	Senior Investigator	CPB C
R. Riccardi	Assistant Professor	Catholic Univ. of Rome
J. Holcenberg	Professor	Children's Hosp. of LA
J. Grygiel	Investigator	CPB C
P. Gormley	Senior Investigator	LCHPH C
P.K. Narang	Investigator	Pharm CC
D. Chatterji	Investigator	Pharm CC
R. Agarwal	Associate Professor	Boston Univ. Sch. Med.

Objectives:

1. To study the clinical pharmacology of antineoplastic agents used in the treatment of pediatric malignancies.
2. To perform pre-clinical and clinical studies on new agents with particular emphasis on those being used to treat pediatric malignancies and those with potential activity against CNS malignancies.
3. To explore a subhuman primate model which provides repetitive access to the cerebrospinal fluid and allows detailed study of the pharmacology and neurotoxicity of chemotherapeutic agents used to treat CNS malignancy.
4. To study the CNS pharmacokinetics of currently employed and potentially useful CNS antineoplastic agents.
5. To attempt to enhance the penetration of systemically administered drugs into the CNS through manipulation of the blood-brain barrier.
6. To assess the neurotoxicity of chemotherapeutic agents used in the treatment of malignancy.
7. To utilize neurophysiologic and neuropharmacologic information gained in the experimental primate system as a basis for designing new clinical approaches to the treatment of CNS malignancy in man.

Methods Employed and Major Findings:A. Clinical Pharmacology of Antineoplastic Agents1. Studies with 6-Mercaptopurine

We have intensively studied the clinical pharmacology of 6-mercaptopurine, the agent most commonly utilized for maintenance chemotherapy in acute lymphoblastic leukemia (see Project No. Z01-CM-06840-08 P). Using the new sensitive and specific high-pressure chromatography assay, we studied the pharmacokinetic effects of orally administered mercaptopurine in monkeys and in patients with acute lymphoblastic

leukemia in an attempt to determine the bioavailability of oral mercaptopurine and the degree of variability in its absorption. Our study demonstrated that the bioavailability of oral 6-MP was extremely limited (16% in man), highly variable, and much lower than had previously been estimated. There was a greater than 5-fold variation in the AUC (a measure of tissue exposure to drug), a greater than 6-fold variation in peak plasma concentration, and an 8-fold variation in the time to peak plasma concentration following p.o. 6-MP. These findings raise important questions regarding whether maintenance chemotherapy in ALL is being optimally delivered. In vitro studies using human leukemic cell lines have demonstrated the optimal cytotoxic concentration of 6-MP to be between 10^{-5} and 10^{-6} molar; their concentrations must be maintained for greater than 12 hours to achieve optimal cell kill. Such concentrations were achieved in only a small fraction of patients following p.o. administration of a standard 75 mg/m² dose of 6-MP, and these levels were maintained for only a brief period. Our in vitro cytotoxicity data provided a substantial rationale for the pursuit of a clinical, Phase I study which is currently under way, evaluating the toxicity and efficacy of prolonged intravenous infusion of 6-MP administered at a dose designed to provide cytotoxic drug levels in the plasma. In addition, we have studied the effect of allopurinol on the pharmacokinetics of both oral and parenteral 6-MP. Based on earlier work, it had been recommended that when allopurinol was given together with 6-MP, that the dose of 6-MP should be reduced by two-thirds to three-fourths. We studied the effect of allopurinol pretreatment on systemic levels of 6-MP achieved following both p.o. and i.v. 6-MP administration in monkeys and man. Our data indicated that pretreatment with allopurinol resulted in a 4-fold increase in the mean 6-MP levels in plasma. In contrast to its effect on p.o. 6-MP, allopurinol had no effect on the pharmacokinetics of i.v. 6-MP. Thus, our data indicated that although allopurinol markedly alters the pharmacokinetics of p.o. 6-MP, it has no effect on i.v. 6-MP. This discrepancy is probably due to the fact that 6-MP has a large first-pass metabolism presumably in the liver. The results of this study have obvious implications for the dosing of 6-MP in the presence of allopurinol.

2. Pre-clinical and Clinical Studies with Tiazofurin

Tiazofurin (TCAR) is a recently developed C-nucleoside which has demonstrated considerable antitumor activity in pre-clinical animal studies. TCAR administration produces guanine nucleotide depletion which results from inhibition of inosine monophosphate dehydrogenase by an active TCAR anabolite, thiazolecarboxamide adenine (TAD). In a pre-clinical study we examined the disposition of TCAR in plasma following doses equivalent to those contemplated for use in human studies. In addition to providing useful pharmacokinetic data this study also revealed excellent penetration of TCAR into the CSF following i.v. administration; the CSF plasma ratio was approximately 25%. With the exception of lethargy, the animals tolerated the experiments without untoward toxicity. These studies suggest that TCAR may have potential in the treatment of CNS malignancy. Our pre-clinical studies have extended to an ongoing

Phase I pediatric study. In addition to studying the toxicity/dose relationship of this compound, this study will evaluate the pharmacokinetics of this drug in children and will attempt to confirm our CSF findings observed in the non-human primates.

B. Pharmacokinetic Studies Using the Subhuman Primate Model

We have developed a subhuman primate system which allows for repetitive sterile sampling of CSF over an extended period of time in unanesthetized animals. This model involves the subcutaneous implantation of an Ommaya reservoir in rhesus monkeys. Numerous studies to date have demonstrated that this model is unique and provides CNS pharmacokinetic data which are similar to that obtained in man. This model has been used in a variety of ways. We have investigated potential methods of improving methotrexate therapy to the central nervous system and have demonstrated that administration of methotrexate by the hyperbaric intrathecal technique results in improved cerebrospinal drug distribution. We have also studied the influence of body position on ventricular cerebrospinal fluid methotrexate concentrations following intralumbar administration, and have shown that maintenance via the flat or Trendelenburg position for at least one hour following intralumbar administration of methotrexate results in substantially greater drug levels than in ventricular CSF. We have also studied the distribution of methotrexate within the CSF following high-dose intravenous methotrexate infusions, an approach currently being studied clinically in man. The pharmacokinetics of a variety of other antineoplastic agents have been studied in this model, not only with respect to their penetration following i.v. administration into the CNS, but also their CSF pharmacokinetics following intrathecal injection. Agents evaluated include L-asparaginase, m-AMSA, dihydroxine anthracine dione, the combination of cytosine arabinoside and tetrahydrouridine, aclacinomycin, and interferon. Examples of studies currently under way include the following:

1. Cerebrospinal Fluid Pharmacokinetics of Intrathecal Diaziquone (AZQ)

AZQ, a recently developed aziridinyl benzoquinone, has demonstrated activity against CNS neoplasms. We have evaluated AZQ for possible use as an intrathecal agent to treat meningeal neoplasia. Following intraventricular administration in monkeys implanted with the Ommaya reservoir, the CSF half-life of AZQ was found to be extremely short (approximately 32 minutes). This rate of AZQ clearance (0.2 ml/min) exceeds that of CSF bulk flow, and indicates that metabolism and/or transcapillary passage may be important clearance mechanisms for this drug. However, in spite of its rapid clearance, substantial AZQ levels were achieved in lumbar CSF following intraventricular injection. Our studies demonstrated that following intraventricular administration, ventricular and lumbar CSF AUCs were 20- and 4-fold higher respectively, than the CSF AZQ AUC achieved by systemic administration of 50 times the intraventricular dose. Furthermore, no acute or chronic neurotoxicity was observed following intraventricular AZQ in monkeys. Our primate studies suggest that there is a substantial pharmacological advantage for the intraventricular administration of AZQ in the treatment of

meningeal neoplasia. These promising results have led to the development of a Phase I-II study of intraventricular AZQ which is currently under way in man.

2. CSF Pharmacokinetics of Cytosine Arabinoside (Ara-C)

We have also utilized the subhuman primate model to study the CSF pharmacokinetics of Ara-C. Preliminary studies in monkeys led to a more comprehensive evaluation in patients. We have demonstrated that following intraventricular administration of Ara-C (30 mg), extremely high CSF levels of Ara-C can be obtained, with undetectable levels of Ara-C in plasma. These therapeutic CSF levels are achieved for a 24 hour period following a single intraventricular administration. Clearance of Ara-C from CSF was noted to be .42 ml/min, suggesting that the drug is primarily cleared by CSF bulk flow. Current studies are evaluating a "Concentration-x-Time" approach via the intraventricular route in both monkeys and in man.

C. Studies on the Neurotoxicity of Methotrexate and/or Cranial Radiation

We have developed a subhuman primate model of methotrexate leukoencephalopathy. Studies in our model have confirmed the synergistic role of methotrexate plus cranial radiation in the pathogenesis of this entity. Current studies are evaluating the uptake of the storage form of methotrexate (methotrexate polyglutamates) in brain following chronic methotrexate administration. These studies performed in animals with and without cranial radiation are designed to elucidate a possible mechanism of methotrexate related CNS damage.

D. Enhancement of Drug Entry into the CNS Following Systemic Administration

The blood-brain barrier constitutes the major obstacle to successful systemic treatment of CNS malignancy. We are exploring, in the subhuman primate model, a variety of methods aimed at enhancing the delivery of systemically administered agents into the central nervous system. Current studies are evaluating the ability of psychotropic agents which have been demonstrated to provide temporary blood-brain barrier opening to enhance the delivery of systemically administered methotrexate and 6-MP to the CNS.

Significance to Biomedical Research and the Program of the Institute:

Rational treatment of pediatric malignancies requires a detailed knowledge of the clinical pharmacology of those antineoplastic agents used therapeutically. Our pre-clinical and clinical studies are providing substantial information of considerable clinical importance. For example, the studies on the bioavailability of 6-MP have raised questions as to the optimal method of delivery of maintenance chemotherapy in ALL. This study has already led to new approaches to chemotherapy in patients with acute lymphoblastic leukemia. Pre-clinical and clinical studies of new agents of potential value in treating pediatric malignancies is an integral and necessary part of any investigative pediatric oncology program. This is particularly true because the majority of agents

used to treat pediatric malignancies have been demonstrated to have different dose toxicity relationships in children than in adults. Our recent Phase I studies on the combination of Ara-A and 2'-deoxycoformycin (see Project No. Z01-CM-06840-08 P) and a current study of Tiazofurin (TCAR) represent clinical studies of two of the most exciting new drugs currently being evaluated for potential use in children.

Optimal treatment of central nervous system neoplasms requires a detailed knowledge of the physiology of the blood-brain barrier in addition to a clear understanding of the CNS pharmacokinetics of antineoplastic agents. Because detailed pharmacologic investigation of humans is limited by the lack of a ready route of access to cerebrospinal fluid, we have developed a unique subhuman primate model which facilitates such studies in a setting that approximates the human situation. This model also provides for the study of chemotherapy and radiotherapy-related neurotoxicity allowing for delineation of factors predisposing to toxicity, as well as for identification of methods useful in monitoring its development. Successful study of the CSF pharmacology of a variety of agents in this subhuman primate model has already led to a number of clinical trials in man which are investigating unique and new approaches to the treatment of both meningeal and non-meningeal CNS malignancy.

Proposed Course:

Our clinical studies of 6-MP will continue and will be expanded. Our data on limited bioavailability of this compound has led to the development of a prospective nationwide study being run by our group which will examine the relationship between the bioavailability of p.o. administered maintenance chemotherapy (both 6-MP and methotrexate) to treatment failure in patients receiving maintenance chemotherapy for ALL. The current intravenous, prolonged 6-MP infusion Phase I study will continue and will be evaluating not only the efficacy of this agent against leukemias, but also its potential against solid tumors. In addition, information generated in studies in our subhuman primate model which suggest the potential possibility of intrathecal administration with 6-MP will be pursued in a clinical, Phase I study. Our Phase I study with Tiazofurin (TCAR) will continue. Particular emphasis will be given to evaluating our findings relating to the apparently favorable CSF:plasma ratio of this compound, and thus its potential to treat CNS malignancy. Use of the primate model to evaluate agents of potential value in treating CNS malignancy will continue. A variety of agents are currently under study. Emphasis is being placed on evaluation of the intravenous approach to the treatment of CNS malignancies, and studies are under way to assess the ability of various compounds to favorably enhance the penetration of intravenously administered compounds into brain tissue. In addition, continuous intra-CSF drug delivery via indwelling continuous infusion pumps has a high priority for study within this project. Several agents are being evaluated for their potential administration and study via this mechanism, both in subhuman primates and man. Finally, continued comprehensive study of post-therapy neurotoxicity is planned, with particular emphasis on uncovering the pharmacologic mechanisms for its development.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06890-04 P
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PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Lymphoma Biology and Epstein-Barr Virus

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)
(Name, title, laboratory, and institute affiliation)
Ian T. Magrath, Senior Investigator, Pediatric Branch

COOPERATING UNITS (if any)
Flow Cytometry Laboratory, George Washington University; Medicine Branch, NCI; Laboratory of Pathology, NCI; Metabolism Branch, NCI; Wistar Institute, Philadelphia; Laboratory of Clinical Investigations, NIAID

LAB/BRANCH
Pediatric Branch, NCI

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5	PROFESSIONAL: 3	OTHER: 2
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CHECK APPROPRIATE BOX(ES)
 (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
 Undifferentiated B-cell lymphomas occur predominantly in children and young adults in a) a geographically delineated form, which is EBV associated, b) a sporadic, widespread form which is not EBV associated, and c) a form arising in certain immunodeficiency syndromes notably that occurring predominantly in homosexual drug abusers, which is also EBV associated. The goals of the present work are to gain information on the epidemiology, pathogenesis and biological differences among the several forms of undifferentiated lymphoma. Links have been established with a number of cancer centers in various parts of the world as part of a concerted effort to characterise, with more precision than has hitherto been achieved, differences in the spectrum of lymphoid neoplasia that occurs in different environments. Biological studies are carried out on cell lines derived from all three of the above categories of undifferentiated lymphomas. Of particular interest is the expression of genes involved in specific chromosomal translocations associated with these tumors, namely the immunoglobulin genes and c-myc oncogene, and changes in the expression of these genes during differentiation of the cell lines. Phorbol esters induce plasmacytoid differentiation, characterized by ultrastructural and immunological changes, in most of the cell lines. Such studies may provide information as to whether c-myc transcription is influenced by promotor or enhancer sequences normally concerned with immunoglobulin gene regulation. A study of the differential expression of surface antigens in different phases of the cell cycle and in different growth phases has been carried out to determine whether antigen expression is altered in cells which are not rapidly proliferating, e.g., by virtue of poor nutrient supply, and to begin to comprehend what factors govern surface antigen expression and how surface antigen density is maintained during cell doubling. The differences between African and American Burkitt's lymphoma are being further characterized. The B2 antigen, for example, present in activated lymphocytes is expressed by African rather than American cell lines. A detailed analysis of differences in complement receptors has been completed.

Personnel List

Hauke Sieverts	Guest Worker	P C
David Benjamin	Visiting Fellow	P C
Oliver Alabaster	Assistant Professor	GWU
Thelma Gaither	Research Biologist	LCI I
George Tsokos	Visiting Associate	A&R A
Jacqueline Whang-Peng	Senior Investigator	MB C
Stanley Korsmeyer	Senior Investigator	MET C
Robert Maguire	Medical Staff Fellow	DCCP C
Carol Janus	Guest Worker	P C
Carlo Croce	Deputy Director	Wistar Inst. Philadelphia

Objectives:

1. To obtain tumor derived cell lines from lymphoma patients and to use these in the study of lymphoma biology.
2. To determine the normal counterpart cell of Burkitt's lymphoma and undifferentiated lymphomas. These studies include characterization of the expression of cell surface proteins, and alterations in phenotype induced by a variety of differentiation-inducing agents including phorbol esters.
3. To study the alteration in cell surface proteins which occur through normal progression through the cell cycle, and also in different growth phases.
4. To investigate the significance of the cytogenetic abnormalities seen in undifferentiated lymphomas.
5. To determine the nature of the association of Epstein-Barr virus (EBV) with Burkitt's lymphoma.
6. To study the nature of regulatory influences on EBV infected cells.

Methods and Major Findings:A. Derivation of New Cell Lines

Two new cell lines have been derived from patients with Burkitt's lymphoma. These patients probably have the acquired immunodeficiency syndrome (AIDS). One of these lines has an 8;22 translocation and the other has an 8;14 translocation. Very little information has been available to date as to the occurrence of karyotypic changes in lymphomas in patients with the AIDS syndrome. The cell line with the 8;22 translocation is of additional interest since it expresses IgM kappa, although Lenoir et al. have reported concordance of translocations involving chromosomes which bear light chain genes, and the type of light chain expressed. Our finding has implications regarding genetic rearrangements in normal cells and the timing of the translocation itself with regard to VJ joining, since one lambda gene is rearranged even though the cells make kappa light chain. The cells also produce EBV.

B. Induction of Differentiation in Undifferentiated Lymphoma Cell Lines

We have shown that phorbol esters (TPA) will induce plasmacytoid differentiation to a greater or lesser degree in the majority of cell lines derived from patients with undifferentiated lymphomas. The cell line most susceptible to TPA is JD 38. TPA induces a marked increase in IgM secretion associated with ultrastructural changes (including the formation of parallel arrays of rough endoplasmic reticulum) consistent with plasmacytoid differentiation. Some alterations in surface antigens are also induced. Chemical studies carried out in collaboration with Dr. Korsmeyer have shown that these changes are accompanied by an increase in the synthesis of new messenger RNA, which is due to an increase in the amount of message specific for secreted IgM. Coincident with the increase in new messenger RNA is a small increase in c-myc RNA. Thus, expression of this oncogene and of the $c\mu$ genes appear to be concordant. This is consistent with the hypothesis that in cells with a translocation, c-myc is under the influence of sequences which enhance immunoglobulin gene expression.

C. Alterations in the Expression of Surface Antigens During the Cell Cycle and Different Growth Phases

Detailed studies have been carried out using a fluorescence activated cell sorter (FACS) on cells simultaneously stained with fluoresceinated antibodies against specific surface proteins, and propidium iodide. By this means the density of certain antigens can be measured simultaneously with the DNA content of individual cells. We have shown that there is a progressive increase in the expression of 4 antigens (IgM, β 2-microglobulin, HLA complex and B75) as cells progress from G1 to G2/M, reaching an approximately 1.6 fold higher value at G2/M, thus maintaining surface antigen density as surface area increases with cell doubling. However, during a period of subculture without medium replenishment there is a progressive decline in the quantity of surface antigens. These changes cannot be accounted for by the reduction in S and G2 M cells which occurs during a 5 day growth period without replenishment of culture medium. In fact analysis by means of DNA content histograms of individual cell cycle phases shows that cells in each phase undergo a progressive reduction in the expression of these antigens. Thus, cells which approach saturation density have significantly lower quantities of several surface antigens. A second finding is that surface IgM and β 2-microglobulin increase in total quantity on individual cells throughout the G1 period, but then do not increase further during the S-phase. During this time, the cell size as shown by dry mass measurements is progressively increasing in a linear fashion. Thus, in late G1 there is an increase in cell surface antigen density with a subsequent decrease in density during S-phase. It is not clear as to whether these changes are occasioned by alterations in the rate of synthesis of surface antigen, or changes in the turnover of these antigens. In any event, it seems possible that cell surface antigen density may be one of the factors regulating antigen synthesis. Preliminary results of the regeneration of surface antigens following pronase treatment of cells indicate that antigen appears on the cell surface simultaneously in all cell cycle phases.

D. Characterization of Complement Receptors

Further studies on complement receptors of Raji cells have indicated that the C3b receptor on this cell line differs markedly from the C3b receptors on normal lymphoblastoid cell lines derived from cord blood lymphocytes or patients with infectious mononucleosis. Unlike the latter cell types, the Raji receptor is capable of interacting with soluble but not cell bound C4b. Further, EAC3b rosette formation was severely limited by increasing ionic strength whereas the binding of EAC3bi was only moderately decreased at physiological ionic strength. In striking contrast, EAC3bi binding to monocytes, polymorphs and human erythrocytes was markedly reduced as ionic strength increased but EAC3b binding to these cells was less sensitive to changes in ionic strength. Thus, even complement receptors which bind the same complement subcomponent differ markedly on different cell types. These differences in binding conditions may have major physiological implications.

E. Collaborative Studies on Genetic Rearrangements in Lymphoma Derived Cell Lines

A number of our cell lines have now been studied by Dr. Carlo Croce with regard to the precise locations of chromosomal breakpoints in Burkitt's lymphoma. It appears that the breakpoints on both the 8th chromosome and the immunoglobulin chromosomes may vary within a given chromosomal region such that reciprocally exchanged material may differ quite markedly from one cell line to another. In the PA682 cell line we have shown that IgM kappa is produced whereas the cell line contains an 8;22 translocation. At the DNA level both kappa genes are rearranged and one of the lambda genes is rearranged. These findings raised questions about the proposed hierarchy of immunoglobulin gene rearrangements and also raises the question as to whether chromosomal translocations may take place unassociated with VJ joining. In this cell line the c-myc gene is not rearranged and it remains to be determined whether it has been translocated from chromosome 8 to chromosome 22. Methods have now been developed using a combination of sephacryl chromatography and ELISA techniques to assay free light chains. Correlations will be made between the synthesis of various immunoglobulin chains and genetic rearrangements in the individual cell lines.

Significance to Biomedical Research in the Program of the Institute:

The detailed characterization of cell lines derived in this laboratory from patients with lymphomas has proved to be extremely fruitful with regard to an increasing understanding of the origins and pathogenesis of undifferentiated lymphomas. In this regard, studies carried out in other laboratories with these and similar cell lines have been pivotal in understanding the nature of the chromosomal translocations which occur in Burkitt's lymphoma. Currently, it appears very likely that alterations in c-myc gene transcription occasioned by either its relocation at another chromosomal site or the alteration in neighboring DNA sequences on chromosome 8 may be an essential component of the pathogenesis of these tumors. Combined molecular, biologi-

cal and immunological studies promise to increase an understanding of the mechanism whereby c-myc gene transcription is altered. The ability to influence the expression of certain genes in these cell lines by means of phorbol esters may also prove invaluable to a further dissection of both the nature of the differentiation block and the events which occur during normal B-cell differentiation. Studies of the regulation of surface antigen density will be important to a rational design of either *in vivo* or *ex vivo* therapeutic manipulations and possibly also to diagnosis utilizing monoclonal antibodies. It will be important to determine whether cells at a specific part of the cell cycle or in a growth phase equivalent to saturation density are protected by virtue of minimal expression of specific antigens. Treatment of cells with cytotoxic agents or monoclonal antibodies will probably also influence the expression of surface antigens. Further, since the surface antigens represent, in many cases, receptors for signals from the immediate cellular environment, it may prove possible to alter cell behavior by binding certain surface antigens with monoclonal antibodies. Anti-IgM, for example, inhibits proliferation of our lymphoma-derived cell lines, but not lines derived from normal lymphocytes.

Proposed Course:

We plan in the future to give greater emphasis to studies correlating molecular changes in immunoglobulin genes and oncogenes, with the expressed phenotypic characteristics of the cell lines. In particular, baseline synthesis of membrane and secreted immunoglobulins including free-light chains as well as the response of the cell to phorbol esters will be correlated with specific genetic rearrangements. We shall develop methods to attempt to determine whether abnormal immunoglobulin molecules are generated, which could interfere with the normal process of cell division and cellular interactions. Attempts will be made to generate new monoclonal antibodies to specific cell surface components, and to study the effect of these antibodies on cellular differentiation and proliferation. The influence of chemotherapeutic agents and the binding of monoclonal antibodies on the expression of several surface antigens will be investigated. Collaborative studies will be undertaken in continued attempts to elucidate the nature of the association of Epstein-Barr virus with Burkitt's lymphoma.

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ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1982 - September 30, 1983

The Radiation Oncology Branch (ROB) of the National Cancer Institute continues in transition. In the summer of 1982, the Branch moved into its new clinical facility. Further space on 1B is in the process of being renovated so that the Physics and laboratory sections can move to 1B before renovations of B3 take place. The renovations on B3 are pivotal before the final staffing situation can be completed. Space has been at a premium in this Department throughout the last 5 1/2 years, since Dr. Glatstein became Chief of the Department.

The three main goals of the Radiation Oncology Branch continue unchanged:

- 1) Major emphasis on clinical trials of combined modality nature, predominantly collaborative with other clinical branches.
- 2) Strong radiation biology program with heavy emphasis on basic science and clinical questions of relevance.
- 3) A training program in radiation therapy, equivalent in stature to the programs in Medical, Surgical and Pediatric Branches within the National Cancer Institute.

At the moment all these goals are being met. The biology program has suffered greatly because of difficulty in optimizing problems with a holding facility for animals. Indeed, this is the major reason why Dr. Travis left the Biology Section in the Spring of 1982. The present B2 animal facility in Building 10 is simply inadequate for long-term experimentation which is the main thrust of the in vivo work for this Branch. It has caused the Radiation Oncology Branch to plan a proportion of its future renovations after the B3 facility is completed to take the 1B space in the future and make it into an animal facility.

The in vitro laboratory program has studied radiation and experimental chemotherapy, radiation modifying agents, and hyperthermia as well. Eventually we hope to experiment in the area of photo sensitivity. Much of the present work has been centered on human CFUC and human tumor cell lines, in collaboration with other Branches.

As far as the training program is concerned, approval has been obtained from the AMA of Residency Review Committee, for the Uniformed Services University of Health Sciences, working through Walter Reed Army Medical Center and the National Naval Medical Center in Bethesda as well as the National Cancer Institute to have a three year training program under the direction of Dr. Eli Glatstein. Half of this will be spent within the National Cancer Institute and half within the military structure. A need for this integrated program reflects the complementary nature of the clinical material of the various hospitals, with gynecologic, head and neck and genitourinary cancers in abundance at the military hospitals, and almost completely lacking within the Cancer Institute base. Two of the four positions for training come from the military.

The clinical program within the Radiation Oncology Branch is centered on combined modality studies. Many of these are collaborated with other Branches, the most important of which are small cell carcinoma of the lung and mycosis fungoides, both in collaboration with the National Cancer Institute Navy Oncology Branch. Ongoing studies suggest benefits of combined modality treatment for these two diseases. However, additional patients are required before final conclusions can be made, since they represent prospective randomized studies. There are also collaborative ventures with the Surgery Branch in soft tissue sarcomas and with the Pediatric Branch in Ewing's sarcoma and rhabdomyosarcoma. Pilot studies have begun on ovarian cancer in conjunction with the Medicine Branch, as well as the studies of lymphomas with the Medicine Branch.

Primary Radiation Oncology Branch studies center around intraoperative radiation therapy. The new facility is not yet completed as far as the intraoperative suite is concerned. Thus, patients are still transported through the hallways under anesthesia when such surgery and intraoperative therapy is planned. Large single doses of electron beam treatment are applied intraoperatively to the tumor bed with critical normal viscera moved out of the way. This has been done in conjunction with misonidazole. These have been extremely difficult management problems, constituting primary pancreatic and gastric cancer and retroperitoneal soft tissue sarcomas. At the present time the treatment definitely appears to be safe. The actual efficacy is still unclear. The studies are considered first steps to later studies which will incorporate chemotherapy as well.

Intravenous misonidazole was studied in some detail and the pharmacology was delineated. The intravenous compound was studied in a randomized fashion with carcinoma of the esophagus and was shown not to be effective. In a one arm study for locally unresectable osteogenic sarcoma and chondrosarcoma there are a few patients who have received misonidazole and have had impressive responses to treatment despite the fact that these tumors are generally considered "radio-resistant". Indeed, one patient has been followed for over four years without any evidence of growth of the tumor mass.

Another major Radiation Oncology Branch study revolves around Stage I and II breast cancer. In this randomized study radical surgery is compared to definitive irradiation with the preservation of the breast. This study, in conjunction with the Surgery Branch has accrued over 115 patients in the first 3 1/2 years. This is considered a major accomplishment, in view of the fact that no prior patient base has been recruited for such a study here at the National Cancer Institute and the difference of the two arms therapeutically makes for difficult randomization. It is far too soon to make any conclusion, but at the moment there is no obvious superiority of either arm suggesting that the long term results of treatment may well be comparable.

Under the direction of Jan van de Geijn, CT scanning has been fully incorporated in our radiotherapy treatment planning. Virtually all patients who are treated with curative intent are now scanned in the treatment position and computerized treatment plans are routinely generated, superimposed on CT cross sections. Dr. van de Geijn has developed a program which allows for adequate dose calculations, even when blocks are placed in the field. It is also possible to account for tissue inhomogeneity

as well. The treatment plans now generated from within the Radiation Oncology Branch are extraordinarily sophisticated compared to what can be done in other medical centers with commercial units. On the other hand, the down time of the CT scanner itself has been a major limitation, along with the relatively small aperture available on the EMI 5005. This unit will soon be supplused.

In the long-range plans, the intraoperative program is considered high priority because it offers a unique opportunity in which to combine sensitizing drugs, and even hyperthermia in the treatment of abdominal neoplasms. It offers precise localization of the tumor and the ability to eliminate critical normal tissues, or at least protect them from high doses of irradiation. We believe it will ultimately prove to be an ideal approach for retroperitoneal nodes as well as pelvic neoplasms.

New areas of investigation include our study with the Medicine Branch on advanced cervix cancer, using radiation therapy and adjuvant chemotherapy. In addition, studies of electron beam therapy for AIDS patients who have Kaposi's sarcoma limited to the skin are also under way. In this investigation, emphasis is placed on identifying a minimal doseresponse curve from which time-dose-fractionation schedules for whole skin electron beam treatment will evolve. In addition, before the end of this fiscal year, the Radiation Oncology Branch expects to have a study going in bladder cancer, in conjunction with the Surgery and Medicine Branches. The main thrust of the Radiation Oncology Branch role will be to investigate the role of interstitial implantation in dealing with bladder cancer. We're particularly anxious to take advanced lesions and implant them with I-125 labelled suture material.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 00650-28 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Service Radiation Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Allen S. Lichter, M.D. Head, Radiotherapy Section, ROB, NCI

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5

PROFESSIONAL:

2

OTHER:

3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objective of this project is to provide expert radiotherapy, consultation, and radiation therapy treatment for Clinical Center patients admitted to services other than the Radiation Oncology Branch of the NCI. Support is given to the Medicine Branch, Surgery Branch, Pediatric Branch, NCI/Navy Medical Oncology Branch, Neurosurgical Service, Endocrine Service, and other Federal Hospitals in the area where technical expertise and technical equipment dictate a need for such consultation.

Project DescriptionProfessional Personnel Engaged on the Project:

Tim Kinsella, M.D.	Senior Investigator	ROB	NCI
Peggie Findlay, M.D.	Senior Investigator	ROB	NCI
Marilyn Glover, R.N.	Clinical Nurse	ROB	NCI
Andrea Zola, R.T.	Medical Radiation Technician	ROB	NCI
Joy Greig, R.T.	Medical Radiation Technician	ROB	NCI
Barbara Kelly, R.T.	Medical Radiation Technician	ROB	NCI
Ardith Wilson, R.T.	Medical Radiation Technician	ROB	NCI
Carol Kieda, R.T.	Medical Radiation Technician	ROB	NCI
Betty Adams, R.T.	Medical Radiation Technician	ROB	NCI

Methods Employed

Formal and informal consultation with referring physicians and application of radiation therapy where appropriate with x-rays and electrons in accordance with standard radiation therapy practice as well as modified programs where necessitated by adjuvant cocomitant therapies.

Major Findings

There were 700 patients seen in formal consultation and an additional 300 (approximately) telephone conversations provided "ad hoc" advice on treatment or general information. Approximately 450 patients will be treated in this fiscal year with the majority of these being protocol patients in the Radiation Oncology Branch or on collaborative studies.

Proposed Course

To continue.

Publications

1. Kinsella, T.J., Fraass, B.A., and Glatstein, E.: Late effects of radiation therapy in the treatment of Hodgkin's disease. Cancer Treat. Rep. 66: 991-1001, 1982.
2. Bader, J.L. and Glatstein, E.: Germ cell tumors of the testis. Comprehensive Therapy 9: 41-49, 1983.
3. Gorden, P., Glatstein, E., and Roth, J.: Acromegaly. In Krieger, D.T. and Bardin, C.W., (Eds.): Current Therapy in Endocrinology. Philadelphia, B.C. Decker, 1983, pp. 43-48.

4. Kinsella, T.J. and Glatstein, E.: Staging laparotomy and splenectomy for Hodgkin's disease: Current status. Cancer Invest. 1(1): 87-91, 1983.
5. Kinsella, T.J. and Glatstein, E.: Management of Acute and Late Effects of Radiation Therapy on Normal Tissues. In Wiernik, P.H., (Ed.): Supportive Care of the Cancer Patient. Futura Publishing Company, 1983, pp. 325-348.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 00684-28 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nonclinical Irradiation Services

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jan van de Geijn, Acting Head, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.4

PROFESSIONAL:

.1

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Radiation Physics and Computer Automation Section provides radiation physics services, equipment, and advice on experiments involving radiobiology. Cells, tissue cultures, mice, rats and dogs were irradiated for radiobiology experiments. Current involvement concentrates on I^{125} dosimetry related to monoclonal antibody studies.

Project Description

Professional Personnel Engaged on the Project:

F. Harrington	Engin. Tech.	ROB	NCI
B. Fraass, Ph.D.	Staff Fellow	ROB	NCI
R. Miller	Health Physicist	ROB	NCI
J. Doolittle	Electronic Tech.	ROB	NCI

Objectives: To provide radiation physics expertise and equipment to researchers involved with radiobiological projects.

Methods Employed

Dosimetric investigations have been made to assist radiobiologists in irradiating cells, tissue culture, mice, rats, and dogs using both linear accelerators and the 250 kVp X-ray unit. Many devices have been fabricated to hold animals in specific positions relative to the radiation beams while shielding certain critical organs.

Major Findings

Cells, tissue cultures, mice, and rats were irradiated. A new ^{60}Co unit was installed and calibrated for radiobiology. Basic methodology was developed.

Significance to Biomedical Research and the Program of the Institute

Radiation physics support is essential to the Radiation Biology Section of the Radiation Oncology Branch.

Proposed Course

To be continued. Continuing technical support will be provided.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 00998-05 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Radiation Sensitizers in Carcinoma of the Esophagus

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James Schwade, M.D. Senior Investigator, ROB, NCI

COOPERATING UNITS (if any)

Surgery Branch, COP, DCT, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project was to study the radiation sensitizing compound misonidazole and its ability to increase the effect of radiation sterilizing neoplastic cells. Patients with carcinoma of the esophagus clinically confined in the mediastinum received radiation with or without intravenous misonidazole, hypoxic cell sensitizer. Survival, freedom from relapse, local control and morbidity were evaluated.

Project Description

Professional Personnel Engaged on the Project:

E. Glatstein, M.D. Chief ROB NCI

Objectives: By assessing the survival, freedom from relapse, and tumor response, we hoped to be able to determine whether or not misonidazole is effective in augmenting radiation effects in patients with carcinoma of the esophagus. If this compound appeared to increase radiation effectiveness, then further work in radiosensitizing compounds would be clearly warranted.

Methods Employed

Patients with previously untreated carcinoma of the esophagus confined clinically to the mediastinum were accessioned and treated with irradiation. Half the patients received, on a randomized basis, intravenously administered misonidazole with each fraction of irradiation.

Major Findings

Twenty-six patients completed this study. There was no improvement in complete response rate or local control or long-term survival in patients who received the radiosensitizer. Indeed, the only two survivors that we have after three years are on the control arm. The radiation fractionation scheme appeared to be excellent from the standpoint of palliation, but little evidence of radiosensitization was seen.

Significance to Biomedical Research and the Program of the Institute

Radiation sensitizing drugs may represent an avenue to increase effectiveness of therapy by augmenting the effects of an existing modality, i.e., radiation. This study represents the only controlled randomized study to assess the efficacy of this compound in carcinoma of the esophagus when administered IV and given with every radiation fraction. Misonidazole showed no suggestion of benefit, and thus the study was terminated.

Proposed Course - Study terminated.

Publications

1. Dunnick, N.R., Schwade, J.G., Martin, S.E., Johnston, M.R., and Glatstein, E.: Interstitial pulmonary infiltrate following combined therapy for esophageal carcinoma. Chest 81: 453-456, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06310-04 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Surgery versus Radiation Therapy in Treatment of Primary Breast Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Allen S. Lichter, M.D. Head, Radiotherapy Section, ROB, NCI

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI; Surgery Branch, NCI; Medicine Branch, NCI; Nursing Department, C.C.

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5

PROFESSIONAL:

2

OTHER:

3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study whether techniques of treatment that preserve the breast (lumpectomy followed by radiation therapy) provide equal survival opportunity when compared to women treated with standard surgical techniques (mastectomy) for primary breast cancer. After a work-up confirms localized disease, the patients are randomized to treatment with either mastectomy or lumpectomy plus radiation therapy. Both groups have axillary nodes dissections and are treated with chemotherapy should the nodes be positive.

Project Description

Professional Personnel Engaged on the Project:

Staff within the Radiation Oncology Branch

Objectives: If survival and recurrence figures are comparable for the two treatments, it should be far more acceptable for women to be treated with less than radical surgical procedures for localized breast cancer. The cosmetic result of localized treatment will be carefully evaluated. The psychological, sexual, and sociological impact of mastectomy versus lumpectomy will be noted. The ability to combine radiation therapy with aggressive chemotherapy in node positive patients will also be assessed.

Methods Employed

Patients with previously untreated carcinoma of the breast clinically and radiographically confined to the breast and axillary lymph nodes will be accessioned into the study. They will be randomized to have treatment with lumpectomy and radiation therapy versus mastectomy. Patients with positive axillary lymph nodes will receive chemotherapy.

Major Findings

This study has been active for 46 months. Currently 118 patients are enrolled and it is too early to assess results. We have shown that it is possible to deliver adjuvant chemotherapy to patients being irradiated for primary breast cancer. We have developed new techniques for radiotherapy treatment planning using CT scans. We have an ongoing assessment of the psychosocial impact of our therapies.

Publications

1. Lichter, A.S., Fraass, B.A., Fredrickson, H.A., Roberson, P.L., and van de Geijn, J.: Computed Tomography in Treatment Planning: Primary Breast Cancer. In Ling, C.C., Rogers, C.C., and Morton, R.J. (Eds.): Computed Tomography in Radiation Therapy. New York, Raven Press, 1983, pp. 99-107.
2. Lippman, M., Lichter, A.S., Edwards, B., Gorrell, C.R., d'Angelo, T., and deMoss, E.V.: The impact of primary irradiation treatment of localized breast cancer on the ability to administer systemic adjuvant chemotherapy. Journal of Clinical Oncology (in press).
3. Schain, W., Edwards, B., Gorrell, C.R., deMoss, E.V., Lippman, M., Gerber, L., and Lichter, A.S.: Psychosocial and physical outcomes of primary

breast cancer therapy: Mastectomy versus excisional biopsy and irradiation. Breast Cancer Research and Treatment (in press).

4. Lichter, A.S., Fraass, B.A., van de Geijn, J., and Padikal, T.N.: A technique for field matching in primary breast irradiation. Int. J. Rad. Onc. Biol. Phys. 9: 263-270, 1983.
5. Srinivasan, G., and Lichter, A.S.: Pleural-based changes on chest x-ray after irradiation for primary breast cancer: Correlation with findings on computerized tomography. Int. J. Rad. Onc. Biol. Phys. (in press).
6. Lichter, A.S.: The current status of treatment of primary breast cancer with local excision plus radiation. Current Concepts in Onc. (in press).
7. Lichter, A.S., and Padikal, T.N.: Treatment Planning in the Treatment of Breast Cancer. In Bleeher, N., and Glatstein, E. (Eds.): Radiation Therapy Treatment Planning. London, Dekker, 1983, pp. 639-662.
8. Roberson, P.L., Lichter, A.S., Bodner, A., Fredrickson, H.A., Padikal, T. N., Kelly, B.A., and van de Geijn, J.: Dose to lung in primary breast irradiation. Int. J. Rad. Onc. Biol. Phys. 9: 97-102, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06313-04 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dose to Lung and Opposite Breast vs. Technique for Primary Breast Irradiation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Benedick Fraass, Staff Fellow, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

Computer Systems Laboratory, DCRT, NIH

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.1

PROFESSIONAL:

.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Treatment planning techniques for primary breast irradiation are investigated to optimize dose to areas at risk while minimizing dose to critical structures. When the high-dose volume is increased to include the internal mammary chain (IMC), dose to lung and opposite breast increase. This effect has been investigated extensively with both treatment planning and dose measurements.

Project Description

Professional Personnel Engaged on the Project:

A. Lichter, M.D.	Senior Investigator	ROB	NCI
J. van de Geijn, Ph.D.	Expert	ROB	NCI

Objectives: To quantify the dose to critical structures, such as lung and opposite breast, as a function of treatment techniques which include the internal mammary chain (IMC) within the high-dose volume.

Methods Employed

Dose distributions for many treatment techniques were simulated using CT scans from 15 patients. Dose to opposite breast was also calculated. Extensive film, thermoluminescent dosimetry (TLD) and ion chamber measurements have been made in water and polystyrene phantoms. These measurements have been used to verify the computer results and have been related to the surface TLD measurements made on patients under treatment.

Major Findings

Typical radiographic verification and simulation films are misleading with respect to the volume of lung irradiated. No single technique is optimal for all patients. Dose to the opposite breast has been quantified. The dose to the opposite breast may be decreased significantly through several different techniques.

Significance to Biomedical Research and the Program of the Institute

An improvement in therapeutic ratio (dose to area at risk/dose to normal tissue) is possible if the treatment technique is determined individually for each patient.

Proposed Course

To continue the investigation of techniques for modifying the dose to the opposite breast and to investigate the feasibility of the combined photon and electron treatment technique.

Publications

1. Lichter, A., Fraass, B., Fredrickson, H., Roberson, P., and van de Geijn, J.: Computed Tomography in Treatment Planning: Primary Breast Cancer. In Ling, C., Rogers, C., Morton, R. (Eds.): Computed Tomography in Radiation Therapy. New York, Raven Press, 1983, pp. 99-107.

2. Roberson, P., Lichter, A., Bodner, A., Fredrickson, H., Padikal, T., Kelly, B., and van de Geijn, J.: Dose to lung in primary breast irradiation. Int. J. Rad. Onc. Biol. Phys. 9: 97-102, 1982.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06319-04 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Use of Prematurely Condensed Chromosomes (PCC) in Biological Dosimetry

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James B. Mitchell, Ph.D., Acting Head, Radiation Biology Section, NCI

COOPERATING UNITS (if any)

Department of Radiation Biology
Colorado State University, Fort Collins, Colorado 80525

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1

PROFESSIONAL:

.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine if the use of premature chromosome condensation (PCC) technique will improve the resolution of the lymphocyte biological dosimetry system for low total doses of radiation (<10 rad). With the PCC technique, chromosomal damage (gross breaks in chromosomes) of interphase cells can be studied immediately following radiation exposure. Assays will be made before the cells have had time to repair many of the initial breaks, thereby increasing the number of breaks counted as opposed to counting aberrations conventionally 24-48 hours after exposure in metaphase I and II.

Project Description

Professional Personnel Engaged on the Project:

J. Bedford, M.D. Department of Radiation Biology, Fort Collins, Colorado

Objective: By scoring radiation damage in chromosomes (gross breaks) immediately following the exposure, we will construct radiation dose response curves which may provide greater resolution in the low dose region (1-10 rad). If this technique does provide greater resolution to the lymphocyte biological dosimeter system, then the determination of small radiation doses to accidentally exposed persons could be done with a considerable amount of confidence.

Methods Employed

Blood lymphocytes and bone marrow will be irradiated with graded doses of gamma photons and fused immediately with mitotic inducer cells. Slight modifications of the Rao and Johnson PCC technique will be used. Gross breaks in whole GI PCC's chromosomes will be scored.

Major Findings

The study is in preliminary stages and the results are presently not available for assessment. This study will be conducted in collaboration with Dr. Joel Bedford of CSU, who is conducting many of the preliminary studies. Once these are finished, PCC using human bone marrow will be assessed.

Significance to Biomedical Research and the Program of the Institute

Determination of low doses of radiation received by persons accidentally exposed to radiation has been an issue of concern over the past 30 years. More precise methods of accessing low doses of radiation would be of value not only for accidental diagnostic exposure but also for environmental exposures to the general population. In addition, these studies should provide better understanding as to the nature of chromosome breakage and repair.

Proposed Course

Project is currently underway.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06320-04 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Response of Mammalian Cells to Drugs and Low Dose Rate Radiation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James B. Mitchell, Ph.D., Acting Head, Radiation Biology Section, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For the past several years, there has been an emphasis in clinical oncology toward multi-modality therapy, in particular, the use of chemotherapy drugs and radiation. Most of the laboratory research has been directed toward the use of drugs in combination with high dose rate radiation. A significant number of tumors are treated with low dose rate irradiation (LDRI) however, by utilizing radioactive implants or applications. There has been little research conducted to explore the possible usefulness of combination chemotherapy drugs and LDRI. This study will evaluate the effectiveness, as measured by cell survival, of selected chemotherapy drugs and LDRI. In addition, the role of reductive cellular compounds and how they may influence cell survival following these therapies will be studied.

Project Description

Professional Personnel Engaged on the Project:

A. Russo	Clinical Associate	ROB	NCI
S. McPherson	Biologist	ROB	NCI
J. Gamson	Biologist	ROB	NCI

Objective: The objective of this project is to determine if there are combinations of continuous low dose rate radiation and chemotherapeutic drugs that will provide more cell-killing of tumor cells (in vitro tumor cell lines) than to normal tissue cell lines.

Methods Employed

In vitro cell cultures will be exposed to the various agents mentioned above and assayed for cellular reproductive integrity using conventional tissue culture techniques.

Major Findings

The project is in the early stages of development. Dose response curves for adriamycin, bleomycin, and LDRI have been conducted. Cell killing may be enhanced for adriamycin and bleomycin following removal of cellular GSH.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of interactions between radiation and drugs, which might be of value to clinical radiotherapy.

Proposed Course

Dose-response curves have been generated for a variety of chemotherapy drugs. The next step will now be to combine the drugs with low dose rate radiation.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06321-04 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Radiosensitization of Aerated and Hypoxic Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James B. Mitchell, Ph.D., Acting Head, Radiation Biology Section, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4

PROFESSIONAL:

2

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There is considerable evidence that the existence of hypoxic cells in human tumors may pose a problem for clinical radiotherapy. The purpose of this project is to study the effects of ionizing radiation delivered at different exposure rates with respect to cell killing, cell cycle status, and cellular redox potential of mammalian cells grown either under aerated or hypoxic conditions. A major portion of this study will be concerned with various means of modulating the cellular redox potential by using drugs that either deplete or elevate cellular glutathione (GSH). In addition, the indirect effects of GSH removal will be assessed by high performance liquid chromatography and gel electrophoresis. These studies should provide a better understanding of the effects of radiation to aerated and hypoxic cells.

Project Description

Professional Personnel Engaged on the Project:

A. Russo, M.D.	Clinical Associate	ROB	NCI
S. McPherson	Biologist	ROB	NCI
J. Gamson	Biologist	ROB	NCI

Objective: The objective of the proposed project is to obtain a better understanding of the nature of lesions and processes leading to cell reproductive death and to study the interrelationships of factors which influence radiosensitivity, with an emphasis on their implications for clinical radiotherapy.

Methods Employed

In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to various dose rates of radiation, either under aerated or hypoxic conditions. Oxygen enhancement ratios (OER) will be determined. Cellular GSH will be measured by spectrophotometric methods.

Major Findings

GSH depletion to values of < 5% are not appreciably lower the OER. Diethyl maleate treated cells, however, exhibited more hypoxic sensitization than did buthionine sulfoximine treated cells.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of the effects of dose rate/fractionation on the OER. Since there is a good deal of information that indicates that hypoxic cells in tumors represent a problem for radiotherapy, these studies could lead to more efficient methods of sterilizing hypoxic cells.

Proposed Course

Using basic hypoxic cells systems, explore effects of low levels of GSH and X-rays at low dose-rate.

Publications

1. Mitchell, J., Russo, A., Biaglow, J., and McPherson, S.: Cellular Glutathione depletion by diethyl maleate or buthionine sulfoximine: No effect of glutathione depletion on the oxygen enhancement ratio. Radiation Research (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06328-03 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Field Configuration in Definitive Radiotherapy of the Intact Breast

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Benedick Fraass, Staff Fellow, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.2

PROFESSIONAL:

.15

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This work has resulted in the development and implementation of a new irradiation technique to produce in a more reliable fashion a uniform dose distribution in the breast tissue and the supraclavicular area. The necessary numerical data for routine application are obtained by using a specially developed computer program.

Project Description

Professional Personnel Engaged on the Project:

A. Lichter, M.D.	Senior Investigator	ROB	NCI
J. van de Geijn, Ph.D.	Expert	ROB	NCI
F. Harrington	Engin. Tech.	ROB	NCI

Objectives: To develop and implement a field arrangement for treatment of cancer of the breast. It is necessary to achieve a uniform dose across the entire treatment volume, while minimizing the dose to adjacent critical structures.

Methods Employed

Extensive experimental work has demonstrated that a new method can be applied to generate a uniform matching of the supraclavicular field with the tangential breast fields.

Major Findings

This method is clinically applicable.

Significance to Biomedical Research and the Program of the Institute

This work makes adequate treatment of all breast tissue routinely possible.

Proposed Course

Continue improvement.

Publications

1. Lichter, A., Fraass, B., van de Geijn, J. and Padikal, T.: Technique for field matching in primary breast irradiation. Int. J. Rad. Onc. Biol. Phys. 9: 263-270, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06329-03 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Radiation Physics Service

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jan van de Geijn, Acting Head, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

7.5

PROFESSIONAL:

2.5

OTHER:

5.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Section provides expert physical and technological support for radiation treatment. This support consists of routine calibration and quality assurance of all radiation equipment and includes special dosimetry studies, computer-assisted treatment planning, and the design and development of special equipment tailored to special clinical needs. Regular checking of dosimetric and technical set-up aspects of radiation treatment to be continued.

1. An efficiently graded quality assurance program, originally developed for the two Siemens linear accelerators, has been adapted and extended for the three Varian accelerators (Clinacs 4, 18, and 20).

2. Adaptation of the new radiation equipment has been performed and special supporting equipment for patient treatment has been developed and implemented.

3. The Clinac 4/100 and Clinac 18 linear accelerators are now fully operational. The Clinac 20 has been accepted, but a great amount of special work is needed to be done in preparation for the temporary transfer of the intra-operative irradiation program to this machine. Also, preparatory work for total skin and total body irradiation is in progress.

4. A special adaptation of an existing operating table has been designed for use in the intra-operative program. Construction is well underway.

5. The computer programs for clinical radiation treatment planning have been further extended in all three subfields: external beam, point-source, and line-source radiation fields.

Project Description

Professional Personnel Engaged on the Project:

F. Harrington	Engin. Tech.	ROB	NCI
B. Fraass, Ph.D.	Staff Fellow	ROB	NCI
R. Miller	Health Physicist	ROB	NCI
K. Yeake	Dosimetrist	ROB	NCI
J. Doolittle	Electronic Tech.	ROB	NCI
J. Caulkins	Health Tech.	ROB	NCI

Objectives: To ensure high quality physics support for radiotherapy.

Methods Employed

A new, efficiently graded system has been developed and implemented for monitoring the performance of two linear accelerators, the simulator, and the CT scanner. Special mechanical supports and measuring devices were developed to quantify the position of patients and to improve the reproducibility of daily patient set-ups. The data acquisition for treatment planning has been simplified and improved. A new method for computer-assisted treatment planning has been introduced. Considerable efforts have been invested in the dosimetry of intra-operative, total-body, and total-skin radiotherapy.

Major Findings

The introduction of beam monitoring jigs enables daily monitoring of output, beam flatness, symmetry, and alignment of light field and X-ray fields for both linear accelerators. The method allows simple documentation of performance. The dosimetry of photon beam total-body irradiation as well as that of total-skin electron beam irradiation for mycosis fungoides requires further attention. Much attention had to be spent on total body irradiation and mycosis fungoides dosimetry.

The most important contribution in computer-assisted treatment planning is the availability of routine interactive optimization and routine multi-slice imaging of dose distributions superimposed on CT scans. An important improvement is the capability to image the effects of irregular shielding blocks which is of essential interest in the treatment of soft-tissue sarcomas and cancers of the esophagus.

The use of locally designed and developed equipment and methodology has proven to be a major factor in quality control of equipment, methodology, and treatment documentation. This is especially important in view of the generally highly complex clinical studies in this branch.

Significance to Biomedical Research and the Program of the Institute

The improvements in quality assurance, patient positioning, and treatment planning are essential as a basis for optimal patient treatment and for meaningful evaluation of treatment protocol studies.

Proposed Course

1. Continuation of adaptation to the new radiation machines.
2. Special attention to the quality assurance aspects of the Microtron, currently under installation.

Publications

1. van de Geijn, J., Harrington, F., Fraass, B., and Glatstein, E.: A graticule for evaluation of megavolt x-ray port films. Int. J. Rad. Onc. Biol. Phys. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06330-03 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Extension of a 3-D Dose Field Model

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jan van de Geijn, Acting Head, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The capability to calculate the distribution of absorbed dose produced by photon beams and electron beams of the most general characteristics is of vital importance in radiotherapy. Conceptually, this model takes as a basis the empirical distributions along three mutually perpendicular reference lines in a "master field" and mathematical expressions to describe the effect of variation effects of field size, depth, and focal distance. This concept is applied to the beam-modifying devices as well. The approach is attractive from a theoretical as well as a practical point of view. The current investigations concern the generalization for irregular fields modified by irregular blocks for photon beams and electron beams including the influence of inhomogeneities. The investigations of the problems posed by irregularly shaped and blocked external beams have been continued and are nearing completion for photon beams. The extension to electron beams is continuing. Of special interest are the implications of the large number of electron energies and the need for flexible application of different energies and field shapes in combination with photon fields. A special subject area is the inclusion of intra-operative dosimetry. The central ray distributions can now be described on the basis of only seven characteristic depth dose data points, for ⁶⁰Co to 18 MV X-rays.

Project Description

Professional Personnel Engaged on the Project:

B. Fraass, Ph.D.	Staff Fellow	ROB	NCI
R. Miller	Health Physicist	ROB	NCI
R. Creecy	Computer Spec.	ROB	NCI

Objectives: To extend a unified calculative model for the description of absorbed dose produced by beams of ionizing radiation, including photon beams as well as electron beams, as a basis for computer-assisted treatment planning.

Methods Employed

1. The variation of relative absorbed dose along the central ray with depth, field size, and source surface distance (SSD) has been studied using published and locally-measured data. Mathematical representations have been established for a range of energies now covering ^{60}Co to 18 MV X-rays.

2. The variation of the relative absorbed dose across the beam has been studied as a function of field size, depth and SSD for many radiation qualities for photons, electrons, and neutrons. Mathematical representations for these variations have been established.

3. Over the present reporting period, special attention has been paid to verification of the model for the local radiation machines and to extension of the model to irregular fields modified by irregular blocks.

Major Findings

It has been found that the modified geometrical projecting concept applies well to the local facilities for regular rectangular beams including the use of wedges.

It has been established that the concept is applicable to irregular fields as well. Preliminary results for electron beams are most promising. The validity for neutron beams has been confirmed by investigators at Fermilab. Most of these results have been incorporated in a clinical treatment planning system.

Significance to Biomedical Research and to the Program of the Institute

The range of validity of the dose field model determines the potential range of applicability of the clinical treatment planning program. In turn, the latter determines the degree of refinement in radiation treatment that can be scientifically documented.

Proposed Course

Continuation, with emphasis on inhomogeneities in photon and electron beams. In regard to electron beams, the influence of oblique incidence, non-standard distances between electron applicators, and patient surface need further attention, especially in view of moving electron beams.

Publications

1. van de Geijn, J.: The Use of the Projective Beam Model for Electron Beams. In Paliwal, B. (Ed.): Proceedings of the Symposium on Electron Dosimetry and ARC Therapy. Madison, Wisconsin, American Institute of Physics, 1982, pp. 137-158.
2. van de Geijn, J. and PoCheng, C.: Routine computation of multi-slice dose distributions in irregular fields modified by irregular blocks. Poster, AAPM Annual Congress. Med. Phys., 1981.
3. van de Geijn, J. and PoCheng, C.: The net fractional depth dose: Concept, physical properties, and computational advantages. Paper Exhibit #RA, AAPM Annual Congress, pp. 549, 1981.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06331-03 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Computer-Assisted 3-D Radiation Treatment Planning

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jan van de Geijn, Acting Head, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this continuing project is the development and clinical implementation of a generalized system for external beam treatment planning. It will enable the optimum utilization of existing treatment facilities. The system is based on a generalized 3-D dose field model which covers photon and electron as well as neutron beams. The computer program and most of its clinical implementation was completed for the photon and electron fields available from the local 6 MV and 12 MV linear accelerators. The current capabilities include interactive simulation of most irradiation techniques, including the effect of most beam modifying devices. The transverse contours are overlaid on corresponding CT scans. Two of the four new radiation machines have been implemented for routine treatment planning. Work is progressing on implementation of a VAX-750 computer in conjunction with the PDP 11/70 system.

Project Description

Professional Personnel Engaged on the Project:

B. Fraass, Ph.D.	Staff Fellow	ROB	NCI
R. Miller	Health Physicist	ROB	NCI
R. Creecy	Computer Spec.	ROB	NCI

Objectives: To develop and implement a generalized system for computer-assisted radiation treatment simulation.

Methods Employed

The dose field model, developed elsewhere by the present principle investigator, was further developed and experimentally tested for the local radiation facilities. The theoretical model was extended to cover irregularly shaped beams as well as irregularly shaped shielding blocks.

The associated computer program for the local PDP 11/70 system was further extended with expert assistance from the Computer Systems Laboratory. A facility has been developed which enables the computation and display of dose distributions in planes perpendicular to the respective beam axes. The capabilities of the graphical input system, the use of the CT images in addition to or instead of mechanically obtained patient contours, the interactive system for the variation of input parameters, and a DEANZA color display system have been further expanded.

Major Findings

The system, although continuing to be further expanded, is in routine use for clinical treatment planning. In comparison to other existing systems, it offers high speed computation and display of complete dose distributions in multiple slices, superimposed on CT images, including effects of wedge filters, irregular shielding blocks and diaphragm rotation. Several modes of display are available. The newly developed Beam's Eye View capability is being implemented for routine use and promises to be very useful. The facility has a major impact on the conceptual understanding of the spatial aspect of radiation treatment dosimetry.

Significance to Biomedical Research and the Program of the Institute

The convenient interactive manipulation of the key beam parameters in combination with fast response is highly valuable in the complicated dosimetry problems encountered in special protocol studies. The facility is also highly effective in the Resident's Training Program.

Proposed Course

1. Implementation of the Beam's Eye View option for regular and irregular electron fields.
2. Establishment of a "Slave Monitor System" to enable the display and limited modification of treatment plans during the daily patient conferences.
3. Extension of the capabilities to compute and display dose distributions in sagittal, coronal and beam's eye view sections of the patient on an interactive basis.
4. Development towards quasi-3-D display of computed dose distributions in relation to CT.

Publications

1. van de Geijn, J., Chien, J., PoCheng, P. and Fredrickson H.: A Unified 3-D Beam Model for External Beam Dose Distributions. In Umegaki, Y. (Ed.): Proceedings of the VIII International Conference on Computers In Radiotherapy. Tokyo, 1980.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06332-03 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Use of a Match-Line Wedge for Radiation Field Matching

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Benedick Fraass, Staff Fellow, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.2

PROFESSIONAL:

.15

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is the development and clinical use of a useful method for matching adjoining megavoltage radiation fields so that the dose distribution through the match region is uniform. A "match-line wedge" has been developed which satisfies the above requirement. Simplicity of use has assured that the wedge is used routinely and effectively.

Project Description

Professional Personnel Engaged on the Project:

J. van de Geijn, Ph.D.	Expert	ROB	NCI
E. Glatstein, M.D.	Chief.	ROB	NCI
F. Harrington	Engin. Tech.	ROB	NCI

Objectives: To find a means of modifying the edges of adjoining radiation fields so that the dose distribution throughout the match region is uniform.

Methods Employed

A match-line wedge has been designed so that a wide pseudo-penumbra is created when the wedge is placed in the edge of the radiation beam. This wide pseudo-penumbra makes the dose distribution in the match region less sensitive to set-up errors and more uniform than is possible with normal matching methods when adjacent fields are matched. The design, mounting, simulation, set-up, and treatment techniques have been developed so that use of the wedge is safe, useful, and simple. The dose distributions which result from variation of different parameters in the system have been studied in detail.

Major Findings

Use of the match-line wedge results in uniform dose distributions in the match region between adjacent radiation fields.

Significance to Biomedical Research and the Program of the Institute

Use of this device improves the uniformity of dose received by patients who are treated with matching fields, thereby improving the accuracy of treatment.

Proposed Course

Implementation of the match-line wedge will continue, including use of improved patient set-up techniques and quality assurance of wedge use. Use of the wedge with a wider range of treatment techniques will be explored.

Publications

1. Fraass, B. and Tepper, J.: Clinical use of a match-line wedge for adjacent megavoltage radiation field matching. Med. Phys. 8: 546, 1981.
2. Fraass, B., Tepper, J., Glatstein, E., and van de Geijn, J.: Clinical use of a match-line wedge for adjacent megavoltage radiation field matching. Int. J. Rad. Onc. Biol. Phys. 9: 209-216, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06333-03 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dosimetry of Total Skin Electron Irradiation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Benedick Fraass, Staff Fellow, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.3

PROFESSIONAL:

.2

OTHER:

.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A detailed study has been made of the dosimetry of total skin electron irradiation. This study has quantified and improved the whole skin treatments received by patients with mycosis fungoides. The treatment technique is being updated and implemented on the new Clinac 20 linear accelerator.

Project Description

Professional Personnel Engaged on the Project:

R. Miller	Health Physicist	ROB	NCI
K. Yeakel	Dosimetrist	ROB	NCI
J. Caulkins	Health Tech.	ROB	NCI
E. Glatstein, M.D.	Chief	ROB	NCI

Methods Employed

The dosimetry system of the MeV XII accelerator has been substantially improved, allowing more precise definition of the total skin dose given. The absolute dose has been calibrated using various ionization chambers and thermoluminescent dosimeters (TLD). Extensive TLD measurements have been made on five patients. These measurements have made possible the mapping of the dose distribution over the whole body, and also have shown the daily variations and patient-to-patient variations which are possible with this treatment technique.

Major Findings

The dose to the skin is fairly uniform over the trunk, but the distribution of dose to legs, arms, and head is significantly different. Daily and patient-to-patient variations in dose are not overly significant.

Significance to Biomedical Research and the Program of the Institute

This work makes adequate treatment for mycosis fungoides possible with the whole-skin irradiation technique.

Proposed Course

Work toward improving the absolute dosimetry is continuing. Also necessary is the improvement of methods to assure uniform dose to the whole skin. The technique will be updated as the new Clinac 20 accelerator is used for this technique.

Publications

1. Fraass, B., Roberson, P., and Glatstein, E.: Whole body electron irradiation - patient skin dose distribution. Radiology 146: 811-814, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06334-03 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dose to Gonads from Radiation Treatment for Lymphomas and Sarcomas

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Benedick Fraass, Staff Fellow, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

Endocrine Branch, NCI
Surgery Branch, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.25

PROFESSIONAL:

.2

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Doses to gonads have been measured on patients who are irradiated as part of their treatment for lymphomas or sarcomas. Ion chamber and thermoluminescent dosimetry (TLD) measurements have been made to verify the measurements on patients. Two very effective gonadal shields have been built and put into routine clinical use.

Project Description

Professional Personnel Engaged on the Project:

T. Kinsella, M.D.	Senior Investigator	ROB	NCI
K. Yeakel	Dosimetrist	ROB	NCI
R. Shering, M.D.	Medical Officer	Endocrine Branch	NCI
E. Shapiro, M.D.	Clinical Associate	Surgery Branch	NCI
J. Caulkins	Health Tech.	ROB	NCI

Objectives: To accurately determine the gonadal doses received by lymphoma and sarcoma patients who are treated with radiation, and to decrease that dose if possible.

Methods Employed

Thermoluminescent dosimetry (TLD) measurements have been made to determine the dose to testes and ovaries of patients treated with mantle, para-aortic, pelvic and leg radiation fields. Extensive ion chamber and TLD measurements have been made to verify the validity of the TLD measurements on patients.

Major Findings

Gonadal doses can now be calculated retrospectively, as long as there is adequate information about radiation field and patient geometry. A gonadal shield useful for the above categories of patients has been developed.

Significance to Biomedical Research and the Program of the Institute

Gonadal doses have been correlated with fertility and hormonal function tests obtained by the Surgery and Endocrine Branches. The results quantify the effects of radiation on fertility. Shielding which reduces the complications of these radiation treatments is clearly of major importance to the patients and is very useful.

Proposed Course

The patient TLD and verification measurements will continue. Doses received by previously treated patients will be calculated. Fertility and hormonal function test results will continue to be correlated with gonadal doses.

Publications

1. Fraass, B. and van de Geijn, J.: Peripheral dose from megavoltage beams. Med. Phys. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06337-03 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Real-Time Radiotherapy Treatment Monitor

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Benedick Fraass, Staff Fellow, Radiation Physics and Computer Automation, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.05

PROFESSIONAL:

.02

OTHER:

.03

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the project is to develop a real-time monitor for radiation treatments. Although routine quality assurance is the immediate aim, continued development will make feasible many projects which rely on real-time patient dose monitoring.

Project Description

Professional Personnel Engaged on the Project:

J. Doolittle Electronic Tech. ROB NCI

Objectives: To design and develop a real-time monitoring device for use with radiotherapy.

Methods Employed

A two-dimensional array of radiation-sensing diodes is used to monitor the radiation which is transmitted through the patient during a treatment. From knowledge of the transmitted intensity, patient dose information is obtained. A microcomputer-based system is used to accumulate and analyze the data from the diode array.

Major Findings

Initial hardware interfacing, computer programming, and diode selection have been accomplished. The system has been tested and found to be promising. Optimization of the many factors affecting system performance is now underway.

Significance to Biomedical Research and the Program of the Institute

This project is expected to improve the quality of radiotherapy in the Radiation Oncology Branch. Further development will lead to innovative and more precise types of treatments.

Proposed Course

To be continued. The present data acquisition system will be developed and refined. Design of the system to be used for automatic monitoring and recording of patient treatments will proceed. Investigation into the use of the diode array system for beam symmetry, quality, and calibration checks, compensating filter design, dynamic radiotherapy treatments, quality control, and real-time treatment analysis will continue.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06343-03 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phase I Study of Intravenous Bromodeoxyuridine (BUDR) (NSC3A297)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Timothy J. Kinsella, M.D. Senior Investigator, ROB, NCI

COOPERATING UNITS (if any)

Clinical Pharmacology Branch, COP, DCT, NCI;
Surgical Neurology Branch, NINCDS

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH Bethesda, MD 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bromodeoxyuridine (BUDR), a known radiosensitizing drug is given as a continuous intravenous infusion and patients with high-grade brain tumors and other poorly radioresponsive tumors. The drug is infused for either 12 or 24 hours daily for up to 14 days with some patients receiving two separate infusions of BUDR. To date 16 patients with primary brain tumors and eight patients with other poorly radioresponsive tumors have been treated with a combination of BUDR and conventional fractionated radiation.

Project Description

Professional Personnel Engaged on the Project:

Angelo Russo, M.D.	Clinical Associate	ROB	NCI
James B. Mitchell, Ph.D.	Expert	ROB	NCI
E. Glatstein, M.D.	Chief	ROB	NCI
C. Meyers, M.D.	Chief, Clinical Pharmacology Branch		NCI
P. Kornblith, M.D.	Chief, Surgical Neurology Branch		NINCDs

Objective: This is a Phase I study designed to assess the toxicity of BUDR given as a continuous intravenous infusion along with concomitant conventional fractionated irradiation. Both local (within the radiation field) and systemic toxicity are being evaluated. The pharmacokinetics of the drug is actively being studied. Additionally, incorporation of the drug into normal bone marrow, normal skin, and selective tumors is being studied with the use of a monoclonal antibody to BUDR and immuno-histochemistry.

Methods Employed

Patients with high-grade primary brain tumors and other primary or metastatic poorly responsive tumors are given BUDR by a central venous catheter for either 12 or 24 hours daily for up to 14 days. The BUDR infusion is started approximately 5 to 7 days prior to initiation of conventional fractionated irradiation. In select patients, a percutaneous bone marrow aspirate from the posterior iliac crest is done prior to and immediately following the 14 day infusion to assess drug uptake by human bone marrow CFUC. Additionally, selective patients undergo pharmacology studies during a brief inpatient visit with serial determinations of arterial and venous plasma levels of BUDR during the infusion period. In patients with skin nodules from metastatic tumors, a punch biopsy of the tumor nodule as well as adjacent normal skin is done to assay directly the incorporation of BUDR using the monoclonal antibody.

Major Findings

We have determined significant systemic toxicity of intravenous Bromodeoxyuridine given as a 12-hour infusion daily for up to 14 days both in the bone marrow and skin. Typically patients will manifest myelosuppression particularly thrombocytopenia approximately 10 days following initiation of the infusion with a nadir approximately 2-3 days following completion of the infusion. Bone marrow recovery occurs within 7-10 days of the nadir. Bone marrow toxicity has been seen in all patients receiving an infusion of greater than 600 milligrams per meter squared for 12-hour infusion. Severe myelosuppression occurs with infusion of greater than 750 milligrams per meter squared per 12 hours. Skin toxicity is the second major systemic toxicity with approximately half the patients having a limited maculopapular rash primarily in sunlight or white light exposed areas. Two patients have developed a diffuse epidermolysis requiring hospitalization. Both patients recovered. Pharmacology studies have determined that we are able to achieve steady state arterial levels in the range of 1 to 2×10^{-6} molar with infusions of 650 to

700 milligrams per meter squared. With this infusion schedule, the systemic toxicity appears tolerable. Local toxicity with an enhancement of skin reaction has occurred in approximately a third of the patients but has been severe enough to require a treatment break in only 5 patients. Tumor incorporation into melanoma nodules has been demonstrated with up to 50% with the cells incorporated into the drug following a 10-14 day infusion.

Significance to Biomedical Research and the Program of the Institute

The Radiation Oncology Branch continues to be involved in the evaluation of the halogenated pyrimidine analogs as radiosensitizers. In using these intravenous infusions, we have demonstrated that we are able to achieve significant arterial levels to result in radiosensitization of both normal tissues such as bone marrow and skin as well as tumors. BUDR will be particularly useful in tumors which are dividing more rapidly than the surrounding normal tissues and primary brain tumors continue to be of major interest.

Proposed Course

We plan to continue evaluation of intravenous Bromodeoxyuridine given as a continuous 24-hour infusion and compare the toxicities to the 12-hour infusion schedule. Within the next 3 to 4 months, we also start to evaluate Idodeoxyuridine in a Phase I study similar to Bromodeoxyuridine.

Publications

1. Mitchell, J.B., Kinsella, T.J., Russo, A., McPherson, S., Rowland, J., Kornblith, P.L., Smith, B., and Glatstein, E.: Radiosensitization of hematopoietic precursor cells CFUS in glioblastoma patients receiving intermittent intravenous infusions of bromodeoxyuridine (BUDR). International Journal of Radiation, Oncology, Biology, Physics 9: 457-463, 1983.
2. Kinsella, T.J., Russo, A., Mitchell, J.B., Rowland, J., Jenkins, J., Schwade, J.G., Meyers, C.E., Collins, J.M., Kornblith, P., Smith, B., Kufra, C., and Glatstein, E.: A phase I study of intermittent intravenous bromodeoxyuridine and conventional fractionated irradiation. International Journal of Radiation, Oncology, Biology, Physics (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06345-03 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Radiosensitizer, Misonidazole (NSC 261037)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

E. Glatstein, M.D. Chief, ROB, NCI

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacy, DTP, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Radiation Therapy

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pharmacokinetic and toxicity studies have been performed on patients undergoing treatments with misonidazole, a nitroimidazole radiosensitizing agent. The studies have allowed better delineation of toxicity and pharmacology of these compounds, and was obtainable with the previously evaluated oral compounds.

Project Description

Professional Personnel Engaged on the Project:

J. Strong, Ph.D. Senior Staff Fellow DTP NCI

Objective: Assessment of the pharmacokinetics and toxicity of misonidazole.

Methods Employed

Patients receiving radiation therapy were treated with misonidazole in escalating doses twice weekly for five weeks.

Major Findings

Toxicity of misonidazole was found to correlate with area under the curve of concentration versus time. A dose of 1.5 gm/m² twice a week for five weeks was found to be a well-tolerated dose with only minimal peripheral neuropathy.

Significance to Biomedical Research and the Program of the Institute

Radiation sensitizing drugs are thought to increase the effectiveness of radiation by allowing an increased effect of radiation on hypoxic cells, while not effecting well-oxygenated cells. The Radiation Oncology Branch at NCI has been one of the leading groups involved in evaluating these very promising and innovative compounds.

Proposed Course

The Radiation Oncology Branch is currently evaluating other nitroimidazole radiosensitizers, such as desmethylmisonidazole.

Publications

1. Schwade, J.G., Strong, J.M., and Gangi, D.: I.V. misonidazole (NSC 261037): Report of initial clinical experience. Cancer Clinical Trials 44: 33-39, 1981.
2. Schwade, J.G., Makuch, R.W., Strong, J.M., and Glatstein, E.: Dose-response curves for predicting misonidazole - Induced peripheral neuropathy. Cancer Treatment Reports 66: 1743-1750, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06348-02 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactive Linear-source Brachytherapy Dosimetry Program

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

R. W. Miller Commissioned Officer, ROB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:

.1

PROFESSIONAL:

.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to develop an interactive computer program for calculating dose distributions in an arbitrary plane from arrays of filtered, linear radioactive sources used primarily for intercavitary radiotherapy. The sources used are ¹³⁷Cs capsules with stainless steel walls. Dose distributions are calculated using the Sievert Integral with experimentally determined attenuation coefficients.

Project Description

Professional Personnel Engaged on the Project:

J. van de Geijn, Ph.D. Expert ROB NCI

Objective: To develop an interactive computer program for the computation and display of dose distributions associated with linear radioactive sources as used in brachytherapy, in order to enable interactive optimization of source strength and geometric distribution.

Methods Employed

- 1) The algorithm is adopted from an existing model based on a special development of the Seivert Integral, elsewhere developed by van de Geijn.
- 2) The computer program is in part based on an existing program, developed elsewhere by van de Geijn. This program is being adapted and extended for interactive operation on the PDP 11/70 system, making use of current manipulative and imaging technology.
- 3) Coordination of I/O methodology with separate and external beam therapy.
- 4) Comparison of computed distributions with experimental results.

Major Findings

The developments have now reached a level where they are applied to clinical problems in the treatment of cervical cancer. An especially important asset is found to be the facility to manipulate the relative spatial position of a source configuration and the possibility to simulate the effects of changing, removing or adding sources.

Significance to Biomedical Research and the Program of the Institute

The existence of a versatile program of this kind, enabling interactive adjustment to the individual clinical problem at hand is highly important. The potential for adding together dose distributions from external beam and internally applied sources, which is currently being effectuated, is especially attractive in the context of various clinical research protocols.

Proposed Course - ContinuationPublications - None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06349-02 R0

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Relationship of Cellular Redox State and Thermotolerance

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James B. Mitchell, Ph.D., Acting Head, Radiation Biology Section, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4

PROFESSIONAL:

2.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hyperthermia is currently being evaluated as a potential cancer treatment modality. The mechanism(s) of hyperthermia killing and the induction of thermal resistance (thermotolerance) are not known. We will examine the role of the cellular reduction potential during and after heating to determine its role or alteration during thermal stress. This will be accomplished by using drugs which either bind glutathione (GSH) or prevent its synthesis. There appears to be a relationship between the synthesis of heat shock proteins and the induction of heat resistance. The effect of thiol modulation will be studied in the context of heat shock proteins. Recently, several compounds have been introduced which elevate cellular GSH. These compounds will be synthesized and evaluated in regard to thermal response.

Project Description

Professional Personnel Engaged on the Project:

A. Russo, M.D.	Clinical Associate	ROB	NCI
S. McPherson	Biologist	ROB	NCI
J. Gamson	Biologist	ROB	NCI

Objectives: To determine how the cellular redox state is altered during thermal stress.

Methods Employed

In vitro cell cultures will be exposed to heat and assayed for reproductive integrity using conventional tissue culture techniques and assayed for various biochemical compounds important in maintaining the cellular redox state.

Major Findings

There is a relationship in elevating glutathione and the induction of thermotolerance. Thermotolerance may be prevented by lowering cellular GSH or preventing its synthesis.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of how heat kills cells, which might be of value to further clinical efforts.

Proposed Course

Continue studying the relationship of glutathione (a cellular reducing compound) and thermotolerance.

Publications

1. Mitchell, J., Russo, A., Kinsella, T., and Glatstein, E.: Glutathione elevation during thermotolerance induction and thermosensitization by glutathione depletion. Cancer Research 43: 987-991, 1983.
2. Mitchell, J. and Russo, A.: Thiols, thiol depletion, and thermosensitivity. Radiation Research (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06350-01 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
A Phase I Trial of the Hypoxic Radiosensitizer, SR-2508

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Steven L. Hancock, M.D. Senior Investigator, ROB, NCI

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, NCI Lab/Branch

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is Phase I trial of the radiation sensitizer, SR-2508. This nitroimidazole derivative has been selected for toxicity evaluation because it is less lipophilic than misonidazole and desmethylmisonidazole and appears to be markedly less neurotoxic than the earlier compounds used in clinical trials.

Project Description

Objective - Determination of the maximally tolerated dose of SR-2508 when administered intravenously 3 to 5 times a week in conjunction with standard fractionated radiation therapy. Pharmacokinetic evaluation of the drug is also being obtained. This is a co-operative study involving 5 institutions and conducted under the auspices of the Radiation Therapy Oncology Group.

Methods Employed

Patients receive 3 to 5 doses of SR-2508 weekly in conjunction with standard radiation therapy of locally advanced or metastatic cancer. Drug is initially to be administered for a 3 week period of time. A six week schedule of administration may be adopted once the drug tolerance has been ascertained.

Major Findings

This Phase I trial is in its initial weeks of patient accrual and, thus far, 12 patients have participated in the study. No adverse symptoms have been reported with doses up to 2.0 grams per meter squared 3 times a week. Elevation of liver function test was noted in 1 patient following 3 doses of drug. The significance of this is currently under investigation. Pharmacokinetic evaluation of the first patient treated in this division indicates distribution and elimination patterns similar to desmethylmisonidazole and an estimated plasma half life of 6 1/2 hours.

Significance to Biomedical Research in the Program of the Institute

The development of an effective radiation sensitizer drug may facilitate improved local control of tumors with radiation therapy without augmenting normal tissue toxicity.

Proposed Course

The Phase I trial of SR-2508 will be continued at this and other participating institutions. Further evaluation of the agent will depend upon the observed toxicity.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06351-01 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Response of Human Hematopoietic Precursor Cells to Halogenated Pyrimidines

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James B. Mitchell, Ph.D., Acting Head, Radiation Biology Section, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

When certain halogenated pyrimidines such as bromodeoxyuridine (BUdR) and iododeoxyuridine (IUdR) are incorporated into cellular DNA, the cells become more sensitive to ionizing radiation. This observation has led to several clinical studies over the years and recently at the NCI to evaluate whether selective sensitization of tumors could be achieved by BUdR/IUdR infusion followed by radiation. An important question arises in these studies regarding whether or not the drug actually is incorporated into cells. This study proposes to obtain information regarding this question by using:

a) cell survival determinations of pre and post infusion bone marrow precursor cells; b) whether or not sister chromatid staining can be observed in bone marrow stem cells; and c) use of a BUdR/IUdR monoclonal antibody and HPLC assays to actually quantitate the amount of BUdR/IUdR in tumor compared to normal tissue.

Project Description

Professional Personnel Engaged on the Project:

A. Russo, M.D.	Clinical Associate	ROB	NCI
T. Kinsella, M.D.	Senior Investigator	ROB	NCI
G. Morstyn, M.D.	Clinical Associate	ROB	NCI
S. McPherson	Biologist	ROB	NCI
J. Gamson	Biologist	ROB	NCI

Objectives: To determine if BUDR/IUDR infusions in patients actually radiosensitize bone marrow cells and quantitate the amount of BUDR/IUDR in tumor vs. normal tissue.

Methods Employed

In vitro techniques to culture human bone marrow precursor cells (CFUc) will be used. A monoclonal antibody for BUDR/IUDR and HPLC assays will be used to quantitate incorporation of BUDR/IUDR in tissues.

Major Findings

Infusion of BUDR intermittently for 12 hours every 24 hours for 14 days radiosensitizes human bone marrow to X-rays, indicating that by this drug delivery adequate levels of drug are achieved for radiosensitization. These studies should provide a better understanding as to quantities of BUDR/IUDR required to radiosensitize cells from tumor and normal tissue in a clinical setting.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding as to quantities of BUDR/IUDR required to radiosensitize cells from tumor and normal tissue in a clinical setting.

Proposed Course

Evaluate bone marrow response for continuous infusion of BUDR/IUDR and continue work on cellular quantitations of BUDR/IUDR.

Publications

1. Mitchell, J., Kinsella, T., Russo, A., McPherson, S., Rowland, J., Kornblith, P., and Glatstein, E.: Radiosensitization of hematopoietic precursor cells (CFUc) in glioblastoma patients receiving intermittent intravenous infusions of bromodeoxyuridine (BUDR). Int. J. Rad. Oncol. Biol. Phys. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06352-01 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relaxation Agents for NMR Diagnostic Imaging

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Otto A. Gansow, Ph.D. Senior Investigator, ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.75

PROFESSIONAL:

0.75

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nuclear Magnetic Resonance imaging is fast becoming a most powerful method for the non-invasive diagnosis of disease. A fundamental limitation of the technique derives from the fact that images are constructed from T1 relaxation time measurements of protons in the various biological "compartments". If T1 values for differing soft tissue types are similar, the type will not, in general, be resolvable in the images. A potential method for improving this situation is the development of relaxation agents which specifically alter T1 relaxation rates in tissues where they may be concentrated. We propose to design and construct such in vivo relaxation agents.

Project Description

Professional Personnel Engaged on the Project:

Richard Knop, M.D.	Clinical Associate	LCP	NIADDK
John Weinstein, M.D.	Senior Investigator	LTB	NCI

Objectives: We propose to construct paramagnetic molecules that localize in certain biological compartments in order to reduce T1 relaxation times of water in the area. The method of construction is well known. We plan to attach paramagnetic metal chelates to proteins found to localize where desired in the body. The idea is that since paramagnetics alter local T1 values, by concentrating them in differing tissue types, we could induce resolution in NMR images. For example, paramagnetic labels attached to blood proteins, which circulate freely, such as albumin, could alter T1 values in flowing blood, thus allowing imaging of cardiac function and blood flow. A second example, would be to label tumor associated monoclonal antibodies. In recent work done in this section, it has proven possible to localize in tumors radioisotopes attached to antibodies by using metal chelates.

Methods Employed

Bifunctional metal chelates or cryptates capable of securely binding paramagnetic metals like iron, chromium or gadolinium will be prepared and attached to the proteins described above. The effect of these paramagnetic relaxation agents on T1 values will be measurable by conventional inversion, recovery methods. Those found to have a measurable effect on normal saline proton T1 values will be injected into normal and tumor bearing animals and NMR images taken.

Major Findings

Preliminary studies have shown that paramagnetic chelates may be attached to antibodies or albumin without affecting the biological properties of the proteins.

Proposed Course

The T1 measurements required to determine whether labeled paramagnetic proteins could be of use in vivo are in progress.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06353-01 RO

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metal Conjugated Monoclonal Antibodies for Tumor Diagnosis and Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Otto A. Gansow, Ph.D. Senior Investigator, ROB, NCI

COOPERATING UNITS (if any)

Johns Hopkins Medical School, Baltimore, Maryland
Argonne National Laboratory, Argonne, Illinois

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tumor-associated monoclonal antibodies are potential therapeutic agents as selective carriers of cytotoxic agents to malignant cells. We are testing this hypothesis in two animal model systems: a tumor virus induced leukemia of mice and human tumor xenographs in nude athymic mice.

The various cytotoxic agents being employed are radioisotopes. Their relative therapeutic efficacy when conjugated to antibodies is being assayed and compared to that of monoclonal antibodies alone. The isotopes to be employed include the highly tumoricidal alpha emitting parent radioisotopes ^{212}Pb or ^{212}Bi . The syntheses of different chelates and radiochemical separations required for these objectives are being devised and reduced to clinical practice. Results from isotopic therapy are being compared with those obtained by use of antibody conjugated toxins or drugs with respect to tumor growth, regression or cure.

These studies will provide for human medicine a basis for design of rational therapy of malignancies by selectively targeting cytotoxic agents to tumors as well as metastases.

Project Description

Professional Personnel Engaged on the Project:

Robert Atcher, Ph.D.	Expert	ROB	NCI
David Colcher, Ph.D.	Senior Investigator	LCMB	NCI

Objectives: The specific goal of these studies is to investigate in vitro and in animal tumor models the therapeutic efficacy of tar-getted cytotoxic agents attached to tumor associated monoclonal antibodies. These studies encompass the synthesis of new bi-functional chelates and cryptands designed for therapy employing a variety of radioisotopes and radiation types. Investigations of antibody conjugated toxins and drugs will also be performed.

Methods Employed

Methods for covalently conjugating metal isotopes in bifunctional chelates to monoclonal antibodies are being devised and developed. The inorganic chemistry of new complexing agents for metal isotopes thought to be useful in tumor diagnosis or therapy is being explored. The objectives of the research must thereby of necessity include: (a) the synthesis and characterization of new bifunctional chelates and their metal complexes, both before and after protein conjugation; (b) the evaluation of currently available chelates for use as carriers of isotopes familiar in clinical environments (e.g., ^{99m}Tc) and of less common, but potentially serviceable radionuclides (e.g., ^{68}Ga , ^{111}In , ^{212}Bi), and (c) the development of chemical procedures (protocols) for routine and reproducible preparations of rigorously stable radiometal chelate conjugated monoclonal antibodies which retain their inherent biological specificity and activity.

Major Findings

The problems which have been addressed in the early months of this grant are: (1) the incorporation of Bi-212 into chelates attached to antibody; and (2) the evaluation of two DTPA chelates for use in antibody modification.

1. Initial attempts in our laboratory to incorporate Bi-212 proceeded as follows. A thorium-228 generator was installed and constructed to run in an automated manner. The desired isotopes were collected on a Dowex-50 resin thus providing a source for approximately 100 microcuries of Bi-212 in equilibrium with Pb-212. This column was eluted with 0.5 M HCl to give reasonably pure Bi-212. An assay method based on a Th-228 standard purchased from Amersham, Inc. was devised to quantitate isotopic yields. The bismuth obtained was taken to dryness in a vacuum centrifuge for use in the incorporation protocol. Next, the isotope was taken up in a sodium citrate solution at pH 3 and reacted with antibody that had been chemically modified with a DTPA chelate. After a thirty minute reaction, the solution was over a column to remove unchelated Bi-212. The fractions containing bismuth bound antibody were dialysed

versus citrate-NaCl for two hours and showed no loss of bismuth. Assay of incorporation showed 20% of bismuth now present bound to antibody. However, the several hours required for this procedure lowered the yield of bound bismuth relative to that obtained from the generator to only 4%. This is because Bi-212 has only a one hour half-life.

In subsequent experiments, it has been found that the yield of bismuth incorporation may be increased dramatically. If one calculates the relative equilibrium constant of bismuth versus lead at pH 3.5 in citrate buffer (0.05M), the important observation is that little lead (<0.1%) will be in the DTPA while all the bismuth will be chelated. Thus it seemed theoretically possible to incorporate Bi-212 in the presence of its long lived parent, the ten hour half-life Pb-212. When the Thorium-228 generator was eluted with 2.0M HCl, both the lead and bismuth isotopes were obtained in good yield. Subsequent incorporation into antibody by the same protocol now make it possible to routinely incorporate bismuth in about 60-70% yield.

2. Two derivatives of DTPA have proven most useful for chemical modification of antibody. They are the dianhydride of DTPA and the mono-iobutyl-carboxy carbonic anhydride of DTPA. Since the yield of isotopic incorporation must depend on the amount of chelate ligand attached to antibody, isotopically tagged versions of the above named two molecules were prepared. A study of the pH and concentration dependence of protein binding of chelating ligand was then performed by using bovine IgG as a model for monoclonal antibody. Simply said, conditions for maximal ligand attachment to IgG have been determined. Tritium or carbon-14 enriched DTPA obtained from commercial sources were employed for ligand syntheses.

Publications

1. Gansow, O. A., Atcher, R., Friedman, A., Antersen, W., and Strand, M.: Linking metals to monoclonal antibodies: The evolution of a new method for tumor diagnosis and therapy. Advances on Chemistry Series (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06354-01 R0

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Iron-57 Nuclear Magnetic Resonance: A New Tool for Biomedical Research

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Otto A. Gansow, Ph.D. Senior Investigator, ROB, NCI

COOPERATING UNITS (if any)

LCP, NIADDK

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We propose to develop Iron-57 Nuclear Magnetic Resonance (NMR) as an experimental method for use in the Biomedical Sciences.

Numerous molecules essential to life are constructed about iron containing central cores. Among these are hemoglobin, ferridoxin and the cytochromes. To date, no physical chemical methods have allowed direct study of the central metal environment of these proteins. Iron-57 nmr, i.e. the direct detection of the iron nmr signal, holds the promise of being that method.

Project Description

Professional Personnel Engaged on the Project:

Lars H. Baltzer, Ph.D. Visiting Fellow LCP NIADDK

Objectives: We plan to measure the Iron NMR signals from a number of biological, inorganic and organometallic compounds. The goal of the project is to define the experimental conditions and parameters necessary for the direct detection of iron NMR in order to explore its utility for studies of biological processes.

To develop the method, a knowledge of two physical parameters of Iron NMR signals must be obtained. They are chemical shift values and T1 relaxation times. By observing resonances of inorganic and organometallic model compounds, it is possible to define the chemical shift scale for the iron nucleus. This data serves to define the resolution of the method. Similarly, by measuring T1 relaxation times of these compounds, we will learn how to optimize chemical environments and experimental conditions required to detect iron resonance. With that information in hand, a rational selection of biological problems amenable to study by this method can be effected.

Methods Employed

A specially constructed nmr probe for observing iron nmr has been built and tested successfully. It is available for use in Building 2, NIH. Enriched iron-57 proteins such as myoglobin and hemoglobin are now being synthesized.

Major Findings

We have accomplished the first direct detection of iron-57 nmr in biological molecules. We have undertaken the first systematic study of iron-57 chemical shift values. Initial results indicate a range of > 5000 ppm. This extraordinary resolution shows the great promise of the technique for investigations of structure and function of iron centers.

Significance to Biomedical Research and the Program of the Institute

Investigations of the role of iron in fundamental chemical and biological processes may now be attempted by observing the chemistry at the iron center itself. This has not heretofore been possible. We have a new method.

Proposed Course

Chemical shift and relaxation studies will be completed. Measurement of iron nmr in proteins will be accomplished.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06355-01 RO °

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Total Skin Electron Beam Radiation for AIDS associated Kaposi's Sarcoma

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Peggie Ann Findlay, M.D. Senior Investigator, ROB, NCI

COOPERATING UNITS (if any)

National Institute of Allergy and Infectious Disease Clinical Center

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.25

PROFESSIONAL:

.75

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NCI and NIAID of the NIH are currently investigating and treating patients with the newly described acquired immune deficiency syndrome (AIDS). About 30% of the patients with AIDS have Kaposi's Sarcoma (KS) a skin malignancy that has the capacity to spread to lymph nodes and internal organs. A significant proportion have KS limited to the skin and oropharyngeal mucous membranes. In patients without AIDS, KS has been very responsive to radiation therapy. In order to avoid the immunosuppressive effects of chemotherapy in those patients with limited disease, and in an attempt to prevent visceral spread by gaining control over skin disease, we are engaged in a trial of electron beam radiation to the entire skin. With this technique, the penetration of ionizing radiation will be limited to a depth of the patient, of less than 1 cm., which should not have an adverse effect on these patients already compromised immune systems.

Project Description

Objective: To determine the lowest effective dose of ionizing radiation in patients with KS associated with AIDS; to evaluate the safety and efficacy of total skin electron beam in the syndrome; to determine what effect control of skin lesions will have on the natural history of KS associated with AIDS; and to determine the effect of this treatment on the immunologic abnormalities in these patients.

Methods Employed

Patients with AIDS and KS limited to the skin and oropharyngeal mucous membranes who have failed Interferon therapy will be treated with 3.9 MeV electron beam total skin radiation therapy using the techniques developed for the treatment of mycosis fungoides. The first six patients will have individual skin lesions irradiated with graded test doses which will be assessed for complete response one month later. This information will be utilized to determine the dose to be prescribed to the skin of these and subsequent patients. Immunologic studies to assess the P and B cell populations in these patients will be performed prior to the initiation of therapy and at one month post completion of treatment.

Major Findings

This trial has just been initiated. Several patients have received test doses of radiation to various individual skin lesions. At this point there is not enough data for even preliminary analysis.

Significance to Biomedical Research and the Program of the Institute

This study should provide a better understanding of the course of Kaposi's Sarcoma and AIDS. In addition, we hope to gain insight into the interaction of radiation and the immune system.

Proposed Course

The project is currently underway.

Publication

None

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1982 to September 30, 1983

Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Prospective randomized protocols in the treatment of soft tissue sarcomas have demonstrated the effectiveness of adjuvant chemotherapy with adriamycin, cytoxan and high dose of methotrexate in the treatment of patients with high grade soft tissue sarcomas of the extremities. Disease-free and overall survival rates appear to be doubled in patients receiving chemotherapy. Another prospective randomized trial has demonstrated that limb-sparing surgery is as effective as amputation in the local management of most of these patients. Preliminary results from a third prospective randomized trial indicates that a short course of adriamycin cytoxan is as effective as a long course of treatment with these drugs in preventing recurrences in patients with high grade soft tissue sarcomas of the extremities. Similar protocols are in progress evaluating the role of adjuvant chemotherapy for the treatment of soft tissue sarcomas of the trunk and head and neck.

Another prospective randomized trial in the Surgery Branch is evaluating the use of intraperitoneal 5FU as an adjuvant in the treatment of poor prognosis patients with colorectal cancers. A prospective randomized trial is in progress in the treatment of esophageal cancer that is evaluating whether pre and post-operative chemotherapy can improve the prognosis of these patients. A prospective randomized trial is in progress evaluating the use of an implantable continuous infusion pump compared to standard therapy for the treatment of patients with hepatic metastases. Over 100 patients have been randomized to a prospective randomized protocol comparing breast conservation surgery compared to modified radical mastectomy in the treatment of patients with breast cancer. Other Surgery Branch trials are evaluating nutritional support in cancer patients, intraoperative radiation therapy for the treatment of pancreatic and gastric cancer and the resection of hepatic metastases.

Laboratory efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients. Techniques have been developed for isolating cloned lymphoid cells that are capable of curing mice of selected syngeneic solid and disseminated tumors. Preliminary clinical trials have been performed studying the use of activated killer cells for the treatment of human cancers. Studies are in progress to isolate lymphoid cell lines and long-term lymphoid cloned cell lines capable of reacting against human tumors that may be effective for the adoptive immunotherapy of cancer. An IND has been obtained for the use of interleukin 2 *in vivo* in cancer patients and in patients with Acquired Immuno-deficiency Syndrome and these trials are about to begin. In experimental animals the *in vivo* use of IL-2 has been shown to enhance immune responses. Other Surgery Branch laboratory efforts are devoted to studying methods for selectively delivering chemotherapeutic agents to hepatic metastases.

Extensive studies are in progress to evaluate tumor host metabolic interactions and to evaluate basic metabolic effects of total parenteral nutrition on the tumor host relationship. Other Surgery Branch laboratory studies involve the study of immunosuppressive factors produced by tumors and the development of monoclonal antibodies against human sarcomas and pancreatic cancer.

TABLE I
Cases - General Surgery
April 1, 1982 - March 31, 1983

<u>General:</u>	Abdominal-perineal resection	3
	Aortocaval lymph node dissection and biopsy for ovarian cancer staging	10
	Appendectomy	1
	Colon/bowel resection	39
	Cholecystectomy	8
	Colonoscopy	9
	Denver shunt	3
	Endoscopy, UGI	12
	Exc. pelvic A/V malformation	1
	Exploratory laparotomy	78
	Feeding gastrostomy	5
	Gastrectomy	2
	Gastrosocopy	4
	Hepatic resection	15
	Hepatic resection w/insertion infusaid pump	16
	Herniorrhaphy/hernia repair	4
	Laparoscopy	5
	Major soft tissue or muscle group excision	38
	Pancreatectomy - partial/total	7
	Peritoneoscopy	5
	Polypectomy with sigmoidoscopy/sigmoid bx/EUA	9
	Revision of colostomy	2
	*Radiation therapy - intraoperative	9
	Splenectomy	6
	Staging laparotomy + splenectomy for lymphoma	19
	Total pelvic extenteration	2
<u>Surgery for</u>		
<u>Melanoma:</u>	Excision solitary nodules/nevi	3
	Lymph node dissection: Axillary	2
	Wide excision with/without STSG	3
<u>Head & Neck:</u>	Radical neck + other resection	4
	Tracheostomy	5
<u>Plastic:</u>	Debridement	3
	Skin grafting, STSG	24
	Lattisimus dorsi/myocutaneous flap reconstruction	13
	Revision breast reconstruction	2
<u>GYN:</u>	Hysterectomy	2
	D & C	1

* Actual operative procedure listed elsewhere

TABLE I (continued)

<u>GU:</u>	Aortocaval/retroperitoneal lymph node dissection	3
	Cystoscopy + other procedures, biopsy	72
	Debulking procedure	6
	Hypospadias repair	2
	Neophrostogram	1
	Nephrostomy/nephrolithotomy/nephrectomy/pyeloplasty	5
	Orchiectomy/orchiopexy	7
	Prostatic biopsy	4
	Transureteroprostatectomy	6
	Testicular biopsy	3
	Urethral dilation	1
<u>Breast:</u>	Axillary node dissection with biopsy	20
	Breast biopsy	75
	Modified radical mastectomy	21
	Simple mastectomy	2
<u>Orthopedics:</u>	Above elbow amputation	2
	Above knee amputation	12
	Below knee amputation	4
	Bone biopsy	15
	Coccygectomy	1
	Finger amputation	1
	Forearm amputation	2
	Forequarter amputation	6
	Hemipelvectomy	11
	Hip disarticulation	2
	Ray amputation, hand	1
Stump revision	1	
<u>Endocrine:</u>	Adrenalectomy	6
	Excision pheochromocytoma	1
	Mediastinal exploration	4
	Parathyroidectomy	29
	Parathyroid autograft implant/removal	5
	Thyroidectomy - complete/subtotal	14
<u>Vascular:</u>	A-V shunt/shunt revision/shunt removal	3
	Broviac/Hickman catheter placement	64
	Ligation IVC	1
	Subclavian CVP line placement	4

TABLE I (continued)

<u>Thoracic:</u>	Unilateral thoracotomy	
	Biopsy nodules	57
	Chest wall resection	2
	Pneumonectomy	2
	Lobectomy	4
	Drainage, empyema/ligation bleeding	3
	Perfusion procedure	1
	Bilateral thoracotomy/median sternotomy	35
	Bronchoscopy	22
	Esophagoscopy/dilatation/ <u>±</u> bronchoscopy	17
	Esophagogastrectomy	10
	Esophageal bypass/colon interposition	4
	Rib biopsy, open	10
<u>Minor:</u>	Biopsy: Node	144
	Incision and drainage of abscess	17
	Biopsy: Tissue mass, NOS	75
	Insertion/removal abdominal catheter for chemotherapy	24
	Wound repacking/debridement	16

TABLE II
Consultants - Surgery
April 1, 1982 - March 31, 1983

<u>Plastic</u>		
<u>Surgery:</u>	Excisional biopsy	1
	Major wound debridement	3
<u>Vascular:</u>	Creation A-V fistula	6
	Embolectomy	1
<u>Gynecological:</u>	D & C	1
	Total abdominal hysterectomy	1
<u>Orthopedic:</u>	Exc. 2nd and 3rd metacarpals	2
	Removal/insertion finger pins	3
	Bone Biopsy	1
	Prosthetic limb replacement (Tikoff-Linberg)	1
	Arthroscopy w/synovial biopsy	25
	Carpal tunnel release	4
	Syovectomy	2
	Below knee amputation	1
	Muscle group excision	2
<u>ENT:</u>	Antrostomy	5
	Laryngoscopy/pharyngoscopy/esophagoscopy	
	bronchoscopy	4
	Myringotomy, bilateral	5
	Polypectomy	3
	Excisional biopsy	5
	Septoplasty	4
	Debridement	1
	Tonsillectomy	2
	Tracheostomy	3
	Orbital/facial exenteration	1
	Esophageal dilation	1
	Glossectomy	1
	Frontal sinus trephination	1
	Total laryngectomy	1
	Closure oral-antral fistula	1

TABLE 111

STATISTICAL REPORT

SURGICAL SERVICES DEPARTMENT
 April 1, 1982 - March 31, 1983

Patient	NCI	NHLBI	Neuro.	NINCOS Muscle	NIAMDD	NIAID	NIDR	NIMH	NICHHD	NEI	Consult- ants	Total # Pts Operated
NCI Major	880	2	24	1	-	-	2	-	-	-	27	936
NCI Minor	287	-	1	2	-	-	-	-	-	2	-	292
NHLBI Major	29	270	1	-	-	-	2	-	-	-	4	306
NHLBI Minor	8	26	-	-	-	-	-	-	-	-	1	35
NINCOS Major	14	1	80	-	-	-	1	-	-	-	2	98
NINCOS Minor	6	-	6	18	-	-	-	-	-	-	9	39
NIAMDD Major	72	-	4	-	-	-	-	-	-	-	38	114
NIAMDD Minor	12	-	-	1	-	-	-	-	-	-	7	20
NIAID Major	55	-	4	-	-	-	-	-	-	3	17	79
NIAID Minor	26	-	-	5	-	-	-	-	-	1	1	33
NIDR Major	-	-	3	-	-	-	1	-	-	-	-	4
NIDR Minor	-	-	-	-	-	-	-	-	-	-	-	0
NIMH Major	1	-	-	-	-	-	-	-	-	-	-	1
NIMH Minor	-	-	-	-	-	-	-	-	-	-	-	0
NICHHD Major	12	-	6	-	-	-	2	-	-	-	2	22
NICHHD Minor	2	-	4	-	-	-	-	-	-	-	-	6
NEI Major	1	-	-	-	-	-	-	-	-	15	1	17
NEI Minor	-	-	-	-	-	-	-	-	-	-	1	1
MAJOR TOTAL	1064	273	122	1	0	0	8	0	0	18	91	1577
MINOR TOTAL	341	26	11	26	0	0	0	0	0	3	19	426
GRAND TOTAL	1405	299	133	27	0	0	8	0	0	21	110	2003*

*Of these 2003 surgical procedures 179 patients were on OPD status and 21 procedures were performed on the nursing units. There were 179 emergency procedures and 149 non-emergency procedures added.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03800-13 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Surgical Consultants & Collaborative Research Involving Surgical Services at NIH

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: S. A. Rosenberg Chief of Surgery, NCI Surg NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.0

PROFESSIONAL:

4.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients for elective consultations as well as all emergency general surgical problems. Many collaborations on clinical studies have resulted from these consultative efforts.

Cooperating Units (if any)

Entire staff	Surgery Branch, NCI	Surg	NCI
G. D. Aurbach	Chief, Metabolic Dis. Br.	MD	NIAMDD
J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
E. Glatstein	Chief, Rad. Oncol. Br.	RO	NCI
J. Robbins	Chief, Clin. Endocrin. Br.	CE	NIAMDD
L. Liotta	Lab. of Pathology	LP	NCI
R. C. Young	Chief, Medicine Branch	M	NCI
P. Pizzo	Chief, Ped. Oncol. Br.	PO	NCI
J. Gardner	Chief, Digestive Dis. Br.	DD	NIAMDD

INTRODUCTION

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of surgical procedures performed by the Surgery Branch is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

Over 1000 consultations were received last year from other NCI Branches as well as other NIH Institutes.

Project Description: Selected projects are presented below to provide examples of the nature of Surgery Branch collaborative efforts.

- Part I. Endocrine Surgery
- Part II. Thoracic and Vascular Surgery
- Part III. Nutritional Support
- Part IV. Ovarian Cancer
- Part V. Tenckhoff Catheters
- Part VI. Vascular Access

Part I. Endocrine Surgery

In response to requests by the Endocrinology Group, the Surgery Branch has designated one surgeon to perform all the endocrinological surgery. From July 1982 through July 1983, 32 neck and/or mediastinal explorations have been performed for primary hyperparathyroidism. Most of the patients had failed prior surgery but approximately 30% were virgin cases. Ninety-four percent of these patients underwent successful procedures and were rendered euparathyroid or

or hypoparathyroid. Seven patients rendered hypoparathyroid underwent cryo-preserved autologous parathyroid autografts, and five grafts have functioned.

Twenty-three patients with cold thyroid nodules were aspirated and underwent thyroid surgery. Thyroid lobectomy was performed on all cases, ten patients had thyroid cancer and underwent total thyroidectomy.

In conjunction with Digestive Diseases a new protocol has been developed for the management of patients with Zollinger-Ellison syndrome. After biochemical testing, these patients undergo radiological procedures to localize their tumors, and laparotomy to remove them. Eight patients have had surgery, and the tumor was removed in five. In two patients the postoperative basal and stimulated gastrin levels were normal suggesting a surgical cure.

Part II. Thoracic and Vascular Surgery

Consultative services for thoracic and vascular surgical problems are handled through the Surgery Branch. One hundred thirty-two major and thirty-five minor thoracic surgical procedures were performed between 4/1/82 and 3/1/83.

Part III. Nutritional Support

The Surgery Branch continues to mount a major effort in supporting patients nutritionally by intravenous feeding (TPN). The number of patients supported by TPN has almost doubled from the previous year. The Surgery Branch has been responsible for 1,955 days of TPN involving 93 patients. In addition, four patients have been started and managed on home parenteral nutrition. This has been achieved with a low complication rate (3%).

Part IV. Ovarian Cancer

Studies of ovarian carcinoma are undertaken in Medicine and Radiation Oncology Branch protocols with the cooperation of the Surgery Branch. Adjuvant systemic melphalan chemotherapy is being compared with intraperitoneal radioactive phosphorus for high-risk patients following complete surgical tumor resections. Combination chemotherapy is being utilized to treat advanced-stage patients. Intraperitoneal chemotherapy is evaluated both as an adjuvant in early-stage patients and as definitive therapy in certain patients with advanced disease. Patients with incomplete surgical tumor resections or with disseminated disease are treated with various combinations of systemic chemotherapy, sometimes including radiation therapy. The Surgery Branch collaborates with the Medicine and Radiation Oncology Branches in ovarian cancer studies by providing surgical evaluations and services, as well as performing definitive resections, staging laparotomies, explorations for complications or failures of treatment, and peritoneal catheter placements. During 1982, the Surgery Branch performed 36 operative procedures on patients in ovarian cancer protocols.

Part V. Tenckhoff Catheters

Tenckhoff catheters have been used for several years for peritoneal dialysis in patients with chronic renal failure. Using these catheters, direct

administration of chemotherapeutic agents is possible into the peritoneal cavity. The conduct of these studies has been under the general direction of Dr. Charles Myers, Chief of the Clinical Pharmacology Branch. Phase I and Phase II trials of intraperitoneal 5-FU and adriamycin have been completed for ovarian cancer. An adjuvant 5-FU trial for poor risk patients who have had a resection for colon and rectal cancer is currently in progress. Phase I trials with intraperitoneal misonidazole for peritoneal implants with ovarian or colorectal cancer have begun. The Surgery Branch is responsible for the insertion and removal of Tenckhoff catheters on protocol patients. Twenty catheters were inserted 4/1/82 - 3/31/83.

Part VI. Vascular Access

The Surgery Branch accepts consults from the Clinical Center to provide means of vascular access. Renal dialysis, plasmapheresis, drug and blood product infusion and blood withdrawal are some of the indications for vascular access procedures in selected patients. Between April and March, 64 Hickman or Broviac catheters were placed to facilitate vascular access in Clinical Center patients.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03801-13 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies in Cancer Surgery

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: S. A. Rosenberg Chief of Surgery, NCI SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

10.6

PROFESSIONAL:

7.6

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant disease. The major emphasis of these studies is in the treatment of malignant melanoma, soft tissue sarcomas, osteogenic sarcomas, and colorectal cancer. The major emphasis in Surgery Branch cancer therapy is in adjunctive therapy with emphasis on the use of multiple treatment modalities in addition to surgery.

Cooperating Units (if any)

Entire staff	Surgery Branch, NCI	SURG	NCI
L. Lichter	Lab. of Pathology	LP	NCI
D. Sachs	Chief, Immunol. Br.	I	NCI
R. C. Young	Chief, Medicine Branch	M	NCI
B. A. Chabner	Head, Biochem. Pharm. Sect.	LCHPH	NCI
M. Lippman	Senior Investigator	M	NCI
E. Glatstein	Chief, Rad. Oncol. Br.	RO	NCI
J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
P. Pizzo	Chief, Pediatric Oncol. Br.	PO	NCI

Project Description:

- Part I. Malignant Melanoma
- Part II. Soft Tissue Sarcomas
- Part III. Osteogenic Sarcoma
- Part IV. Urology
- Part V. Colorectal Cancer
- Part VI. Breast Cancer
- Part VII. Endoscopy
- Part VIII. Computer Applications
- Part IX. Esophageal Cancer
- Part X. Intraoperative Radiotherapy
- Part XI. Resection of Pulmonary Metastases
- Part XII. Treatment of Liver Metastases
- Part XIII. Reconstruction of the Breast

Part I. Malignant Melanoma

The Surgery Branch is currently concluding follow-up on patients entered into treatment trial of patients for Stage II melanoma conducted in conjunction with the Immunology and Medical Oncology Branches. Patients were randomized to receive either lymphnode dissection alone or lymphnode dissection followed by treatment with either methyl CCNU, BCG, or BCG plus allogeneic and melanoma cells. 181 patients were randomized into this protocol. Follow-up is continuing. There does not appear to be a difference between any of the adjuvant treatment groups and treatment with surgery alone.

Part II. Soft Tissue Sarcomas

The Surgery Branch is conducting a variety of protocols evaluating the treatment of patients with soft tissue sarcomas. 65 patients have been included in a randomized protocol evaluating the role of adjuvant chemotherapy with adriamycin and cytotoxin in the treatment of patients with soft tissue sarcomas of the extremities. A highly statistically significant improvement has been seen in patients randomized to receive chemotherapy ($p < .007$). Follow-up is continuing. 43 patients have been included in randomized protocols evaluating the role of amputated surgery compared to limb-sparing surgery plus radiation therapy in the treatment of patients with extremity sarcomas. Limb-sparing

surgery appears to be an effective treatment for these patients. No differences have been seen between patients randomized receiving limb-sparing surgery or patients randomized receiving amputated surgery. Seventy-one patients have been randomized in a protocol comparing short course and long course chemotherapy in patients with high grade soft tissue sarcomas of the extremities. Although followup is short no difference exists between the chemotherapy arms of the protocol.

Part III. Osteogenic Sarcoma

We continue to follow the 55 patients entered into studies evaluating the use of high dose methotrexate in patients with osteogenic sarcoma. With a minimum followup of 4 1/2 years and a maximum followup of seven years, the clinical performance of this group of adjuvant chemotherapy treated patients continues to compare quite favorably against our own or any published historical control group. Recurrent free survival appears to plateau at about 35% compared to 18% for our own historical controls. Overall survival is 3-fold better for the study patients (60% at five years) compared to historical controls (20% at five years).

26 patients have been accessioned to our randomized prospective trial comparing the strategy of immediate high dose methotrexate adjuvant chemotherapy following primary tumor resection to that of surgery alone with chemotherapy held in reserve for only those patients who develop recurrence. Analysis of the data thus far fails to show statistically significant better disease-free or overall survival for patients in either arm of the study although a trend favoring patients receiving immediate adjuvant chemotherapy has developed. This study has been supplanted by our participation with the Pediatric Oncology Branch in the Pediatric Oncology Group cooperative study for patients with osteogenic sarcoma. This trial seeks to compare immediate very intensive adjuvant chemotherapy (Rosen's T10 regimen) with no immediate adjuvant treatment. During an eight month accrual period, the cooperative group has put 16 patients on study. Five of these randomized patients have been registered by the NCI.

The Surgery Branch has begun to explore the use of limb salvage operations for patients with humeral and femoral primary osteogenic sarcoma in selected cases in an effort to define the role of this procedure.

Part IV. Urologic Surgery

Urologic surgery of the Surgery Branch provides consultative services for all genitourinary problem throughout the National Institutes of Health. This has included evaluation for hematuria, cystitis, urinary outlet obstruction, nephrolithiasis, impotence, renal massess, ureteral obstruction, and many other conditions. The patients are evaluated, appropriate tests suggested and surgery performed as indicated. The Surgery Branch also performs surgery for primary adrenal lesions such as theochromocytoma and aldosteronoma and for secondary abnormalities such as ectopic ACTH production. The Surgery Branch also performs collaborative studies with the Medicine Branch in the treatment of patients with metastatic testicular carcinoma. The patients are jointly managed and surgery is performed when

indicated.

Part V. Colorectal Cancer

At the present time two protocols for the study of patients with colorectal cancer are operative. The first protocol concerns the postoperative management of patients who have had lymph node positive colon or rectal cancer removed. These patients are carefully followed with monthly CEA assays, 3-monthly abdominal ultrasound and CAT scans, and monthly physical examinations. By so doing we wish to pick up early recurrences for "second look surgery" as well as determine optimal follow-up regimens. In the second protocol the same group of patients are randomized to receive intravenous or intraperitoneal 5-FU. Forty one patients have been entered into these studies. In the second study patients with local or regional colon or rectal recurrences undergo second look surgery randomized to receive or not receive radiation therapy.

Part VI. Breast Cancer

Under Protocol 79-C-111 which began accrual of patients in July 1979, 96 patients have been entered through December 1982. Patients with clinical Stage I or II unilateral breast cancer are randomly assigned to receive total mastectomy with axillary dissection, or excisional biopsy with axillary dissection followed by radiotherapy to the affected breast. Of the 96 patients, 47 have been randomized to the mastectomy group, and 49 to the radiotherapy group. Patients who receive mastectomy will be given an opportunity to have reconstruction after six months. Nineteen mastectomy patients have had reconstruction. Forty-two patients (44%) had at least one positive axillary node and have entered into adjuvant chemotherapy with IV Adriamycin and oral Cytoxan. With a median follow-up of 22 months there have been seven recurrences; six in the mastectomy arm and three in the radiation arm. Three of these patients have died. A increased rate of accrual is hoped for during the next year.

Part VII. Endoscopy

The Surgery Branch continues to collect clinical data to help define the appropriate role for laparoscopy in cancer diagnosis and treatment. Laparoscopic tubal ligation, as a consultative service, is available for appropriate patients. During the past year, 30 patients have undergone laparoscopy on the NCI surgical service. Surgery Branch colon and rectal cancer protocols promise to increase our utilization of this endoscopic procedure in the coming year.

Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The utility of this procedure from both the diagnostic and therapeutic point of view of colon lesions is well established. Twenty-four colonoscopic procedures were performed in the past year. These included 18 diagnostic examinations and 6 snare polypectomies. Direct visual access to and ability to histologically sample tissues from the entire lower GI tract offer excellent opportunities for earlier diagnosis of colon malignancy. Premalignant lesions in patients with ulcerative colitis can be identified. Colonoscopy will play a role in the preoperative assessment of

appropriate patients to undergo colon and rectal surgery.

Part VIII. Computer Applications

Data for Surgery Branch research continued to be collected, stored, and reported for three primary systems: 1) Cancer Patient Research Information System (CAPRI), 2) Serum Inventory, and 3) Surgical Metabolism Studies. The basic objectives underlying the design of the Surgery Branch data systems are to: 1) ensure very high accuracy of the data, and 2) permit information to be easily recorded, keyentered, verified, corrected, retrieved, and analyzed.

The programs and software packages available at the NIH Computer Center on the IBM-370 system and the DECsystem-10 are utilized for data entry, program maintenance, remote job submission, interactive computing, and graphic presentations. The Surgery Branch Data System Office maintains a library of user manuals for these systems. Two high-speed printing terminals (Alanthus T-1222) and one graphics display terminal (Tektronix 4012) provide access to the central computers. The addition of an electrostatic copier attached to the Tektronix terminal makes archivable, working copies of the plots generated on the screen immediately available. An MFE-5000 terminal is used to enter data which has been recorded on cassette tape by data loggers connected to automatic analyzers and radiation counters.

The processing of data by computer continues to play a significant role in assisting Surgery Branch investigators to define and describe the characteristics of protocol populations.

Part IX. Esophageal Cancer

A prospective randomized study to determine the efficacy of pre- and post-operative adjuvant chemotherapy in the treatment of patients with squamous cell carcinoma of the esophagus has been initiated by the Surgery Branch. Patients with carcinoma of the middle or lower third of the esophagus are randomized to receive either pre- and post-operative chemotherapy with cisplatin, bleomycin, and vindesine, or surgery alone. Fifteen patients have been entered into the study since initiation in January of 1983.

Part X. Intraoperative Radiotherapy

The Surgery Branch has initiated investigations of the role of combined surgery and intraoperative radiation therapy for the local control of abdominal malignancies. A series of pilot patients have been treated where the patients underwent surgical resection of a variety of abdominal tumors, were transported under anesthesia from the operating room to the radiotherapy treatment facility, were treated with electron radiation directly to the tumor bed having vital abdominal structures shielded from the radiation beam, and were returned to the operating room for completion of surgery. No technical difficulties were encountered in the pilot series, and therefore investigative prospective randomized protocols were initiated during 1980 to evaluate combined surgical resection and intraoperative radiation in certain poor-prognosis malignancies. Protocols for the treatment of gastric and pancreatic cancers were established,

comparing intraoperative radiotherapy with conventional external beam postoperative radiation in patients able to have all gross tumor surgically resected. Protocol for the treatment of retroperitoneal sarcomas was developed, comparing intraoperative and postoperative radiotherapy with conventional external beam postoperative radiation in patients able to have all gross tumor surgically resected. Protocol for the treatment of retroperitoneal sarcomas was developed, comparing intraoperative and postoperative low-dose external beam radiation with conventional high-dose postoperative low-dose external beam radiation with conventional high-dose postoperative radiotherapy in patients with surgically resectable tumors; in addition, the use of adjuvant chemotherapy is evaluated in patients treated for retroperitoneal sarcomas. Protocol for the treatment of bony sarcomas of the pelvis was initiated, treating patients with combined surgical resection and intraoperative radiation therapy to the tumor bed, comparing disease control in protocol patients with historical experience. Protocol for evaluating an escalating dose schedule for intraoperative irradiation was developed for patients with various malignant diseases that have no conventional surgical or radiotherapeutic treatment options. As of 7/1/83, accrual status of the various intraoperative radiotherapy studies was: gastric carcinoma 37 evaluated, 24 randomized, 15 on study; pancreatic carcinoma 106 evaluated, 41 randomized, 14 on study; retroperitoneal sarcoma 54 evaluated, 25 randomized, 19 on study; pelvic bony sarcoma 7 evaluated, 4 on study; escalating dose study 41 evaluated, 20 on study.

Part XI. Resection of Pulmonary Metastases

The Surgery Branch continues its protocol for the aggressive removal of metastatic sarcoma to the lung. After a thorough work-up to rule out metastatic disease at other sites, patients with locally controlled osteogenic or soft tissue sarcomas are subjected to pulmonary resection. An attempt is made to remove all gross evidence of tumor while preserving as much pulmonary parenchyma as possible. Multiple procedures are often required. Within the past 12 months 76 operations have been performed for resection of pulmonary metastatic disease. Forty-four of these were median sternotomies and 34 were lateral thoracotomies.

Part XII. The Treatment of Liver Metastases

The Surgery Branch continues to maintain an aggressive stance with regard to the treatment of colorectal metastases to the liver. Patients who are referred to the NIH and have metastatic disease to the liver undergo resection if four, or less, suitably located nodules are identified. A protocol (82-C-138) was initiated to address the problem of palliation for colorectal metastatic disease in the liver. This study examines patients with metastatic disease confined to the liver and who are unresectable and is a prospective randomized evaluation of systemic versus intrahepatic continuous infusion of FUdR. Since its inception in August the protocol has accrued 12 patients. Data is too preliminary to be able to allow a statement about which mode is the apparent better method of palliation. But, initial experience suggests that the reported high response rates to intrahepatic drug therapy are indeed accurate. The larger and more important question of which method of drug delivery prolongs survival is yet to be answered, but this data will become available with additional time and additional patient accrual.

Part XIII. Reconstruction of the Breast - Dr. McDonald

A part of the Breast Cancer Treatment Protocol makes available reconstruction of the breast following mastectomy to those patients who randomized to the mastectomy arm. We also make breast reconstruction available to those patients who have had a mastectomy and are being treated off the protocol. A review of the patients to this date reveals that 33.3% of those on the protocol who are offered reconstruction following mastectomy have elected to have it done and 25% of those patients in the treatment off protocol group have elected to have breast reconstruction. The majority of the breast reconstructions have been carried out using flap tissue because a deficiency of tissue on the anterior chest wall obviating simple reconstruction using insertion of an implant. The two methods of flap reconstruction being used are the latissimus dorsi musculocutaneous flap and the transverse rectus abdominis flap. A pilot study of immediate reconstruction was carried out in the past but did not prove satisfactory, so we now use the delayed technique.

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2. Javadpour, N., Soares, T., and Princler, G.L.: In vitro synthesis of alphafetoprotein and human chorionic gonadotropin in testicular cancer. Cancer. 49: 303-307, 1982.
3. Roth, J.A.: Humoral immune responses to human primary and metastatic sarcomas detected by an enzyme-linked immunoabsorbant solid-phase assay. Surgical Forum XXXII, 1981, pages 454-456.
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18. Rosenberg, S.A., Tepper, J., Glatstein, E., Costa, J., Baker, A., Brennan, M., DeMoss, E.V., Seipp, C., Sindelar, W.F., Sugarbaker, P., and Wesley, R.: The treatment of soft-tissue sarcomas of the extremities. Prospective randomized evaluations of (1) limb-sparing surgery plus radiation therapy compared with amputation and (2) the role of adjuvant chemotherapy. Annals of Surgery. 196: 305-315, 1982.
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29. Roth, J.A.: Tumor induced immunosuppression. Surgery, Gyn. Obstet. 156: 233-240, 1983.
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03811-09 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Immunotherapy of Animal and Human Sarcomas

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: S. A. Rosenberg Chief of Surgery Surg NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

8.5

PROFESSIONAL:

5.0

OTHER:

3.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Detailed studies of tumor-host immune interactions in animals and humans with sarcomas are being performed in an attempt to develop new immunodiagnostic and immunotherapeutic techniques for diagnosis and treatment of these tumors. Immune response to murine sarcomas has been extensively evaluated and both tumor-specific and fetal antigens have been identified. Attempts are being made to develop adoptive immunotherapeutic techniques utilizing transfer of cells grown in long term culture in T cell growth factor. Techniques for the prolonged growth of cytotoxic and proliferative T cells have been developed. These cells have been shown to mediate immunologic rejection of allografts and syngeneic tumors and attempts to use these cells for the adoptive immunotherapy of mouse and human tumors are in progress. The systemic administration of Interleukin-2 has been shown to enhance immune responses in vivo and therapeutic trials of IL-2 in humans will be undertaken shortly.

Cooperating Units (if any)

T. Sharp	Clinical Associate	Surg NCI
A. Mazumder	Clinical Associate	Surg NCI
M. Rosenstein	Research Fellow	Surg NCI
L. Grimm	Expert	Surg NCI
J. Donohue	Clinical Associate	SURG NCI
D. Weiland	Clinical Associate	SURG NCI
S. Schwarz	Biologist	SURG NCI
H. Wexler	Biologist	SURG NCI
P. Spiess	Biologist	SURG NCI
C. Hyatt	Biologist	SURG NCI
M. Lotze	Senior Investigator	SURG NCI
F. Chang	Senior Investigator	SURG NCI
A. Rayner	Medical Staff Fellow	SURG NCI

PUBLICATIONS

1. Eberlein, T.J., Rosenstein, M., Spiess, P.J., and Rosenberg, S.A.: The generation of long term T-lymphoid cell lines with specific cytotoxic reactivity for a syngeneic murine lymphoma. J. Natl. Cancer Inst. 69: 109-116, 1982.
2. Rosenberg, S.A., Eberlein, T., Grimm, E., Mazumder, A., and Rosenstein, M.: Adoptive transfer of lymphoid cells expanded in T-cell growth factor: Murine and human studies. In Fefer, A., Goldstein, A.L. (Eds.): The Potential Role of T-Cell Subpopulations in Cancer Therapy, New York, Raven Press, 1982, pp. 161-171.
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4. Grimm, E.A., Mazumder, A., and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. V. Generation of allospecific cytotoxic lymphocytes to nonimmunogenic antigen by supplementation of in vitro sensitization with partially purified T-cell growth factor (PP-TCGF). Cell. Immunol. 70: 248-259, 1982.
5. Grimm, E.A., and Rosenberg, S.A.: Production and properties of human IL-2. In Fathman, G. and Fitch, F.W. (Eds.): Isolation, Characterization and Utilization of T Lymphocyte Clones. New York, Academic Press, 1982, pp. 57-82.
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21. Rosenstein, M., Eberlein, T., Schwarz, S., and Rosenberg, S.A.: Simplified techniques for the isolation of alloreactive cell lines and clones with specific cytotoxic or proliferative activity. J. Immunol. Methods. (in press).
22. Rosenberg, S.A., Spiess, P.J., and Schwarz, S.: In vivo administration of interleukin-2 increases the in vivo generation of cytotoxic cells. Transplantation. (in press).
23. Grimm, E.A., Gorelik, E., Rosenstein, M.M., and Rosenberg, S.A.: The lymphokine-activated killer cell phenomenon: In vitro and in vivo studies. In Cohen, S. and Oppenheim, J. (Eds.): The Proceedings of the Third International Lymphokine Congress. New York, Academic Press. (in press).
24. Mazumder, A., Eberlein, T.J., Grimm, E.A., Wilson, D.J., Keenan, A.M., Aamodt, R., and Rosenberg, S.A.: Phase I study of the adoptive immunotherapy of human cancer with lectin activated autologous mononuclear cells. Cancer. (in press).
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06652-07 SURG

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Immune Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: P. H. Sugarbaker

Senior Investigator

SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The work in this laboratory includes three major projects: 1) Studies involve an assesemnt of cell-mediated immune responses after the host has responded to a variety of alloantigenic stimulation to the same and other antigens. The significance of this work comes from attempts to understand regulatory mechanisms of cellular immune responses. This work has resulted in the development of a new mechanism of T-cell control - the alloantigen elimination hypothesis. 2) Studies on the use of activated killer cells in the destruction of tumor cells have been undertaken. The receptor on tumor cells for killer lymphocytes is the subject of current investigation. An adjuvant immunotherapy attack on cancer cells remaining after surgery is planned using cells activated in vitro to lyse tumor cells. 3) A new mechanims of specific suppression to facilitate the transplantation of tissue and skin allografts is being attempted. The use of antigen specific suicide with ³H-Thymidine to produce clonally depleted cell populations is under investigation.

Cooperating Units (if any)

W. Matthews, Jr.	Chemist	SURG NCI
Y. Roth	Investigator	SURG NCI
M. Kemeny	Medical Staff Fellow	SURG NCI
C. McCullough	Clinical Associate	SURG NCI
F. Gianola	Physician's Assistant	SURG NCI

Publications:

1. McCullough, CS, Sugarbaker, PH, and Matthews, W.: Effects of passive enhancement on graft and host: Graft adaptation by alloantibody as the mechanism of prolonged skin allograft survival. Transplantation. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06654-06 SURG

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies in Malignant Disease

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: W. F. Sindelar Senior Investigator SURG NCI

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5.2

PROFESSIONAL:

3.0

OTHER:

2.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with sarcomas and gastrointestinal carcinomas are studied for evidence of reactivity against tumor-associated determinants expressed on both fresh and cultured syngeneic or allogeneic tumor cells using immunofluorescence and immunoperoxidase staining techniques. Various human malignant cell lines have been established in vitro and are being characterized morphologically and immunologically. An experimental model of pancreatic carcinoma has been developed in hamsters. Tumor-associated antigens have been isolated from both animal and human pancreatic cancers and are being investigated for possible applications to immunotherapy or methods of immunodiagnosis. Tissue-specific antigens have been isolated and are being investigated for possible use in immunotherapy of pancreatic carcinoma. Monoclonal antibodies have been developed to tumor-associated determinants in pancreatic cancers.

Cooperating Units (if any)

C. Kurman	Microbiologist	SURG NCI
C. Hyatt	Biologist	SURG NCI
R. Guohuang	Visiting Fellow	SURG NCI
J. Glenn	Clinical Associate	SURG NCI
J. Chin	Clinical Associate	SURG NCI
T. Kinsella	Senior Investigator	ROB NCI
A. M. DeLuca	Biologist	ROB NCI

PUBLICATIONS

1. Sindelar, W.F.: Cancer of the Small Intestine. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott, pp. 616-642.
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3. Sindelar, W.F., Kurman, C.C.: Nitrosamine-induced pancreatic carcinogenesis in outbred and inbred Syrian hamsters. Carcinogenesis 3: 1021-1026, 1982.
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6. Sindelar, W.F., Tralka, T.S., and Gibbs, P.S.: Evidence for acute cellular changes in human hepatocytes during anesthesia with halogenated agents: An electron microscopic study. Surgery 92: 520-527, 1982.
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14. Tepper, J., Sindelar, W.F., Travis, E., et al.: Tolerance of canine anastomoses to intraoperative radiation therapy. Int. J. Radiat. Oncol. Biol. Phys. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06655-03 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Influencing Host Cellular and Humoral Immune Responses to Neoplasia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: J. A. Roth

Senior Investigator

SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Thoracic Oncology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our laboratory has focused on factors that influence host responses to tumors and may adversely influence responses to immunotherapy. We have identified an immunoregulatory factor produced by a variety of human tumors that profoundly inhibits in vitro cell mediated immune responses. We are in the process of characterizing and purifying this factor and also determining its role in vivo. We are developing sensitive new techniques to measure humoral immune responses to human tumor-associated antigens including an enzyme-linked immunosorbent solid-phase assay using soluble and membrane bound human tumor-associated antigens. Using monoclonal antibodies, we are analyzing the distribution of tumor associated antigens on primary tumors and their metastases. Concurrently, we have established a murine melanoma model with cloned primary and metastatic cell lines to test various therapeutic options. We are preparing hybridoma monoclonal antibodies to human and murine tumor-associated antigens in an attempt to develop specific serotherapy for metastatic tumors.

Cooperating Units (if any)

J. Putnam	Clinical Associate	Surg NCI
P. Scuderi	Staff Fellow	Surg NCI
E. Trahan	Chemist	Surg NCI
R. Ames	Biologist	Surg NCI
B. Funkhouser	Clinical Associate	Surg NCI
H. Pass	Staff Fellow	Surg NCI
S. Leong	Clinical Associate	Surg NCI

PUBLICATIONS

1. Roth, J.A., and Wesley, R.A.: Human tumor-associated antigens detected by serologic techniques: Analysis of autologous humoral immune responses to primary and metastatic human sarcomas by an enzyme-linked immunoabsorbant solid-phase assay (ELISA). *Cancer Res.* 42: 3978-3986, 1982.
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3. Schwartz, J., Roth, J., and Carpentier, B.: Effect of human lung carcinoma extract as an immunosuppressant of allografts in rats. *Afr. J. Clin. Exp. Immunol.* 3: 191-197, 1982.
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5. Roth, J.A.: Collective Review: Tumor induced immunosuppression. *Surg. Gynec. Obstet.* 156: 233-240, 1983.
6. Roth, J.A., Grimm, E.A., Osborne, B.A., Putnam, Jr., J.B., Davidson, D.D., and Ames, R.S.: Suppressive immunoregulatory factors produced by tumors. *Lymphokine Res.* 2: 67-73, 1983.
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8. Roth, J.A.: Detection of a suppressive immunoregulatory factor (IRF) in the sera of sarcoma patients by enzyme-linked immunoassay (ELISA) and correlation with clinical course. *J. Surg. Oncol.* (in press).
9. Roth, J.A., Davidson, D.D., Ames, R.S., and Schneider, P.D.: Tumor stasis factor (TSF): a possible mechanism for the regulation of tumor cell proliferation. *J. Surg. Res.* (in press).
10. Putnam, J.B., Jr., Roth, J.A., Johnston, M.R., Wesley, M.N., and Rosenberg, S.A.: Survival following aggressive resection of pulmonary metastases from osteogenic sarcoma: Analysis of prognostic factors. *Ann. Thor. Surg.* (in press).
11. Keller, S.M., Papazoglou, S., McKeever, P., Baker, A., and Roth, J.A.: Late occurrence of malignancy in a ganglioneuroma 19 years following radiation therapy to a neuroblastoma. *J. Surg. Oncology* (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06656-02 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Definition and Modification of Neoplastic Tissue Sterol Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: P. D. Schneider Senior Investigator SURG NCI

COOPERATING UNITS (if any)

C. M. Gorschboth, Medical Technologist, SURG, NCI
S. B. Edge, Clinical Associate, SURG, NCI
D. A. Potter, Clinical Associate, SURG, NCI
Molecular Disease Branch, NHLBI

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to investigate tumor sterol metabolism in normal human liver as well as hepatoma lines, a serum-free culture system has been established for human hepatocytes. Lipoprotein binding studies have been instituted in a human hepatoma cell line as well as normal human liver. *In vivo* studies of Morris hepatoma 7777 have been initiated with animals receiving sterol synthesis inhibitor called Mevinolin. Two initial studies have been completed to establish the proper dosage interval and to investigate the effect of the drug on a key sterol synthetic enzyme. Additional data in this system includes information about total body sterol synthesis utilizing isotope methods previously established. The interaction of melanoma cells in culture with liposomes of varying molecular content has been investigated in an attempt to disclose unusual aspects of lipid processing by melanoma cells. Publications this year related to this work: (1) Schneider, PD, Gorschboth, CM, Preventing eschemic liver injury by interfering with lysosomal autophagy. *J. Surg. Res.* 34: p.5, 1983. (2) Potter, DA, Gorschboth, CM, Schneider, PD, Enhanced liposome uptake by melanoma: potential for targeted regional drug delivery. Accepted for *Surgical Forum* 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06657-01 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies to Identify a Circulating Factor that Causes Cachexia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P.I. J. A. Norton, M.D. Acting Head, Surgical Metabolism Section SURG NCI

COOPERATING UNITS (if any)

J. Moley, Clinical Associate, SURG, NCI
 R. Edwards, Technologist, SURG, NCI
 Seoras Morrison, Ph.D., Laboratory of Theoretical
 Biology, NCI, NIH

LAB/BRANCH

Surgery Branch

SECTION

Metabolism Section

INSTITUTE AND LOCATION

Cancer Insititute, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An in vitro assay for protein synthesis and breakdown has been developed by incubating weanling rats' extremity muscles in a physiological buffer system. Test sera are added from normal, septic and cachectic animals to determine if there is a factor in the sera which decreases protein synthesis and increases protein catabolism. Such a circulating factor has been reported in the septic state. An in vivo assay has been developed for a cachectic factor by administering sera from cancer bearing animals who are not eating to normal animals, and measuring food intake. If these studies demonstrate a factor, additional studies will be done to determine if it is a tumor derived factor or a host derived factor in response to the tumor, and to biochemically characterize the factor.

Exogenous insulin has been administered to rats bearing a sarcoma. Insulin given to tumor-bearing animals increases spontaneous food intake, nitrogen balance, body weight gain, decreases muscle catabolism (3 methyl histidine) without stimulating tumor growth. Survival studies show no advantage to the insulin treated animals in time of survival, but the animals have greater carcass mass. Compositional analysis indicates that the accrual is both protein and fat similar to normal body composition. Thus exogenous insulin appears to preserve the host without feeding the tumor.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06658-01 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Pineal Gland Hormone Melatonin and Breast Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: D. N. Danforth, Jr. Senior Investigator SURG NCI

COOPERATING UNITS (if any)

Medicine Branch, NCI

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The regulation of estrogen receptor activity and metabolism is being investigated in vitro in MCF-7 human breast cancer cells, and in normal hamster uteri in vivo and in vitro. The enhancement of estrogen receptor activity by melatonin, and its dependence upon protein synthesis, is being studied in whole cell and cell-free systems to determine the mechanism of this regulation. The physiochemical changes and alterations in biological activity of the receptor induced in these cells by melatonin are being characterized using analytical biochemical techniques. Structural differences between estrogen receptors of normal and malignant tissues, and their modification by melatonin, are being studied to better understand the treatment and response of women with hormone-dependent breast cancer. The 24-hour plasma melatonin profiles, plasma steroid hormone levels, and hormonal and familial risk factors have been studied in women with breast cancer, in women at high risk for breast cancer, and in normal subjects. In women with breast cancer the relationship of plasma melatonin to the estrogen and progesterone receptor content of the primary tumor, hormonal risk factors, and extent of disease has been established. Women with hormonal dependent breast cancer have a depression of their plasma melatonin which is independent of all other parameters studied. This abnormality is also present in women at high risk for breast cancer but not found in normal subjects. The possibility of plasma melatonin being an etiologic factor for determining the presence or absence of hormone-dependent breast cancer is being studied in these subjects.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06659-01 SURG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Urologic Malignancy

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

PI: W. M. Linehan Senior Investigator SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Urologic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the past year, a laboratory has been organized to study problems in Urologic Oncology in the Surgery Branch of the National Cancer Institute. A variety of problems related to cancer of the urogenital system are being addressed. Studies in humans and animal models of the metabolic abnormalities associated with prostatic cancer are in progress. The etiology and metabolic basis for osteomalacia in patients and in animals with prostate cancer are being evaluated. The factors controlling the growth of prostatic cancer both in vivo and in tissue culture are being studied. In addition, studies have begun investigating the endocrine responsiveness of bladder cancer and on the influence of LHRH agonists on the growth of a hypercalcemia producing rat lytic cell tumor of the testis.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE BIOLOGICAL RESPONSE MODIFIERS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1982, through September 30, 1983

INTRODUCTION

The Biological Response Modifiers Program (BRMP) is a comprehensive program of the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), involved in clinical and laboratory research with both extramural and intramural components to investigate, develop and bring to clinical trials potential therapeutic agents that may alter biological responses important in the biology of cancer growth and metastasis. This program was conceived as a focused approach in DCT to support further basic research in biologicals and biological response modifiers (BRM) and to rapidly apply potential leads from that research to the treatment of cancer in man.

The BRMP was initiated in concept in October 1978. At that time, the DCT Board of Scientific Counselors established a Subcommittee on Biological Response Modifiers whose overall mission was to review the current clinical and laboratory experience and to develop guidelines for developing biological materials for clinical use. The Associate Director for the BRMP was identified in 1980 and officially came on board October 20, 1980. Much of the initial groundwork, personnel actions, equipment purchases and other arrangements to begin the program were under way at the time of the announcement by Department of Health and Human Services of the official establishment of the BRMP on April 27, 1981. Presently, the BRMP is a fully functioning program pursuing a focused coordinated approach toward achieving the objectives identified by the DCT Board of Scientific Counselors.

Both extramural and intramural components comprise the BRMP. The extramural Biological Resources Branch (BRB) supports, through a balanced program of grants and contracts, preclinical and clinical biological response modifiers research in the biomedical community. The branch monitors Phase I and early Phase II clinical studies, which assess biological effects of BRM in cancer patients and correlate changes in the biological modifications with anti-tumor activity. The BRB has established a preclinical screening program for the selection and preclinical assessment of the efficacy of BRM. A resource distribution system involving information acquisition and assessment as well as agent acquisition and testing has been established.

The intramural program consists of the Biological Therapeutics Branch and the Laboratory of Molecular Immunoregulation. The Biological Therapeutics Branch 1) performs research on the cellular and humoral components of the immune response that may be involved in resistance to tumor growth; 2) studies growth factors and other biological response modifiers that may be involved in the regulation of tumor growth; 3) develops new biologicals and BRMs and investigates the effects of selected BRMs on the host and on tumor growth; and 4)

develops protocols for optimal biological response modification and evaluates the therapeutic efficacy of these substances in experimental animal tumor systems and in cancer patients.

The Laboratory of Molecular Immunoregulation 1) investigates at a molecular level the intercellular and intracellular processes that regulate host defense mechanisms, including isolation of RNA and the cloning of DNA that code for lymphokines and cytokines; 2) studies the lymphokine/cytokine modulation of cellular functions that participate in host defense; 3) devises new diagnostic tests to better define immune status and pursue more critical evaluation of BRM; 4) evaluates the effects of BRM on immunoregulatory pathways and host defense mechanisms and 5) generates new BRM that modify host defense mechanisms.

ACCOMPLISHMENTS - EXTRAMURAL PROGRAM

Grants Program

In FY 1983, active grants numbered approximately 79 (including six grants funded for their terminal year in 1982). Approximately \$11.0 million (73 grants) was awarded in FY 1983. This includes \$9.7 million to fund 57 ROIs, one R23, and five POIs. An additional \$1.3 million was awarded to fund continuation costs for ten grants activated in response to four RFAs in FY 1982.

Requests for Applications

In FY 1982, the Biological Response Modifiers Program issued four Requests for Applications (RFAs): Monoclonal Antibody in Cancer Therapy, Monoclonal Antibody in Animal Tumor Models, Therapeutic Use of Lymphokines, and Animal Tumor Models for Antipeptide Growth Factor and Maturation Factor Therapy. Seventy-two applications were received in response to the RFAs. Ten applications were funded for \$1.3 million.

The BRMP did not issue any new RFAs in FY 1983. It is estimated that the FY 1983 continuation costs for the ten grants awarded in FY 1982 will be approximately \$1.3 million.

Program Announcements

In FY 1983, the BRMP issued four Program Announcements: Use of Tumor Associated Antigens as Immunogens; Development of Genetically Engineered Cell Products; Use of Growth Factors, Maturation Factors and Antigrowth Factors in Animal Tumor Models, and Development of Cell Lines Producing Lymphokines and Cytokines. Grant applications are still being received for these Program Announcements. Eighteen grant applications have been already reviewed and, of these, three have been funded.

Contract Program

The branch staff initiates requests for proposals and provides programmatic direction, evaluation and monitoring for contracts supported by the BRB. In FY 1983, 32 contracts were active. During FY 83, the BRB awarded approximately \$2.7 million to 13 extramural contracts, including \$1.3 million for seven new clinical task order contracts and \$1.4 million in nine nonclinical contract

non-Hodgkin's lymphoma, one with Hodgkin's disease, one with myeloma and one with melanoma. Biological activity was noted in the form of stimulation of NK cell activity with low doses and suppression at high doses. A suggestion of stimulation of monocyte activity and suppression of hematopoiesis and erythropoiesis in the bone marrow was also demonstrated. The usual side effects associated with interferon such as fatigue, fever, anorexia, chills and weight loss were noted along with mild leukopenia and liver cell function abnormalities. The higher doses with this product also induced central nervous system malfunction in that two patients had seizures, and several became confused and unable to perform normal intellectual functions. All side effects were self limiting. Currently ongoing at UCLA, Duke, Memorial Sloan-Kettering, Georgetown, and Wisconsin are Phase II studies evaluating potential efficacy of this product in patients with breast carcinoma, renal cell carcinoma, melanoma, myeloma and colon carcinoma.

Clinical trials evaluating the potential for MVE-2 as a biological response modifier were performed at Ohio State University and Vanderbilt University. Investigators at Vanderbilt evaluated a weekly schedule and those at Ohio State a biweekly schedule. Twenty-two patients were studied at Vanderbilt and 16 at Ohio State. They demonstrated that maximally tolerable doses are in the range of 600-900 mg/m² and maximal cumulative doses in the range of about 2.5 gms/m². The material was reasonably well tolerated with the exception of a high incidence of proteinuria in patients receiving a total dose of over 1500 mg/m². As the total cumulative dose increased the incidence of proteinuria likewise increased. There was one antitumor response in a patient with melanoma noted and some suggestion of stimulation of NK cell activity and of antibody dependent cellular cytotoxicity. However, there was no solid evidence for strong biological response modification. The agent was supplied by Adria Laboratories and it will be their decision as to whether to pursue this product or not in Phase II studies.

Two thymosin products were supplied by Hoffmann-La Roche for evaluation. Fraction 5 is a mixture of approximately 20 polypeptides obtained by extraction and purification of material from a calf thymus. Alpha 1 is a purified polypeptid obtained from fraction 5. These products were studied at 4 institutions; the University of California at San Francisco, a member of the Northern California Oncology Group, Memorial Sloan-Kettering, the Fred Hutchinson Cancer Research Center, and the University of California in San Diego.

Phase I studies have determined that doses as high as 960 mg/m² of fraction 5 and 9.6 mg/m² of alpha 1 are tolerable. Essentially no toxicity was seen with either of these products over a wide dose range up to the doses indicated above. One-hundred-seventy-eight patients were evaluated in four Phase I studies with fraction 5 and over 100 patients in three studies of alpha 1. Beneficial responses were seen in 3 patients with renal cell carcinoma, using fraction 5. No objective antitumor responses were seen in patients receiving alpha 1. George Washington University also performed a randomized study in which patients received alpha 1 following the administration of radiation therapy for lung carcinoma. The study was designed to determine whether alpha 1, administered after radiation therapy prolonged a response. Patients receiving alpha 1 did indeed have a longer duration of response than those receiving placebo; however, the number of patients treated was very small. This prolongation was associated with improvement in lymphocyte function.

awards: two of these were new awards, four received incremental funding, one was recompleted and two were for the purchase of biologicals. These contracts provided for 1) the collection, storage, testing and quality assurance of BRM, 2) initial exploration into the monoclonal antibody and cytokine areas, 3) technical support for the review and evaluation of BRMs, and 4) Phase I/II clinical trials.

Clinical Phase I/II Studies

The BRMP established a Master Agreement mechanism in FY 1980 whereby 27 institutions were identified as highly qualified for performing clinical studies using BRM. The studies are closely monitored by the BRB to assess significance of changes in biological responses observed in patients under treatment with BRM. Toxicity, biological response and therapeutic effects are monitored simultaneously.

In previous fiscal years, fourteen task orders for testing interferons, thymosins and MVE-2 were awarded under this Master Agreement. Phase I trials for the alpha interferons, thymosins and MVE-2 have been completed. Ongoing Phase II trials with lymphoblastoid (alpha) interferon (Wellferon) are continuing to define the biologic activity of this highly purified, extracted alpha interferon. Both "high" and "low" doses are being tested in a broad spectrum of cancer patients to determine the effects on biological responses as well as the clinical activity.

Clinical trials were performed evaluating three interferon products. Two naturally extracted alpha interferons were purchased by contract from Warner-Lambert and from Meloy Laboratories. The Warner-Lambert material was tested by the Dana-Farber Institute. They studied 46 patients, 23 of whom had breast cancer, and determined that a dose of 18 MU/day, given intramuscularly, was the maximal tolerable dose. Beneficial clinical effect was noted in three patients with breast cancer and two with renal cancer. Actual objective responses were not seen. Alpha interferon obtained from Meloy was evaluated at Stanford and at Georgetown Universities. The Stanford investigators also established 18 MU/day given intramuscularly as the maximum tolerable dose of this material. The group at Georgetown evaluated a single dose administering up to 60 MU/m² (total dose approximately 100 MU) without toxicity. In these two trials clinical benefit was noted in one patient with chronic leukemia, one with bladder carcinoma, one with renal cell carcinoma, one with melanoma, one with head and neck cancer and one with a non-Hodgkin's lymphoma. Biological response modifying activity, i.e. NK cell stimulation was noted at the higher single doses by the Georgetown group. Toxicity with each of these products consisted of fatigue, fever, anorexia, weight loss, chills and to a lesser degree mild leukopenia and liver function abnormalities.

A Phase I study with naturally-extracted but highly-purified lymphoblastoid alpha interferon purchased from Burroughs-Wellcome, Ltd. was performed at Duke University and UCLA. The Duke group established that a dose of 15 MU/m², administered three times a week over a 5 week period was the maximum tolerable dose of this material administered by this schedule. Investigators at UCLA administered this interferon by a twice a week schedule for 7 days followed by a three week rest. On this schedule 18-30 MU was the maximally tolerable dose. Objective responses were seen in one patient with renal cell, one with

This significant lead is to be followed up by a prospectively randomized trial performed by the Radiation Therapy Oncology Group. The patients will receive either alpha 1 or placebo in an attempt to definitively establish whether or not alpha 1 is therapeutically beneficial to such patients. Additionally, patients with renal cell carcinoma will be further evaluated in Phase II trials to determine the true efficacy of fraction 5.

In FY 1982, three task orders to study a monoclonal antibody recognizing antigens on malignant T cells and on chronic lymphocytic leukemia cells were initiated and these studies carried on in FY 1983. These escalating-dose, Phase I/II trials will determine the toxicity and biological activity of these anti-T monoclonal antibodies. Some initial assessment of therapeutic effects will accrue from these studies as well. The task order mechanism continues to be a useful way to fund extramural research for short term biological research.

A relationship with the DCT Cancer Therapy Evaluation Program (CTEP) has been developed regarding the development, evaluation and monitoring of biologicals and BRM during preclinical and clinical development. The BRMP has the responsibility for preclinical and early clinical development, i.e., Phase I trials to establish maximum tolerable dose, toxicity and biological response modifying capabilities. Responsibility extends into early Phase II trials should biological response modifying capabilities need further definition. Once data are available to establish toxicity, potential efficacy, maximum tolerable dose and biological response modifying dose from these Phase I and early Phase II trials, an intra-Division committee made up of members from both BRMP and CTEP makes recommendations regarding further clinical trials for development in efficacy Phase II and combination Phase III trials. Examples of this to date include the establishment of a maximum tolerable dose, a probable biological response modifying dose and potential areas of effectiveness in Phase I and early Phase II trials for Wellferon. Recommendations of the committee will be sought for the further evaluation of this agent. Thymosin fraction 5 and alpha 1 are other examples in that these agents have been developed through early Phase II trials. Recommendations have been made to CTEP that these agents be studied additionally in cooperative groups, primarily in renal cell carcinoma for Fraction 5 and the non-oat cell lung cancer area for alpha 1. The former is currently being studied within the Northern California Oncology Group (NCOG) and the latter is beginning trials within the Radiation Therapy Oncology Group (RTOG).

Dr. Smalley, Chief, BRB, has served on the protocol review committee of CTEP. His primary responsibility has been to serve as reviewer for the protocols involving biologicals and to provide expertise to the committee on any protocols regarding biological agents submitted by the cooperative groups and the CTEP contractors.

Additional areas of cooperation between CTEP and BRMP have been within the clinical trials monitoring contract held by CTEP. This contract also serves to monitor the BRMP trials, and a cooperative relationship for this has been established.

PHARMACEUTICAL COMPANY RELATIONSHIPS

The BRMP has established relationships with several pharmaceutical companies. Perhaps the most well-developed and productive relationship to date has been with Burroughs-Wellcome, in which the company and the Program cooperated fully in evaluating the lymphoblastoid interferon, Wellferon, through Phase I and biological response modifying trials. This agent will now be developed more fully by CTEP in several efficacy trials within cooperative groups. Additional relationships exist with Hoffmann-La Roche and Schering, the former primarily as a result of the development of the thymosin agents but indirectly through the evaluation of recombinant interferon alpha in an intramural Phase I trial. Additional relationships with Biogen, Cetus-Shell, Genentech and Becton Dickinson have all been established, and each of these companies is in the process of supplying agents for clinical trials.

Additional task orders were initiated and awards made in FY 83 for studies evaluating a monoclonal antibody that recognizes antigens on normal suppressor cells. This study is aimed to determine both the tolerability and feasibility of administering this monoclonal antibody and to study the potential effect it may have on decreasing suppressor cell activity and thereby increasing immunocompetence of patients with malignancy. Additional trials evaluating recombinant beta and recombinant gamma interferon as well as natural beta interferon were also funded and initiated during FY 83. These trials should be completed in FY 84 and should establish the tolerability, toxicity and biological modifying capabilities of these new interferons.

Preclinical Screening Program

Over the past two years, a screening program to detect and evaluate active biologicals for the treatment of cancer has been developed by the BRMP. This screening program was formulated based on BRMP staff recommendations, the drug screening program and the recommendations of the BRMP Subcommittee, as well as on the recommendations of many experts in the field. The common track screen consists of a series of sequential assays through which BRM agents are evaluated for therapeutic potential. The screen was developed to define the effects of BRM on murine T cell, B cell, natural killer (NK) cell and macrophage functions. The sequence of testing consists of 1) in vitro activation: in vitro testing, 2) in vivo activation: in vitro testing and 3) in vivo activation: in vivo testing. The sequence allows the parameters of dose, schedule, route, duration and maintenance of activity, adjuvanticity and synergistic potential to be explored in an orderly fashion for each BRM. Specific tracks for analysis and evaluation of BRM such as monoclonal antibodies, lymphokines and growth and maturation factors are currently under development.

Since October 1981, the following agents have been entered in the BRM screen:

1. MVE-2
2. MDP
3. Azimexon
4. Thymosin alpha 1
& Fraction 5
5. Interferon - Human Gamma (recombinant & natural), Murine Gamma (recombinant & natural), Human Alpha (natural lymphoblastoid), Murine Alpha/Beta (natural) & Human/Mouse Alpha (recombinant A/D hybrid)

- | | |
|---------------|---------------------------------------|
| 6. N 137 | 11. Bestatin |
| 7. Poly IC:LC | 12. Tumor Necrosis Factor
(Murine) |
| 8. Lentinan | 13. Alkyl-lysophospholipid |
| 9. Picibanil | 14. FK-565 Peptide |
| 10. Tuftsin | |

Testing is nearly complete on MVE-2, MDP, Azimexon, thymosin alpha-1 and fraction 5, the interferons and N-137. The other agents are at various stages of completion within the screen program. At present, thymosin alpha-1 and fraction 5 are the best T-cell immunomodulators found requiring about equidose of alpha 1 to equal the activity of fraction 5. They have also demonstrated excellent therapeutic capabilities in selected animal model systems. MDP has also demonstrated good T-cell activating properties. Among interferons, several human and murine natural and recombinant forms have been examined. The natural and recombinant human alpha interferons were without activity in the mouse, while the human/mouse recombinant alpha interferon was an excellent NK cell activator. Subsequent studies into hyporesponsiveness did not demonstrate the induction of tolerance observed clinically with interferon. Murine gamma interferon was found to be much more active than the alpha form in NK and macrophage activation. Poly IC:LC has been found to be the best long-term NK-cell and macrophage-activating agent studied. It has also been found to be an excellent interferon inducer of long duration. No evidence of induction of hyporesponsiveness has been found. This agent has also been shown to have excellent therapeutic effects. MVE-2 and Picibanil were also found to activate NK cells and macrophages in vivo but were somewhat less impressive when compared with poly IC:LC. Several other agents that have been entered into the screen and tested showed moderate to poor activity in the various screen components. Azimexon, for example, gave moderate activity in reducing spontaneous metastases while giving only a weak effect on T-cell activation. N-137 showed moderate activity in eliminating both experimental and spontaneous metastases, and Lentinan gave evidence of moderate in vivo NK activation.

Technology Transfer and Information Dissemination

At the request of the DCT Board of Scientific Counselors, the BRMP has sponsored a series of scientific retreats with the overall objective of gaining input from the extramural community. For each retreat, BRMP staff identified specific goals and objectives to be met. Information obtained from the retreats, where possible, is used to assess the priorities for future program priorities. Summary documents, prepared from each session, have been widely distributed to the scientific community. The retreats held during the past year are as follows:

- Assessment of Monoclonal Antibodies in Therapeutic Trials
- State-of-the-Science of Lymphokines and Cytokines in the Treatment of Cancer
- A Proposed Acquisition/Distribution System for Biologics
- Identification of Possible New Immunomodulators for the BRMP Screen

- The Development of a Specific Track Screening Program for Monoclonal Antibodies, Tumor-Associated Antigens, Lymphokines and Growth/Matured Factors.

In FY 83, the BRB also sponsored a conference on interferon in clinical trials to summarize the clinical activities and BRM effects of the alpha interferons tested by our university task order contractors. Plans are underway to host a national conference on ex vivo immunoabsorbants in the fall of 1983.

ACCOMPLISHMENTS - INTRAMURAL PROGRAM

The Biological Therapeutics Branch (BTB) and the Laboratory of Molecular Immunoregulation (LMI) currently comprise the intramural component of the BRMP. The BTB was formed as a result of a merger in December 1981 of the Biological Development Branch of the BRMP with the Laboratory of Immunodiagnosis, DCBD, NCI. At that time, the entire staff of the Laboratory of Immunodiagnosis, headed by Dr. Ronald Herberman, moved to the NCI-Frederick Cancer Research Facility and the staffs of both branches were merged. In FY 1983, Dr. Joost J. Oppenheim, formerly of NIDR, joined the BRMP to direct the operations of three sections of the BTB (Biochemistry, Lymphokines and Basic Mechanisms). This component then officially became the Laboratory of Molecular Immunoregulation (LMI) on February 2, 1983. Dr. Ronald Herberman continues as Chief of the BTB and its four sections (Clinical Investigations, Monoclonal Antibody/Hybridoma, Natural Immunity and Immunopharmacology).

1. Biological Therapeutics Branch

The Clinical Investigations Section of the BTB is responsible for the investigation of the therapeutic efficacy of biologicals and BRM and the analysis of biologic response modification and toxicity of these agents and approaches. This section of the BRMP was established to facilitate the early clinical trials of biologic products with potential as anticancer agents. Located at Frederick Memorial Hospital, the facilities of the intramural clinical program were opened to the public in May 1981 and include a four-bed inpatient unit, a ten-bed outpatient unit and a cytopheresis unit. Agents tested initially included interferons, lymphokines, immunomodulators, and monoclonal antibodies. The Clinical Investigations Section is particularly concerned with in-depth Phase I and II trials of biologicals and BRM involving small numbers of patients. Optimal immunomodulatory doses, as well as maximum tolerated doses, of these new agents are being determined.

For the past two years, the Clinical Investigations Section has continued to concentrate on Phase I trials of various types of interferon and has initiated or completed an intralesional interferon study, a protein A column study for breast cancer, a Phase I cis-retinoic acid study, Phase II studies of recombinant alpha interferon treatment of lymphoma and breast cancer and Phase I monoclonal antibody studies for treating chronic lymphocytic leukemia, T-cell lymphoma and malignant melanoma. The most important results of these studies include:

Interferon (IF) - Within the area of interferon testing, the research program has focused its attention on the three classes of interferons, namely, alpha, beta and gamma.

• Alpha IF - A major part of the work of this Section during the first year involved early clinical trials of two representative alpha interferons: highly purified nonrecombinant lymphoblastoid interferon (provided to the NCI by the Burroughs Wellcome Company) and highly purified recombinant leukocyte A interferon (provided to the NCI by Hoffmann-La Roche, Inc.). These two interferon preparations are currently available in large quantity and could conceivably be tested on a long-term basis in cancer therapy. Other clinical trials of representative interferons initiated during the past two years included a Phase I trial of immune interferon in cancer patients and a Phase I trial of genetically engineered fibroblast interferon. In this manner, the Clinical Investigations Section is studying, in at least Phase I testing, an interferon of each of the three major types.

Phase I trials of highly purified recombinant and nonrecombinant alpha interferons were completed during the first and second year. Recombinant leukocyte A interferon was tested in two multiple-dose Phase I trials, in which dosages were escalated in groups of 5 or more patients. Patients were extensively monitored for toxicity, antitumor effect, immunologic function and serum interferon activity. Eighty-one patients were entered by the Clinical Investigations Section on a three times weekly multiple-dose Phase I trial of this agent. The BRMP also participated in a collaborative manner with the Baltimore Cancer Research Center in a similar multiple-dose trial of this agent on a twice daily schedule of administration. These trials showed that recombinant leukocyte A interferon could be administered safely in doses up to 54×10^6 units daily and 118×10^6 units three times weekly. The toxicities resembled those previously reported for nonrecombinant leukocyte interferons of considerably lower specific activity, suggesting that such toxicities are probably a direct result of the interferon molecule itself. Antitumor effect was seen in 9 out of 81 patients (partial remissions) with diagnoses of non-Hodgkin's and Hodgkin's lymphoma, chronic lymphocytic leukemia, breast cancer and melanoma. Serum interferon activity was found to be comparable to that previously reported for similar doses of natural Cantell alpha interferon. Analysis of immunologic monitoring of patients revealed that most patients had no change or a decrease in their natural killer cell-mediated cytotoxic activity. Approximately 80% of the patients demonstrated increased monocyte function as measured in a growth inhibition assay. Lymphocyte blastogenesis was uniformly decreased in patients treated with this agent, a reflection of its antiproliferative action.

Highly purified nonrecombinant lymphoblastoid interferon was tested in an escalating dose trial by both intravenous and intramuscular routes of administration. Twenty-nine patients were treated by the intravenous route and ten patients by the intramuscular route. A maximum tolerated dose has been defined for the intravenously treated patients at 30×10^6 units daily for five days. Very high levels of serum interferon activity were seen with the intravenously treated patients. Antitumor effect (partial responses) was seen in three patients with non-Hodgkin's lymphoma, undifferentiated carcinoma and hypernephroma.

We have completed a Phase II trial of recombinant alpha interferon for the treatment of breast cancer. Eighteen patients with breast cancer were treated at a dose of 50 million units/m² three times weekly intramuscularly. This was the maximum tolerated dose as determined from our Phase I trial of the

same recombinant alpha interferon. Sixteen patients progressed during therapy and two remained stable.

We are currently performing a Phase II trial for lymphoma and chronic lymphocytic leukemia (CLL) using recombinant alpha interferon at 50 million units/m² three times per week intramuscularly. We have treated 30 patients with lymphoma, 23 with favorable histology; seven patients with unfavorable histology lymphoma, seven patients with chronic lymphocytic leukemia and nine patients with cutaneous T-cell lymphoma. Sixty percent of the patients with favorable histology lymphoma have had an objective response. All of these patients had been heavily pretreated with chemotherapy and/or radiation therapy and were resistant to these agents. Three of seven patients with chronic lymphocytic leukemia have had a partial remission and six of nine patients with cutaneous T-cell lymphoma have had a partial remission. Only two of seven patients with unfavorable histology lymphoma have had responses. Overall, recombinant alpha interferon appears to be a promising agent for the treatment of resistant favorable histology lymphoma and cutaneous T-cell lymphoma. We anticipate that this study will be completed in early FY 84.

The Clinical Investigations Section has also conducted a study of recombinant alpha interferon by intralesional injection in a variety of tumor types. Of 20 patients treated, two patients with malignant melanoma responded to therapy. None of the three patients with breast cancer have had responses.

- Beta IF - A Phase I study of recombinant beta interferon was initiated in FY 83. Six patients were treated; however, the study was discontinued because it was determined that the interferon lacked stability. This study will be resumed in the future.

- Gamma IF - A Phase I dose-escalation study of nonrecombinant gamma interferon is nearing completion. The maximum tolerated dose appears to be in the range of 50 million units/m² given on a twice weekly basis intravenously. We have not witnessed any clinical responses.

A Phase I study of recombinant gamma interferon (Genentech) has just been initiated. This first trial of recombinant gamma will establish its effects in man and analyze the BRM activity of this agent.

Ex Vivo Immunoabsorbants - We have initiated a Phase I/II trial of plasma perfused over immobilized protein A in patients with breast cancer. Perfusion is performed twice weekly with 50 ml of plasma over 0.6 mg of protein A with eventual escalation to 100 ml of plasma over 1.25 mg of protein A. Of the three initial patients who have completed this study, no toxicity or clinical responses have been observed.

13 Cis-Retinoic Acid - A Phase I trial of 13 cis-retinoic acid has been completed. Sixteen patients were studied. Toxicities included pruritus, dryness of mouth and skin, fatigue, fever and anorexia. We have not witnessed responses in any patients at dosages between 5 and 100 mg/m².

Monoclonal Antibody - In June of 1982, we began a Phase I trial of T101 monoclonal antibody. This antibody binds to a 65,000 molecular weight antigen found on the cell surface membrane of normal and malignant T lymphocytes and malignant B lymphocytes in CLL. Twelve patients with CLL have so far been

treated. We have observed transient reductions in the circulating leukemia cell count without reductions in lymph nodes or organs. We have identified T101 staining of circulating cells and bone marrow cells. Toxicity has included fever, urticaria and shortness of breath. The shortness of breath appeared secondary to rapid intravenous infusion of 50 mg or more of T101 antibody over two hours. This toxicity was completely eliminated when infusions were slowed to 1 to 2 mg per hour. We have also treated eight patients with cutaneous T-cell lymphoma with T101 antibody and have seen five minor skin responses. One patient had the disappearance of what appeared to be a malignant lymph node. We have not seen a reduction in organs infiltrated by tumor. We have identified T101 localization in cutaneous skin lesions in vivo.

We have also treated eight patients with malignant melanoma with the 9.2.27 monoclonal antibody which is an IgG_{2a} antibody directed toward the 250,000 molecular weight antigen that is almost entirely restricted to melanoma cells. Toxicity has included urticaria, fever and serum sickness in one patient. Unlike the T101 antibody trial, we have not seen any pulmonary toxicity. Patients were treated with five doses between 1 and 200 mg. We have identified 9.2.27 antibody localization in melanoma skin lesions at all dosages equal to or greater than 50 mg, but we have not seen objective clinical responses to antibody alone.

The future direction of the Clinical Investigations Section includes continuation of Phase I and Phase II studies of various interferons and the interferon inducer poly IC:LC, monoclonal antibody trials and protein A column perfusion. We expect in the next year to begin trials with monoclonal antibodies conjugated to radionuclides, drugs and/or toxins.

In FY 83, research emphasis was changed within the Monoclonal Antibody/Hybridoma Section to increase the focus on 1) production of murine monoclonals to tumor-associated antigens, primarily in colorectal cancer, and 2) immunohistochemical techniques for the characterization of monoclonal antibodies. The research within the Section represents a broad spectrum of research in solid tumors, leukemias and lymphomas, all of which is motivated by a need to improve current treatment of human cancer. The patient monitoring of the two clinical trials with monoclonal antibodies has been carried out within the Monoclonal Section. In the context of the T101 trial, a major finding has been the definition of antigenic modulation on the patient's leukemic cells. This observation has initiated a number of laboratory studies on the role of monocytes and cytokines in enhancement of modulation, and the potential utility of this finding for immunoconjugate treatment is being pursued in both in vitro and in vivo animal models. Regarding the 9.2.27/melanoma trial, we have established that antibody traffics to and specifically binds to cutaneous melanoma cells and is retained in tumor sites for up to 10 days following infusion. We have also established that with the protocol utilized, antiglobulin reactivity is only transient and does not deter localization in tumor sites. The clinical trial is closely tied to a nude mouse-human melanoma xenograft model with spontaneous metastasis, in which we are exploring the optimization of serotherapy with conjugated and unconjugated antibody and with effector cells. These in vivo studies are made possible by continuing more basic studies in the Section on immunoconjugate production. Within this setting, we have been one of the first groups successful in scale up of immunoconjugate studies, from in vitro to animal model systems, where we have shown in the

guinea pig L10 model that established primary tumor and metastases can be successfully treated.

Our basic program for production of murine monoclonals to tumor-associated antigens has been highly successful in producing highly specific monoclonals through the use of novel immunochemically defined immunogens. In addition, we have initiated a similar set of fusions in large cell carcinoma of the lung along with continuing efforts on human melanoma. These efforts are required for generation of future agents for diagnosis and treatment. The Section, in this regard, has shown an excellent capability of eliciting, characterizing and scaling up to clinical trial status with monoclonal reagents. This is typified by the antibody 9.2.27, which has considerable potential for the diagnosis and treatment of human melanoma, and the monoclonal antibody-defined 100K dalton MAA, which has demonstrated usefulness in monitoring tumor burden in melanoma patients. Within the fiscal year, the Section has been actively addressing the problems involved in raising murine monoclonal antibodies for therapeutic evaluation to idiotypic determinants of surface immunoglobulin-bearing human B-cell leukemias. These studies have been hampered in the past by the lack of a stable source of idiotype secreting cells. By the combination of pretreatment of leukemic cells with tumor promoters followed by fusion with a mouse myeloma and by improved methods of immunoglobulin isolation and immunization, we have produced at least six candidate antibodies for three different patients. We are now expanding the number of patients and evaluating the subclass of antibodies that will be scaled up for treatment. Despite the clinical orientation of the research in the Section, more basic efforts have not been ignored. A good example is the study of occult leukocyte antigens which was made possible by the development of a technique for analysis of internal antigens by flow cytometry. These studies will necessitate a reassessment of leukocyte differentiation schemes and leukemic cell classification.

The research activities of the Natural Immunity Section are primarily focused on the role of natural effector cells in resistance against cancer. There have been extensive studies on NK cells, with emphasis on the characteristics of these cells, the factors regulating their activity and their in vivo role in resistance against tumor growth.

A major advance in the characterization of NK cells has come from the finding of their close association with a subpopulation of lymphoid cells, termed "large granular lymphocytes" (LGL). Human LGL have been isolated with high purity and extensively characterized. Most recently, several antibodies that react with the Fc receptor on LGLs and granulocytes have been shown to be valuable reagents for enumeration and separation of LGL.

In studies with rat LGL, the OX-8 monoclonal antibody has been shown to be a highly selective reagent for this population in nude rats. This has permitted a comprehensive survey of the histological localization of LGL. Large numbers of LGL have been found in inflamed and/or infected tissues and in the subcutaneous and pulmonary sites of tumor growth.

Studies have continued to determine the nature of the specificity of NK cells. In experiments with human LGL, it has been possible to begin a detailed biochemical analysis of the target structures recognized by NK cells, by measuring the ability of solubilized materials to inhibit the binding of LGL to target cells. The target structures from the K562 cell line have been purified more

than 200-fold and have been demonstrated to be high molecular weight glycoproteins. In another aspect of our studies of the specificity of human NK cells, primary solid tumor cells, including autologous tumor cells, have been shown to be susceptible to cytotoxicity by purified LGL.

There have been some recent advances in our understanding of the mechanism of lysis by NK cells. Highly purified populations of human LGL have been shown to produce high levels of soluble cytotoxic factors, which have the ability to lyse NK-susceptible target cells. In addition, the granules from rat LGL leukemias have been isolated and shown to have high levels of cytotoxic reactivity against a variety of tumor target cells. These studies are expected to rapidly lead to new insights into the mechanism of killing by NK cells.

Interferon has been shown to have a variety of effects on immune reactivity, including the ability to rapidly augment the reactivity of NK cells. To obtain better insight into the nature of the diversity of the biological effects of interferon, various preparations of human natural, recombinant, and hybrid recombinant alpha interferon were tested for their ability to augment the reactivity of NK cells. These studies are providing considerable insight into the portions of the interferon molecule associated with each biologic activity and offer the potential for constructing interferon molecules with highly selective biologic effects.

In addition to interferon, interleukin-2 (IL-2) potently augments human NK activity. This boosting by IL-2 appears to be mediated at least in part by stimulation of endogenous production of IFN- γ by the LGL. The studies of the effects of IL-2 on NK cells have revealed that in addition to this cytokine being a growth factor, it can augment cytotoxic reactivity in the absence of growth promotion.

A continuing issue of central importance is the in vivo relevance of NK cells and other natural effector cells, particularly in regard to resistance against tumor growth. There are increasing indications that NK cells play a central role in resistance against metastatic spread of tumors. The rat has provided a very useful model system for investigating this issue in detail, since it is possible to depress NK activity in vivo and selectively reconstitute the animals with highly enriched populations of LGL. Such studies have indicated the ability of LGL to partially or fully restore cytotoxic reactivity against NK-radiolabeled tumor cells from the lungs, and the ability to inhibit pulmonary metastases from a mammary adenocarcinoma. These results provide the first direct evidence that NK cells are involved in in vivo resistance to tumors, particularly in the elimination of potentially metastatic tumor cells from the circulation and capillary beds.

During this year, efforts within the Immunopharmacology Section have focused on an examination of the immunological and pharmacological mechanisms by which BRM modulate host reactivity and on development of protocols for optimal and sustained modulation of host resistance in attempts to develop more effective therapy of cancer.

Several BRM were shown to augment NK activity in various tissue sites, with appreciable differences depending on route of administration. These BRM were also shown to induce a variety of cytokines in vivo or in vitro, including interferon, colony stimulating factor and prostaglandin E2. After multiple

treatments with some BRM, substantial hyporesponsiveness for augmentation of NK activity was noted, whereas no such hyporesponsiveness for activation of macrophages was seen.

Protocols have been developed that provide substantial preventive or therapeutic effects against lung metastases. Combined treatment of chemotherapeutic agents plus BRM was substantially more effective than therapy by either type of agent alone. Similarly, treatment with BRM immediately following surgical removal of large mouse mammary carcinomas resulted in a decreased number of lung metastases and a prolongation of survival time. The data indicate that BRM treatment may be particularly effective when utilized to augment effector cell activity for elimination of tumor cells remaining after reduction of tumor burden by chemotherapy or surgical therapy.

2. Laboratory of Molecular Immunoregulation

Studies of a human lung tumor-associated antigen (LTA) have continued in the Biochemistry Section, with emphasis on the improvement of immunoassays for quantitating the tumor antigen and the application of these assays in the evaluation of LTA as a marker for lung cancer. An enzyme-linked immunoabsorbent assay (ELISA) has been developed that is based on an immobilized, highly purified form of LTA and a second generation goat anti-LTA serum. The goat antiserum continues to react specifically with LTA with considerable binding activity. The ELISA is a more convenient and antigen-economical assay than the former RIA and has proven effective in several aspects of the overall program. For example, the ELISA is being used as a screening assay for the detection of monoclonal antibody, for monitoring during antigen purification and for assessing the clinical utility of LTA in patients specimens. With regard to this last item, LTA levels in the serum of three terminal lung cancer patients were monitored and LTA levels were found to be an effective indicator of disease recurrence and, in fact, more predictive than CEA. The presence of LTA in bronchial washings from lung cancer patients was also observed and studies are in progress to evaluate this as a potentially useful diagnostic marker.

Research efforts concerning human transforming growth factors (TGF) have increased significantly during this fiscal year to the point that this area has now become a major project within the Biochemistry Section. A form of TGF of characteristic size (30,000 M.W.), which appears to be greatly elevated in the urine of cancer patients, is being studied. The factor(s) is being isolated and has already been highly purified in an attempt to compare its properties to those of known growth factors such as epidermal growth factor (EGF) and tissue culture-derived growth factors. To facilitate these studies, a rapid and sensitive assay has been developed which measures binding competition for labeled EGF to immobilized membranes containing EGF receptors. This assay offers many advantages over the previously used soft agar growth assay. Using this assay and reverse-phase high-performance liquid chromatography, 5 species of urinary TGF were identified. Although functionally similar, they are chemically distinct from growth factors produced by cultured cells. Both qualitative and quantitative transforming differences have been observed in TGFs isolated from cancer patients relative to normal individuals. The diagnostic utility of these findings is being assessed. In the process of performing the above studies another activity was detected in the urines from normal individuals and from patients. This factor induced murine thymocyte and human

fibroblast proliferation and in its biochemical characteristics resembles interleukin 1. This observation may provide a ready source and reveal a physiological role for this macrophage and epidermal cell-derived mediator of inflammation.

The Lymphokines/Cytokines Section investigates "hormone-like" products of cells (cytokines), especially the products of lymphocytes (lymphokines), which may regulate host defense mechanisms capable of mediating tumor destruction in cancer patients. Investigators in the section are involved in the production and purification of lymphokines, development and standardization of bioassays and treatment protocols in animal models and cancer patients.

A continuing effort to identify sources of lymphokines and cytokines has yielded adequate amounts of some materials such as interleukin-2 in the form of crude material and highly purified material from the MLA-144 cell line; interferons alpha, beta and gamma, of both mouse and human origin, including recombinant alpha interferon; and tumor necrosis factor. It is one of the Section's goals to establish assays either in-house or through contractors with the program to enable us to investigate a variety of lymphokine/cytokine-containing materials. At this point, in-house assays for interleukin-1, interleukin-2, alpha, beta and gamma interferons, colony stimulating factor, migration inhibitory factor and macrophage activating factor are performed routinely.

Monoclonal antibodies have been obtained to human alpha interferon, gamma interferon, CSF and IL-1 from collaborators and are being used to study the modes of action of these lymphokines/cytokines.

In collaboration with the Monoclonal Antibody/Hybridoma Section, BTB, human/human T cell hybridomas are being developed, which constitutively secrete lymphokines such as human interleukin-2 and colony stimulating factor. These hybridomas are then adapted to growth in serum-free medium and may be a good source for isolating lymphokines for future studies.

Significant progress has been made in defining the relationship between migration inhibitory factor (MIF) and interferon. Using a micro-adaptation of the MIF assay that employs human monocytes purified by centrifugal elutriation as the indicators, it was shown that antibody to gamma interferon did not remove MIF activity from a lymphokine preparation. Gamma interferon was shown, however, to be a potent inhibitor of human monocyte migration, and work is continuing to define how many migration inhibitory factors exist and the physiological roles of each factor.

A model system for studying the control of T-cell growth in normal and neoplastic T cells by the lymphokine IL-2 (TCGF) has been developed. Cell lines have been established that are sensitive to glucocorticoid-mediated control of TCGF required for cell growth. These lines are being used to study the autocrine nature of TCGF action on normal and neoplastic T cells. Cell lines that respond to variants of TCGF with distinctly different biochemical properties have been derived. These lines are being used to help define the mechanisms of TCGF action on T lymphoid cells.

In collaboration with the BTB, we are evaluating the effects of various biological response modifiers on the release of the lymphokine GM-CSF by human monocytes and bone marrow cells and by murine macrophages. Poly-IC:LC, human and mouse interferons, BM41.332 and LPS could induce GM-CSF secretion. With the exception of human interferon, these BRMs also induced secretion of prostaglandin E, which acts as a feedback inhibitor of GM-CFU-C colony formation. The inhibitory pathway could be blocked by Indomethacin, resulting in the accumulation of larger amounts of GM-CSF.

Studies of the relationship of neuroendocrine hormones and cytokines have been initiated. One research question is, Do the endocrine receptors and lymphokines operate through similar intracellular mechanisms, and are they related to one another at the molecular level? Published evidence indicates that some subpopulation of human lymphoid cells, including the LGL population, analogous to the murine NK cells, exhibits receptors for the endocrine hormone beta-endorphin. This information opens new doors to a more unified theory of cellular regulation.

An application of lymphokines is the enhancement of in vitro human immune responses through potentiating lymphocyte sensitization to antigens in vitro. Progress was made during this year in obtaining in vitro human antimelanoma immune responses using lymphocytes from melanoma patients undergoing treatment with a monoclonal antimelanoma antibody of mouse origin. Cells from the in vitro immunization were then used to produce human/human fusions in collaboration with the Monoclonal Antibody/Hybridoma Section. These fusions show promise as continuous sources of human antitumor antibody. Preliminary evidence shows that crude preparation of human lymphokines can enhance the sensitization of human lymphocytes in vitro, and experiments are now concentrating on defining the optimum way in which these peptides may be used to produce strong responses and provide large pools of antigen-specific B lymphocytes for use in hybridoma fusions.

During the past year there have been a number of findings within the Immunobiology Section that help delineate the nature of the host-tumor interaction and that provide information about the molecular and cellular events involved in these responses. These findings are as follows:

From studies of the relative role of macrophages and NK cells in the host response to tumor growth, it appears that NK cells may inhibit the formation of metastases during the bloodborne phase, whereas macrophages ($M\phi$) probably only exert antitumor activity at local tissue sites after extravasation of tumor cells from the bloodstream. By utilizing antiserum to asialo GMI, which preferentially depletes NK killer activity when administered in vivo, an in vivo model has been established for selectivity studying $M\phi$ -mediated antitumor effects in normal or BRM treated mice, without the complication of NK activity. In related in vitro experiments, we have shown that murine recombinant gamma interferon (γ IFN) supplies both the priming and triggering signals for $M\phi$ activation. This means that γ IFN may play an important role in the generation of $M\phi$ -mediated antitumor events in vivo.

Because of the major role that RNA plays in any process of cellular differentiation, the changes in RNA metabolism that occur during the transition from resting to tumoricidal (activated) macrophages have been analyzed. There was a direct correlation between expression of tumoricidal activity and decrease

in RNA synthesis. Molecular analysis of the species of RNA affected revealed a selective inhibition of the 28S ribosomal RNA in activated macrophages. This was observed in macrophages that had been activated in vitro by endotoxin, lymphokines or interferon, or in vivo by endotoxin or *Corynebacterium parvum*. Based on these observations, it was established that agents which inhibit RNA synthesis activate macrophages to a tumoricidal state. Furthermore, increasing evidence has been obtained on the positive effects of inhibitors of RNA synthesis on macrophage activation by demonstrating synergy between these agents and suboptimal doses of BRM.

A variety of agents have been examined for their ability to activate human monocytes to a tumoricidal state. Macrophage activation factor (MAF) produced by human lymphocytes after activation with sepharose bound concanavalin A can activate monocytes to become tumoricidal. MAF has been shown to be distinct from γ IFN. However, IFN in some experimental conditions can also be demonstrated to have the capacity to activate macrophages to be tumoricidal. Soluble MAF or MAF encapsulated into multilamellar liposomes will induce a tumoricidal state, although the latter method is much more effective. Liposomes can also be used to activate macrophages by muramyl dipeptide or MTP-PE, a lipophilic derivative of muramyl dipeptide. Using rodent and human MAF, it has been shown that species-specific surface receptor binding can be bypassed using liposome-encapsulated MAF. MAF may have at least two separate "functional" parts of the molecule: one for cell surface binding and a second for initiating the activation process.

Changes in lipid of composition of macrophage membrane and lipid metabolism have been examined. Mouse macrophages that have been activated in vivo by adjuvants express higher levels of the glycolipid asialo GMI. Biochemical analysis indicated that there are other major shifts in neutral glycolipids and gangliosides when macrophages go from resting to activated states. In lymphocytes, α and β IFN caused an increase in arachidonic acid as well as in other polyunsaturated fatty acids in the surface phospholipids, resulting in a more unsaturated pattern in their fatty acid composition. These changes result in an alteration of the plasma membrane motional state, which may promote certain membrane-dependent cell functions such as cytotoxicity. Similarly, there were changes in the lipids of the NK-susceptible tumor target cells, K562. These changes correlate with increased sensitivity of K562 to lysis by NK cells after treatment with IFN. IFN also inhibits phospholipid (PL) methylation in mononuclear cells, an effect that might contribute to alterations in the properties of the membrane of IFN-treated cells. Other studies of methylated non-polar lipids in monocytes have revealed an inverse relationship between activation of the oxidative burst and the rate of phospholipid and nonpolar lipid methylation. The inhibitory effects on lipid methylations in stimulated monocytes suggest a relevant role of these pathways in the response of monocytes to stimuli.

Functions of accessory cells, which are required for the process of lymphocyte activation, have also been examined. DR antigen expression can be increased on human monocytes by treatment with γ IFN in vitro, whereas exposure to prostaglandin decreases DR expression. Since only DR+ monocytes can mediate lymphocyte activation, these agents regulate the capacity of monocytes to present activating agents to lymphocytes. Paraformaldehyde fixed human monocytes can activate T lymphocytes only if they are preincubated with the stimulant for several hours at 37°C and subsequently supplemented with IL-1. Exposure of the

monocytes to lysosomotropic agents during this preincubation period inhibits their ability to activate lymphocytes, suggesting that stimulant processing at the lysosomal level is necessary for accessory cell function. Several nonmonocytic cell types such as B lymphocytes and some NK cells that express DR antigen were demonstrated to produce IL-1 and shown to function as accessory cells. Finally, the cascade of cell-cytokine interactions required to promote lymphocyte activation has been further delineated. Exposure to exogenous stimulants initiate a sequence of factor-cell interactions that amplify the immune response as follows: colony-stimulating factor stimulates macrophages or monocytes to produce IL-1. IL-1 in turn enhances lymphocyte production of IL-2, which in turn promotes the production immune interferon (γ IFN). γ IFN has a multiplicity of immunoenhancing consequences through its differentiation effects on specific and NK lymphocytes and macrophages and also exerts a positive feedback effect by enhancing monocyte DR/Ia antigen expression of augmenting lymphocyte activation.

SUMMARY REPORT

BIOLOGICAL RESOURCES BRANCH

October 1, 1982 Through September 30, 1983

INTRODUCTION

The Biological Resources Branch (BRB) of the Biological Response Modifiers Program (BRMP) is composed of two sections:

1. Procurement, Formulation and Preclinical Trials Section (PFPTS).
2. Clinical Trials Section (CTS).

The BRB supports, through a balanced program of grants and contracts, preclinical and clinical biological response modifiers (BRM) research in the biomedical community. The branch monitors Phase I and early Phase II clinical studies which assess biological effects of BRM in patients and correlate changes in the biological modifications with antitumor activity. The BRB has established a preclinical screening program for the selection and preclinical assessment of efficacy of BRM. A resource distribution system encompassing both information acquisition and assessment, as well as agent acquisition, and testing has been established.

BRANCH PERSONNEL Offices of the Branch are in Building 426 at the Federick Cancer Research Facility, Frederick, Maryland.

OFFICE OF THE CHIEF

Chief, BRB -----	Richard V. Smalley, M.D.
Program Analyst -----	James C. Vennetti
Secretary -----	Paula F. Wolfe

PRE-CLINICAL SECTION

Head, PFPTS -----	Cedric W. Long, Ph.D.
Health Scientist Admin.--	Gary B. Thurman, Ph.D.
Health Scientist Admin.--	Andrew J. Vargosko, Ph.D.
Clerk-Steno (PPT) -----	Cynthia J. Earp

CLINICAL SECTION

Head, CTS -----	Vacant
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BIOLOGICAL RESPONSE MODIFIERS PROGRAM, PROCUREMENT, FORMULATION AND PRECLINICAL TRIALS SECTION (PFPTS)

Cedric W. Long, Ph.D. is the Section Chief. This section is responsible for the identification of BRM of interest to the BRMP through literature reviews in addition to coordinating information access in such a manner that relevant information on potential agents is directed toward appropriate program personnel and working groups. The PFPTS also assists in the development of BRM through screening of potential agents, animal toxicology and therapeutic trials, and serves as a liaison for this activity to the Developmental Therapeutics Program. Other responsibilities include the development of appropriate experimental systems for detection and evaluation of potential BRM and the coordination, planning and monitoring of detailed evaluations of biological response modifiers in relevant systems. The Section Head serves as Project Officer on all BRB contracts in the preclinical area, as Administrative Program Director on BRB grants and as Program Director on Cell Surface Immunology grants. Dr. Gary Thurman serves as Program Director for Molecular Immunology grants and Dr. Andrew Vargosko as Program Director for Cellular Immunology grants in the BRB.

BIOLOGICAL RESPONSE MODIFIERS CLINICAL TRIALS SECTION

The BRM Clinical Trials Section monitors Phase I clinical trials involving the use of BRM and administers clinical grants. An important aspect of this function is the close liaison with CTEP in assessing correlations between changes in immunological reactivity and clinical efficacy and toxicity in these studies.

The Head of the Clinical Section left the program in April, 1983, to return to academia. The Branch is actively recruiting to fill the position of Head of this section. Monitoring of Phase II clinical trials with CTEP personnel is an ongoing arrangement in which the BRMP and CTEP work conjointly.

SUMMARY OF FY 83 ACTIVITIES

During this year the BRB has been involved in the following:

- ° Issued 4 Program Announcements and received 18 grant applications as a result. Approximately 3 awards were made as a result of the Announcement.
- ° Maintained 10 contracts testing BRM in Phase I clinical trials.
- ° Initiated 2 Request for Proposals (RFPs) and awarded 2 new contracts in FY 1983.
- ° Initiated the recompetition of the Clinical Trials Master Agreement.
- ° Awarded 7 new clinical Task Order Contracts under the Master Agreement held by the 27 institutions. (See Table III)

- Monitored 12 compounds in the BRM preclinical Screen.
- Sponsored 5 meetings dealing with BRM.

GRANTS AND CONTRACT ADMINISTRATION

A significant accomplishment of the BRB has been the successful development of a system of grant and contract support and administration. The current and future initiatives of the extramural program should establish the BRMP as a major support mechanism for preclinical and clinical research into BRM. The branch staff maintains liaison with all pertinent peer review groups involved in grant and contract review. It also provides planning, direction, implementation, and evaluation of research supported by grants and contracts.

Liaisons have been established and maintained with other programs in the NCI, including, the Immunology and Tumor Biology Programs in the Division of Cancer Biology and Diagnosis (DCBD), to minimize overlap in the grant and contract area. A regular working relationship is maintained with the Developmental Therapeutics Program. A cooperative clinical evaluation system with the CTEP has been established to coordinate Phase I and Phase II BRM Clinical Trials.

GRANTS PROGRAM

In FY 1983 active grants numbered approximately 79 (including 6 grants funded for their terminal year in 1982). Approximately \$11.0 million (73 grants) was awarded in FY 1983. This includes \$9.7 million to fund 57 ROIs, one R23, and five POIs. An additional \$1.3 million was awarded to fund continuation costs for ten grants activated in response to four RFAs in FY 1982.

The grants can be categorized in specific areas as follows:

Cell Surface Immunology

Adjuvants; Antibodies; Antigens

Molecular Immunology

Interferons; Thymic Factors; Lymphokines

Cellular Immunology

Lymphoid cells; Growth and Maturation Factors; Miscellaneous Approaches to Biological Response Modification (bone marrow transplantation, immunization by various altered cells, organ transplants, viral components and immune or necrosis factors); Complications, Adverse Effects and Related Phenomena Attending the Use and Evaluation of BRM in Cancer Therapy. (Includes studies relevant to the safety of BRM when used therapeutically.)

Table 1 lists each grant title by referral area category.

REQUESTS FOR APPLICATIONS

In FY 1982 the Biological Response Modifiers Program issued four Requests for Applications (RFAs): Monoclonal Antibody in Cancer Therapy; Monoclonal Antibody in Animal Tumor Models; Therapeutic Use of Lymphokines; and Animal Tumor Models for Antipeptide Growth Factor and Maturation Factor Therapy. Seventy-two applications were received in response to the RFAs. Ten applications were funded for \$1.3 million.

The BRMP did not issue any new RFAs in FY 1983. It is estimated that the FY 1983 continuation costs for the ten grants awarded in FY 1982 will be approximately \$1.3 million.

PROGRAM ANNOUNCEMENTS

In FY 1983 the BRMP issued four Program Announcements: Use of Tumor Associated Antigens as Immunogens; Development of Genetically Engineered Cell Products; use of Growth Factors, Maturation Factors and Antigrowth Factors in Animal Tumor Models; and Development of Cell Lines Producing Lymphokines and Cytokines. Grant applications are still being received for these Program Announcements. Eighteen grant applications have been already reviewed and, of these, three have been funded.

CONTRACT PROGRAM

The branch staff initiates requests for proposals and provides programmatic direction, evaluation and monitoring for contracts supported by the BRB. In FY 1983, 32 contracts were active. During FY 83 the BRB awarded approximately \$2.7 million to 13 extramural contracts, including \$1.3 million for seven new clinical task order contracts and \$1.4 million in nine non-clinical contract awards; two of these were new awards, four of which received incremental funding, one was recompeted and two were for the purchase of biologicals. The contracts active and projected for funding in FY 1983 are shown in Table II. These contracts in general, provided for (1) the collection, storage, testing and quality assurance of BRM, (2) initial exploration into the monoclonal antibody and cytokine areas, (3) technical support for the review and evaluation of BRM, and (4) Phase I/II clinical trials.

CLINICAL PHASE I/II STUDIES UNDER A MASTER AGREEMENT

The BRMP established a Master Agreement mechanism in FY 1980 whereby twenty-seven institutions were identified as capable of performing clinical studies using biological response modifiers (BRM). The studies are closely monitored by the Biological Resources Branch (BRB) to assess significance of changes in biological responses observed in patients under treatment with BRM. Toxicity, biological response and therapeutic effects are monitored simultaneously.

In previous fiscal years, fourteen task orders for testing interferons, thymosins and MVE-2 were awarded under this Master Agreement (Table IV). Phase I trials for the alpha interferons, thymosins and MVE-2 have been completed. Ongoing Phase II trials with lymphoblastoid (alpha) interferon (Wellferon) are continuing to define the biologic activity of this highly purified extracted alpha interferon. Both "high" and "low" doses are being tested in a broad spectrum of cancer patients to determine the effects on biological responses as well as the clinical activity.

Clinical trials were performed evaluating three interferon products. Two naturally extracted alpha interferons were purchased by contract from Warner-Lambert and from Meloy Laboratories. The Warner-Lambert material was tested by the Dana-Farber Institute. They studied 46 patients, 23 of whom had breast cancer, and determined that a dose of 18 MU/day, given intramuscularly, was the maximal tolerable dose. Beneficial clinical effect was noted in three patients with breast cancer and two with renal cancer. Actual objective responses were not seen. Alpha interferon obtained from Meloy was evaluated at Stanford and at Georgetown Universities. The Stanford investigators also established 18 MU/day given intramuscularly as the maximum tolerable dose of this material. The group at Georgetown evaluated a single dose administering up to 60 MU/m² (total dose approximately 100 MU) without toxicity. In these two trials clinical benefit was noted in one patient with chronic leukemia, one with bladder carcinoma, one with renal cell carcinoma, one with melanoma, one with head and neck cancer and one with a non-Hodgkin's lymphoma. Biological response modifying activity, i.e. NK cell stimulation was noted at the higher single doses by the Georgetown group. Toxicity with each of these products consisted of fatigue, fever, anorexia, weight loss, chills and to a lesser degree mild leukopenia and liver function abnormalities.

A Phase I study with naturally-extracted but highly-purified lymphoblastoid alpha interferon purchased from Burroughs-Wellcome, Ltd. was performed at Duke University and UCLA. The Duke group established that a dose of 15 MU/m², administered three times a week over a 5 week period was the maximum tolerable dose of this material administered by this schedule. Investigators at UCLA administered this interferon by a twice a week schedule for 7 days followed by a three week rest. On this schedule 18-30 MU was the maximally tolerable dose. Objective responses were seen in one patient with renal cell, one with non-Hodgkin's lymphoma, one with Hodgkin's disease, one with myeloma and one with melanoma. Biological activity was noted in the form of stimulation of NK cell activity with low doses and suppression at high doses. A suggestion of stimulation of monocyte activity and suppression of hematopoiesis and erythropoiesis in the bone marrow was also demonstrated. The usual side effects associated with interferon such as fatigue, fever, anorexia, chills and weight loss were noted along with mild leukopenia and liver cell function abnormalities. The higher doses with this product also induced central nervous system malfunction in that two patients had seizures, and several became confused and unable to perform normal intellectual functions. All side effects were self limiting. Currently ongoing at UCLA, Duke, Memorial Sloan-Kettering, Georgetown, and Wisconsin are Phase II studies evaluating potential efficacy of this product in patients with breast carcinoma, renal cell carcinoma, melanoma, myeloma and colon carcinoma.

Clinical trials evaluating the potential for MVE-2 as a biological response modifier were performed at Ohio State University and Vanderbilt University.

Investigators at Vanderbilt evaluated a weekly schedule and those at Ohio State a biweekly schedule. Twenty-two patients were studied at Vanderbilt and 16 at Ohio State. They demonstrated that maximally tolerable doses are in the range of 600-900 mg/m² and maximal cumulative doses in the range of about 2.5 gms/m². The material was reasonably well tolerated with the exception of a high incidence of proteinurea in patients receiving a total dose of over 1500 mg/m². As the total cumulative dose increased the incidence of proteinurea likewise increased. There was one antitumor response in a patient with melanoma noted and some suggestion of stimulation of NK cell activity and of antibody dependent cellular cytotoxicity. However, there was no solid evidence for strong biological response modification. The agent was supplied by Adria Laboratories and it will be their decision as to whether to pursue this product or not in Phase II studies.

Two thymosin products were supplied by Hoffmann-La Roche for evaluation. Fraction 5 is a mixture of approximately 20 polypeptides obtained by extraction and purification of material from a calf thymus. Alpha 1 is a purified polypeptide obtained from fraction 5. These products were studied at 4 institutions; the University of California at San Francisco, a member of the Northern California Oncology Group, Memorial Sloan-Kettering, the Fred Hutchinson Cancer Research Center, and the University of California in San Diego.

Phase I studies have determined that doses as high as 960 mg/m² of fraction 5 and 9.6 mg/m² of alpha 1 are tolerable. Essentially no toxicity was seen with either of these products over a wide dose range up to the doses indicated above. One-hundred-seventy-eight patients were evaluated in four Phase I studies with fraction 5 and over 100 patients in three studies of alpha 1. Beneficial responses were seen in 3 patients with renal cell carcinoma, using fraction 5. No objective antitumor responses were seen in patients receiving alpha 1. George Washington University also performed a randomized study in which patients received alpha 1 following the administration of radiation therapy for lung carcinoma. The study was designed to determine whether alpha 1, administered after radiation therapy prolonged a response. Patients receiving alpha 1 did indeed have a longer duration of response than those receiving placebo. The number of patients treated was very small, however. This prolongation was associated with improvement in lymphocyte function. This significant lead is to be followed up by a prospectively randomized trial performed by the Radiation Therapy Oncology Group. The patients will receive either alpha 1 or placebo in an attempt to definitively establish whether or not alpha 1 is therapeutically beneficial to such patients. Additionally, patients with renal cell carcinoma will be further evaluated in Phase II trials to determine the true efficacy of fraction 5.

In FY 1982, three task orders to study a monoclonal antibody recognizing antigens on T cells and on chronic lymphocytic leukemia cells were initiated and these studies carried on in FY 1983. These escalating dose, Phase I/II trials will determine the toxicity and biological activity of these anti-T monoclonal antibodies. Some initial assessment of therapeutic effects will accrue from these studies as well.

During FY 1983 the following task orders were awarded under a Master Agreement for Phase I/II Clinical Trials of Biological Response Modifiers:

1. Phase I Study to Evaluate the Efficacy of Anti-T Suppressor Cell Antibody in Cancer Therapy (one award)
2. Phase I Study to Evaluate the Biological Response Modifying Activity and Potential Antitumor Activity of Recombinant Human Beta and Gamma Interferon (five awards)

The Biological Resources Branch is in the process of recompeting the Master Agreement for Phase I/II Clinical Trials. It is anticipated that Master Agreements will be awarded early in FY 1984.

A relationship with CTEP has been developed regarding the development and the evaluation and monitoring of biologicals and biological response modifiers during preclinical and clinical development. The BRMP has the responsibility for preclinical and early clinical development i.e. phase I trials to establish maximum tolerable dose, toxicity and biological response modifying capabilities. Responsibility extends into early phase II trials should biological response modifying capabilities need further definition. Once data is available to establish toxicity, potential efficacy, maximum tolerable dose and biological response modifying dose from these phase I and early phase II trials, an intra-Division committee made up of members from both BRMP and CTEP makes recommendations regarding further clinical trials for development in efficacy phase II and combination phase III trials. Examples of this to date include the establishment of a maximum tolerable dose, a probable biological response modifying dose and potential areas of effectiveness in phase I and early phase II trials for Wellferon. Recommendations of the committee will be sought for the subsequent further evaluation of this agent. Thymosin fraction 5 and alpha 1 are other examples in that these agents have been developed through early phase II trials. Recommendations have been made to CTEP that these agents be studied additionally in cooperative groups, primarily in renal cell carcinoma for Fraction 5 and the non-oat cell lung cancer area for alpha 1. The former is currently being studied within the NCOG and the latter is beginning trials within RTOG.

Dr. Smalley has served on the protocol review committee of CTEP. His primary responsibility has been to serve as reviewer for protocols involving biologics and to provide expertise to the committee on any protocols regarding biological agents submitted by the cooperative groups and the CTEP contractors.

Additional areas of cooperation between CTEP and BRMP have been within the clinical trials monitoring contract held by CTEP. This contract also serves to monitor the BRMP trials and a cooperative relationship for this has been established.

PHARMACEUTICAL COMPANY RELATIONSHIP

The BRMP has established relationships with several pharmaceutical companies. Perhaps the most well-developed and productive relationship to date has been with Burroughs-Wellcome in which the company and the Program cooperated fully in evaluating the lymphoblastoid interferon Wellferon through phase I and biological response modifying trials. This agent will now be developed more fully by CTEP in several efficacy trials within cooperative groups. Additional relationships exist with Hoffmann-La Roche and Schering, the former primarily as a result of the development of the thymosin agents but

indirectly through the evaluation of recombinant interferon alpha in an intramural phase I trial. Additional relationships with Biogen, Cetus-Shell, Genentech and Becton Dickenson have all been established. Each of these companies are in the process of supplying agents for clinical trials.

Additional task orders were initiated and awards made in FY 83 for studies evaluating a monoclonal antibody which recognizes antigens on normal suppressor cells. This study is aimed to determine both the tolerability and feasibility of administering this monoclonal antibody and to study the potential effect it may have on decreasing suppressor cell activity and thereby increasing immunocompetence of patients with malignancy. Additional trials evaluating recombinant beta and recombinant gamma interferon as well as a trial evaluating natural beta interferon were also funded and initiated during FY 83. These trials should be completed in FY 84 and should establish the tolerability, toxicity and the biological modifying capabilities of these new recombinant interferons.

PRECLINICAL CONTRACTS

The following preclinical contracts are planned for award in FY 1983.

1. Characterization and Analysis of Protein Materials. The purpose of this contract is to develop qualitative and quantitative methods to ascertain purity, identity and quality of biologicals and drugs. Reports of characterization and analysis are used to assess suitability of products for evaluation and development in preclinical and clinical trials.

This work is currently being performed under contract to the University of Iowa. It is being recompeted and the work scope is being increased in the recompetition because of the need to conduct additional immunological testing. It is projected that this recompetition will be awarded in the fourth quarter of FY 1983.

2. Technical Support for Review and Evaluation of Biological Response Modifiers.

This contract is to provide for the collection, storage, compilation and organization of preclinical and clinical data on Biological Response Modifiers considered of interest to the BRMP.

Contract proposals are currently under review, and it is anticipated that an award for this project will be made this fiscal year.

3. Chemical Coupling Cytotoxic Agents to Monoclonal Antibody.

This contract, which is held by Hybritech, Incorporated, of San Diego, California, will be entering the second year of funding in FY 1983. The program plans to increase the number of monoclonal antibodies coupled to various cytotoxic agents during the fourth quarter of this year.

4. Feasibility Study for the Acquisition and Distribution of BRM.

The purpose of this contract is to assist the BRMP in determining the usage requirements and availability of biologicals within the research community and to define the most effective size and scope of an acquisition/distribution program for biologics. This contract will be awarded late in FY 1983 or early in FY 1984, depending upon the procurement process.

5. Agents for Pre-Clinical and Clinical Evaluation.

The BRB is in the process of purchasing monoclonal antibody (anti-leu 2a) and recombinant mouse alpha interferon for use in clinical and pre-clinical trials.

NEW INITIATIVES

- ° The BRB plans to release a Program Announcement late in FY 1983 for grant awards in FY 1984, entitled "Determination of the Therapeutic Usefulness of Purified Cytokines and Anti-Cytokine Monoclonal Antibodies in Cancer Models."
- ° A Request for Cooperative Agreement Applications entitled "Discovery of Strategies for the In Vitro and In Vivo Preclinical Testing of Monoclonal Antibodies and Their Immunoconjugates for Biologic and Therapeutic Effectiveness" will be released in FY 1984.
- ° A new RFP for the production of Liposome Pharmaceutical is being prepared for release in FY 1984.
- ° The following Clinical Task Orders are planned for FY 1984, pending the availability of funds:

- Phase I Study of TNF
- Phase I Study of Liposome Encapsulated MDP
- Phase I Study of Liposome Encapsulated MAF
- Phase I Study of Genetically Engineered IL-2
- Phase I/II Study of Anti-Melanoma Immunoconjugate
- Phase I/II Study of Anti-T Cell Immunoconjugate
- Phase I Trial of a Combination of Beta and Gamma Interferon

- ° In FY 1984, the BRB will recompute the projects for the Collection, Storage, Quality Assurance and Distribution of Biological Response Modifiers. This activity is currently being performed under contracts to Litton Bionetics, Inc. and Meloy Laboratories, Inc.

Preclinical Screening Program

Over the past two years, a screening program to detect and evaluate active biologicals for the treatment of cancer has been developed by the BRMP. This screening program was formulated based on BRMP staff recommendations, the drug screening program and the recommendations of the BRMP Subcommittee, as well as

on the recommendations of many experts in the field. The common track screen consists of a series of sequential assays through which BRM agents are evaluated for therapeutic potential. The screen was developed to define the effects of BRM on murine T cell, B cell, natural killer (NK) cell and macrophage functions. The sequence of testing consists of 1) in vitro activation: in vitro testing, 2) in vivo activation: in vitro testing and 3) in vivo activation: in vivo testing. The sequence allows the parameters of dose, schedule, route, duration and maintenance of activity, adjuvanticity and synergistic potential to be explored in an orderly fashion for each BRM. Specific tracks for analysis and evaluation of BRM such as monoclonal antibodies, lymphokines and growth and maturation factors are currently under development.

Since October 1981, the following agents have been entered in the BRM screen:

- | | |
|--|---------------------------------------|
| 1. MVE-2 | 7. Poly IC:LC |
| 2. MDP | 8. Lentinan |
| 3. Azimexon | 9. Picibanil |
| 4. Thymosin alpha 1
& Fraction 5 | 10. Tuftsin |
| 5. Interferon - Human Gamma (recombinant
& natural), Murine Gamma (recombinant
& natural), Human Alpha (natural lympho-
blastoid), Murine Alpha/Beta (natural)
& Human/Mouse Alpha (recombinant A/D
hybrid) | 11. Bestatin |
| | 12. Tumor Necrosis Factor
(Murine) |
| | 13. Alkyl-lysophospholipid |
| 6. N 137 | 14. FK-565 Peptide |

Testing is nearly complete on MVE-2, MDP, Azimexon, thymosin alpha-1 and fraction 5, the interferons and N-137. The other agents are at various stages of completion within the screen program. At present, thymosin alpha-1 and fraction 5 are the best T-cell immunomodulators found requiring about equidose of alpha 1 to equal the activity of fraction 5. They have also demonstrated excellent therapeutic capabilities in selected animal model systems. MDP has also demonstrated good T-cell activating properties. Among interferons, several human and murine natural and recombinant forms have been examined. The natural and recombinant human alpha interferons were without activity in the mouse, while the human/mouse recombinant alpha interferon was an excellent NK cell activator. Subsequent studies into hyporesponsiveness did not demonstrate the induction of tolerance observed clinically with interferon. Murine gamma interferon was found to be much more active than the alpha form in NK and macrophage activation. Poly IC:LC has been found to be the best long-term NK-cell and macrophage-activating agent studied. It has also been found to be an excellent interferon inducer of long duration. No evidence of induction of hyporesponsiveness has been found. This agent has also been shown to have excellent therapeutic effects. MVE-2 and Picibanil were also found to activate NK cells and macrophages in vivo but were somewhat less impressive when compared with poly IC:LC. Several other agents that have been entered into

the screen and tested showed moderate to poor activity in the various screen components. Azimexon, for example, gave moderate activity in reducing spontaneous metastases while giving only a weak effect on T-cell activation. N-137 showed moderate activity in eliminating both experimental and spontaneous metastases, and Lentinan gave evidence of moderate in vivo NK activation.

BRMP - DECISION NETWORK COMMITTEE (BRMPDNC)

The BRMPDNC has responsibility for guiding program staff in the overall development of the program and for reviewing and selecting agents to be evaluated in the screen and to be studied clinically. The membership of this committee consists of representatives from the Division of Cancer Treatment, the Division of Cancer Biology and Diagnosis, the Division of Cancer Cause and Prevention, the National Institute of Allergy and Infectious Diseases, Frederick Cancer Research Facility (FCRF), the DCT Board of Scientific Counselors and its BRMP Subcommittee. Members have been included who have expertise in (1) toxicology (2) regulatory policies (3) basic research (4) pharmacology and (5) clinical medicine. Four of the ten rotating member slots are reserved for extramural representation. The committee has enlisted the prospective participation of a large number of consultants who have agreed to serve on a per request basis and are available for consultation in specific areas of expertise.

Technology Transfer and Information Dissemination

At the request of the DCT Board of Scientific Counselors, the BRMP has sponsored a series of scientific retreats with the overall objective of gaining input from the extramural community. For each retreat, BRMP staff identified specific goals and objectives to be met. Information obtained from the retreats, where possible, is used to assess the priorities for future program priorities. Summary documents, prepared from each session, have been widely distributed to the scientific community. The retreats held during the past year are as follows:

- Assessment of Monoclonal Antibodies in Therapeutic Trials
- State-of-the-Science of Lymphokines and Cytokines in the Treatment of Cancer
- A Proposed Acquisition/Distribution System for Biologics
- The Development of a Specific Track Screening Program for Monoclonal Antibodies, Tumor-Associated Antigens, Lymphokines and Growth/Matured Factors.

In FY 83, the BRB also sponsored a conference on interferon in clinical trials to summarize the clinical activities and BRM effects of the alpha interferons tested by our university task order contractors. Plans are underway to host a national conference on ex vivo immunoabsorbants in the fall of 1983.

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3. Smalley, R.V., Long, C.W., Sherwin, S.A., Oldham, R.K.: Biological Response Modifiers: Current Status and Prospects as Anticancer Agents. In Clinical and Basic Tumor Immunology, (eds.) Herberman, R.B., Martinus, N., The Hague, 1983, (In Press).
4. Foon, K.A., Smalley, R.V., Riggs, C.W., Gale, R.P.: The Role of Immunotherapy in Acute Myelogenous Leukemia. Arch Int Med, 1983, (In Press).
5. Oldham, R.K., Smalley, R.V.: Immunotherapy: The Old and the New. J Biol Resp Mod, 2:1-37, 1983.
6. Smalley, R.V., and Oldham, R.K.: Biological Response Modifiers: Preclinical Evaluation and Clinical Activity. CRC Critical Reviews in Oncology/Hematology, 1983, (in press).
7. Mayer, D., Smalley, R.V.: Interferon: A Review Including Implications for Nursing Care. Onc Nursing Forum, 1983, (in press).
8. Balch, C.M., Smalley, R.V., Bartolucci, A.A., Burns, D., Present, C., and Durant, J.R.: A Randomized Prospective Clinical Trial of Adjuvant C. Parvum Immunotherapy in 260 Patients with Clinically Localized Melanoma. Cancer. 49:1079-1084, 1982.
9. Mayer, D.M. and Smalley, R.V.: Hodgkin's Disease and the Non-Hodgkin's Lymphomas. Fundamentals of Internal Medicine. (Eds.) Kaye, D. and Rose, L.F., C.V. Mosby Co., St. Louis, MO, 1983, 383-391pp.
10. Present, C.A., Bartolucci, A.A., Smalley, R.V., and Vogler, W.R.: Malignant Melanoma: Intravenous Corynebacterium Parvum with DTIC and Cyclophosphamide. Immunotherapy of Human Cancer: Present Status of Trials in Man. (Eds.) Terry, W., Rosenberg, S., Excerpta Medica, New York, 1982, 285-288pp.
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12. Thurman, G.B., Stull, H.B. and Oldham, R.K. 1983. The effects of human interferon on the migration of guinea pig peritoneal exudate cells. IN: Interleukins, Lymphokines and Cytokines, Oppenheim, J.J. and Cohen, S., eds. Academic Press. In press.
13. Stevenson, H.C., Favilla, T., Beman, J.A., Akiyama, Y., Miller, P., Herberman, R.B., Oldham, R.K., Stull, H. and Thurman, G.B. A system for obtaining large numbers of highly purified cryopreserved human monocytes and monocyte-depleted lymphocytes by leukoporesis/counter-current centrifugal elutriation (CCE). J. Immunol. Methods. In press.
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15. Suk, W.A., Snead, R.M. and Long, C.W. Inhibition of Retrovirus Expression and Cell Cycle Progression by Amino Acid Analogs. In Vitro, 1983. In press.
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ABSTRACTS

1. Velez-Garcia, E., Moore, M., Marcial, V., Ketcham, A., Liu, C., Bartolucci, A., Smalley, R., Vogel, C.: A US Southeastern Cancer Study Group Adjuvant Study for Women with Operable Breast Cancer and Positive Axillary Lymph Nodes. EORTC Conference, 1983.
2. Velez-Garcia, E., Moore, M., Marcial, V., Vogel, C., Bartolucci, A., Liu, C., Ketcham, A., Smalley, R.: Post Operative Adjuvant Chemotherapy with or without Radiation Therapy in Patients with Stage II Breast Cancer - A Southeastern Cancer Study Group Study. Proc. ASCO 2:111,1983.
3. Abrams, P.G., Sherwin, S.A., Ward, J., Heyman, M.R., St. John, R., Fein, S., Bottino, G.C., Smalley, R., Oldham, R.: A Phase I Trial of Recombinant Leukocyte A Interferon Administered by Intralesional Injection. Proc. ASCO 2:48,1983.

PRESENTATIONS

R.V. Smalley

1. Current Status Monoclonal Antibody Therapy, 6/14/83, Albert Einstein Medical Center, Philadelphia, PA

2. Biological Response Modifier Therapy, 6/10/83, American College of Physicians Course, Atlantic City, NJ
3. Current Status Monoclonal Antibody Therapy, 6/2/83, Medical College of Pennsylvania, Philadelphia, PA
4. Immune Modulation with Interferon, 5/22/83, Burroughs Wellcome Workshop, San Diego, CA
5. Clinical Trials with Interferon, 5/7/83, Pennsylvania Oncologic Society, Philadelphia, PA
6. BRMP Trials with Alpha and Gamma Interferon, 4/22/83, 3rd International Congress of Interferon Biology, Rotterdam, Holland
7. Current Status of Interferon Clinical Trials, 1/19/83, Southeastern Cancer Study Group, Charleston, SC
8. Phase I Clinical Trials with Thymosin, 11/20/82, RTOG, Phoenix, AR
9. Extramural BRM Program - 1983, 11/12/82, Roswell Park MI, Buffalo, NY
10. Management of Metastatic Breast Cancer, 11/5/82, 1st Annual Cincinnati Breast Symposium, Cincinnati, OH
11. Biological Response Modifier Therapy - 1982, 10/14/82, ECOG, West Palm Beach, FL
12. Biological Response Modifier Therapy - 1982, 7/19/82, RTOG, Philadelphia, PA

BRMP DECISION NETWORK COMMITTEE

STANDING MEMBERS

Chairman:

Dr. Richard V. Smalley, Chief
Biological Resources Branch
BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Bldg. 426, Room 1
Frederick, MD 21701
(301)695-1098

Dr. Ronald Herberman, Chief
Biological Therapeutics Branch
BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Bldg. 560
Frederick, Maryland 21701
(301)695-1323

Executive Secretary:

Ms. Virginia Suppers, Special Asst.
Biological Response Modifiers Program
DCT, NCI, NIH
Frederick Cancer Research Facility
Building 567, Room 129
Frederick, Maryland 21701
(301)695-1423

TBA, Chief
Clinical Trials Section
BRB, BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 426, Room 1
Frederick, Maryland 21701
(301)695-1098

Ex Officio Member:

Dr. Saul Schepartz, Deputy Director
Division of Cancer Treatment, NCI, NIH
Building 31, Room 3A51
Bethesda, Maryland 20205
(301)496-6404

Dr. Cedric Long, Chief
Preclinical Trials Section
BRB, BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 426, Room 1
Frederick, Maryland 21701
(301)695-1098

Dr. David Abraham
Investigational Drug Branch
CTEP, DCT, NCI, NIH
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301)496-5725

TBA - Chief
Toxicology Branch, DTP, DCT, NCI
8300 Colesville Road
Silver Spring, Maryland 20205
(301)427-8755

Dr. George Galasso
Development & Applications Branch
MIDP, NIAID, NIH
Westwood Building, Rm. 750
5333 Westbard Avenue
Bethesda, Maryland 20205
(301)496-7051

Dr. Bruce A. Maurer, Chief
Molecular Immunology Section
CBB, DCBD, NCI, NIH
Westwood Building, Room 10A07
5333 Westbard Avenue
Bethesda, Maryland 20205
(301)496-7815

Dr. Daniel Hoth
Investigational Drug Branch
CTEP, DCT, NCI, NIH
Lanow Building
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301)496-5223

Dr. J.A.R. Mead, Deputy Assoc. Dir.
Developmental Therapeutics Prog.
DCT, NCI
Blair Building, Room 5A03
8300 Colesville Road
Silver Spring, Maryland 20205
(301)427-8685

Dr. E. Bruce Merchant, Chief
Immunohematology Branch
DBBP, BOB, FDA
NIH Campus, Building 29, Room 225
Bethesda, Maryland 20205
(301)496-4538

Dr. David Nelson
Metabolism Branch
DCBD, NCI, NIH
Building 10, Room 4 North 117
Bethesda, Maryland 20205
(301)496-5387

Dr. Robert K. Oldham, Assoc. Director
Biological Response Modifiers Program
DCT, NCI, NIH
Frederick Cancer Research Facility
Building 567, Room 129
Frederick, Maryland 21701
(301)695-1416

Matthew Suffness, Ph.D.
Natural Products Branch
DTP, DCT, NCI, NIH
Blair Building, Room 514
8300 Colesville Road
Bethesda, Maryland 20205
(301)427-8716

James E. Talmadge, Ph.D.
Preclinical Screen
NCI/Frederick Cancer Research Facility
P.O. Box B, Building 539
Frederick, Maryland 21701
(301)695-1474

Gary B. Thurman, Ph.D.
Preclinical Trials Section
BRB, BRMP, DCT, NCI
Frederick Cancer Research Facility
Building 426, Room 1
Frederick, Maryland 21701
(301)695-1098

Robert E. Wittes, M.D.
Cancer Therapy Eval. Program
DCT, NCI, NIH
Lanow Building, Room 4C37
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301)496-6138

ROTATING MEMBERS

Dr. Michael A. Chirigos, Chief
Immunopharmacology Section
BDB, BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 560, Room 31-76
Frederick, Maryland 21701
(301)695-1327
Term: 1/82 - 1/84

Dr. Alexander Fefer, Prof. of Med.
Division of Oncology
Department of Medicine
University of Washington,
School of Medicine
Seattle, Washington 98195
(206)543-8557
Term: 1/82 - 1/84

Dr. Freddie Ann Hoffman
Special Assistant for Nutrition
CIB, CTEP, DCT, NCI, NIH
Lanow Building, Room 4B04
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301)496-6056
Term: 1/82 - 1/83

Dr. Michael Mastrangelo, Director
Immunotherapy Clinic
Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111
(215)728-2912
Term: 1/82 - 1/84

Dr. Enrico Mihich, Director
Grace Cancer Drug Center
Roswell Park Memorial Institute
666 Elm Street
Buffalo, New York 14263
(716)845-5860
Term: 1/82 - 1/84

Dr. Jeffrey Schlom, Chief
Experimental Oncology Section
LCMB, DCCP, NCI, NIH
Building 37, Room 1A07
Bethesda, Maryland 20205
(703)354-0417 (Meloy Lab)
Term: 4/82 - 1/84

TABLE I

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> <u>GRANT NUMBER</u>	<u>PRINCIPAL</u> <u>INVESTIGATOR</u>	<u>INSTITUTION AND</u> <u>GRANT TITLE</u>
<u>Molecular Immunology</u>		
<u>INTERFERON</u>		
19061-07	Pitha, P.	Johns Hopkins University "Antitumor Effect of Interferon"
26475-04	Fleischmann, W.R.	University of Texas "Modulation of Antitumor Effect of Interferon"
26966-03	Meyers, J.D.	Fred Hutchinson Cancer Research Ctr. "Interferon for CMV Infection, Leukemia Relapse and GVHD"
29990-03	O'Malley, J.	Roswell Park Memorial Institute "Immune Interferon in Cellular, Viral and Immune Systems"
29991-03	Gupta, S.	Sloan-Kettering "Interferon-Induced Proteins in Mouse and Human Cells"
30517-02	Havell, E.A.	Trudeau Institute "Antitumor Actions of Interferons"
31080-02	Ozer, H.	Roswell Park Memorial Institute "Clinical Phase II Trial of HFIF in Lymphoma and Myeloma"
<u>THYMIC FACTOR</u>		
29943-03	Goldstein, A.L.	George Washington University "Role of Thymosin Peptides in T- Cell Differentiation"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> <u>GRANT NUMBER</u>	<u>PRINCIPAL</u> <u>INVESTIGATOR</u>	<u>INSTITUTION AND</u> <u>GRANT TITLE</u>
<u>LYMPHOKINES</u>		
29145-02	Papermaster, B.	Cancer Research Center "A Lymphokine Fraction for Testing in Cancer Patients"
30172-03	DiSabato, G.	Vanderbilt University "Lymphocyte Factors in Normal and Leukemic Mice"
33429-01	Hilfiker, M.L.	Cleveland Clinic "Lymphokine Immunotherapy for Cancer Treatment"
33449-01	Rinehart, J.L.	Ohio State University Hospital "Liposome-Macrophage Activating Factor for Clinical Use"
35845-01	Sidman, C.L.	The Jackson Laboratory "Purification and Immunobiology of B Maturation Factors"
35999-01	Chiao, J.W.	New York Medical College "Suppression and Maturation Induction of Leukemia Cells"
<u>Cell Surface Immunology</u>		
<u>ADJUVANTS</u>		
15325-08	Gray, G.R.	University of Minnesota "Antitumor Active Components of BCG Cell Walls"
15665-08	Medoff, G.	Washington Univ. School of Medicine "Polyenes as Biologic Response Modifiers"
27922-03	Lamm, D.L.	University of Texas "BCG Immunotherapy of Superficial Bladder Cancer"
29570-02	Stein, J.A.	Bellinson Medical Center "Intralesional BCG Immunotherapy in Malignant Melanoma"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
<u>ADJUVANTS (CON'T)</u>		
31545-01	Enker, W.E.	Sloan-Kettering "Suppressor Cell Elimination as Immunotherapy"
32155-03	Reinisch, C.	Sidney Farber Cancer Institute "Selective Manipulation of T-Cell Subset by Adjuvant"
<u>ANTIBODIES</u>		
15952-08	Reif, A.	Boston City Hospital "Experimental Studies on Immunity to Cancer Cells"
16699-04	Hiramoto, R.	University of Alabama "A Model for Multiple Modality Therapy of Osteosarcoma"
19127-05	Jansons, V.K.	New Jersey Medical School "Liposomes as Carriers for Anti- tumor Agents"
P01 25863-03	Bolognesi, D.	Duke University "Control of Neoplasia by Passive Serum Therapy"
26386-03	Bernstein, I.D.	Fred Hutchinson Cancer Research Ctr. "Monoclonal Antibody Therapy of Cancer"
28740-03	Bast, R.C.	Sidney Farber Cancer Institute "Specific Immunotherapy with Monoclonal Antibodies"
29125-02	Papermaster, B.W.	Cancer Research Center "Mouse Tumor Assays for Lymphokine Immunotherapy"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> <u>GRANT NUMBER</u>	<u>PRINCIPAL</u> <u>INVESTIGATOR</u>	<u>INSTITUTION AND</u> <u>GRANT TITLE</u>
<u>ANTIBODIES (CON'T)</u>		
29160-02	Griffin, T.	University of Massachusetts Medical School "Ricin A Chain/Anti-CEA Antibody Conjugates"
29544-02	Wang, M.C.	Roswell Park "Immuno-Specific Chemotherapies of Prostate Cancer"
30520-02	de Noronha, I.M.	Cornell University "Serotherapy of Virus-Induced Feline Sarcoma or Leukemia"
30573-02	McCune, C.S.	University of Rochester "Specific Immunotherapy of Renal Carcinoma"
31288-02	Primus, J.F.	University of Kentucky "Tumor Localization and Therapy with Immunoliposomes"
31525-01	Merluzzi, V.J.	Sloan-Kettering " <u>In Vivo</u> Modification of Host Immunity after Chemotherapy"
31699-01	Lee, F.H.	Univ. of Southern California "Adriamycin and Antibody Conjugates in Cancer Therapy"
31752-01	Goldenberg, P.	"N.J. Medical and Dental College, Newark "Chemoimmunotherapy of Transplanted Colon Cancer"
31753-01	Hawthorne, M.F.	Univ. of California, Los Angeles "Boren-10-Labeled Antibodies in Cancer Therapy"
33280-01	Kirch, M.D.	Sloan-Kettering "Antibody Therapy of Transplants and 1° Leukemias"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> <u>GRANT NUMBER</u>	<u>PRINCIPAL</u> <u>INVESTIGATOR</u>	<u>INSTITUTION AND</u> <u>GRANT TITLE</u>
<u>ANTIBODIES (CON'T)</u>		
33361-01	Preston, J.F.	University of Florida "Amanitin-Protein Conjugates as Potential Inhibitors"
33397-01	Sato, G.H.	University of California, San Diego "Monoclonal Antibody in Animal Tumor Models"
33399-01	Levy, R.	Stanford University "Human Anti-Tumor Therapy with Monoclonal Antibodies"
33454-01	Neefe, J.R.	Georgetown University "Induction of Antitumor Effectors by Monoclonal Antibody"
33462-01	Bankert, R.B.	Roswell Park "Monoclonal Antibodies Applied to Treat/Diagnose Cancer"
33470-01	Strand, M.	The Johns Hopkins University "Chemically Modified Monoclonal Antibody in Tumor Models"
33477-01	Bernstein, I.D.	Fred Hutchinson "Monoclonal Antibody in Animal Tumor Models"
33573-01(R23)	Merritt, W.	The George Washington University "Anti-Glycolipid Monoclonal Antibodies: Cytotoxin Target"
34394-01	Grant, C.	Harvard University "Monoclonal Antibody Therapy of Feline Leukemia"

TABLE I (CONTINUED)
GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> <u>GRANT NUMBER</u>	<u>PRINCIPAL</u> <u>INVESTIGATOR</u>	<u>INSTITUTION AND</u> <u>GRANT TITLE</u>
<u>ANTIGENS</u>		
28696-03	Oettgen, H.	Sloan-Kettering "Experimental Therapy of Human Melanoma with Vaccines"
28738-02	Bortin, M.	Mt. Sinai Med. Ctr., Milwaukee "Alloimmunization for Induction of Antitumor Immunity"
29597-03	Vosika, G.	University of North Dakota "Bacterial Component Immunotherapy in Cancer"
30276-02	Niederborn, J.Y.	University of Texas "Immunological Modulation of Ocular Tumor Metastasis"
31088-01	Pinsky, C.M.	Sloan-Kettering "Local Immunotherapy for Superficial Bladder Tumors"
<u>Cellular Immunology</u>		
<u>LYMPHOID CELLS</u>		
36669-01	Choi, Y.S.	Sloan-Kettering "Human B-Cell Growth Factors"
25184-03	Klein, E.	Karolinski Institute "Autoreactive Cells in Tumor Patient"
25608-03	Merluzzi, V.J.	Sloan-Kettering "Specific Repair of Drug-Induced Immune Cellular Deficits"
29005-02	Mannick, J.	Peter Bent Brigham Hospital "Adoptive Immunotherapy in Renal Cell Carcinoma Patients"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> <u>GRANT NUMBER</u>	<u>PRINCIPAL</u> <u>INVESTIGATOR</u>	<u>INSTITUTION AND</u> <u>GRANT TITLE</u>
<u>LYMPHOID CELLS (CON'T)</u>		
30558-02	Cheever, M.A.	University of Washington "Specific Immunotherapy of Murine Tumors"
31544-01	Hersh, E.M.	M.D. Anderson "Monocyte and RES Function in Human Cancer"
33035-01	Wing, E.J.	Montefiore Hospital Pittsburgh "Anti-Tumor Activity of Colony Stimulating Factor"
33491-01	Koprowski, H.	The Wistar Institute "Monoclonal Antibody in Cancer Therapy"
<u>GROWTH AND</u> <u>MATURATION</u> <u>FACTORS</u>		
15581-07	Dennert, G.	Salk Institute "Immune Response to Cell Surface Antigens"
27765	Hiramoto, R.N.	University of Alabama "Exploitable Mechanisms in Combin- ation Cancer Therapy"
33932	Deodhar, S.	The Cleveland Clinic "Therapy of Cancer Metastases with C-Reactive Protein"
<u>MISC. APPROACHES</u> <u>TO BIOLOGICAL</u> <u>RESPONSE MODIFICATION</u>		
17393-08	Donahoe, P.	Massachusetts General Hospital "Mullerian Inhibiting Substance"

TABLE I (CONTINUED)
GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
MISC. APPROACHES TO BIOLOGICAL RESPONSE MODIFICATION (CON'T)		
18105-07	Applebaum, F.R.	Fred Hutchinson Cancer Research Ctr. "Immunotherapy Studies of Spontaneous Malignancy"
PO1 18221-06	Storb, R.F.	Fred Hutchinson Cancer Research Ctr. "Marrow Grafting in Treatment of Hematologic Malignancies"
PO1 23766-04	O'Reilly, R.J.	Sloan-Kettering "Marrow Transplantation in Aplastic Anemia and Leukemia"
24553-03	Huang, L.	University of Tennessee "Targeting of Liposomes to Tumor Cells"
25526-04	Papahadjopoulos, D.	University of San Francisco "Liposome Targeting to Tumor Cells <u>In Vivo</u> "
27330-02	Nishioka, K.	University of Texas "Antitumor Activity of Tuftsin"
27615-02	Barth, R.	Ohio State University "Immunity to Tumor Localized Chemically-Modified Drugs"
28630-02	Hoffman, M.	Sloan-Kettering "Activation and Function of QAS + NK Cells"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

<u>CATEGORY</u> <u>GRANT NUMBER</u>	<u>PRINCIPAL</u> <u>INVESTIGATOR</u>	<u>INSTITUTION AND</u> <u>GRANT TITLE</u>
MISC. APPROACHES TO BIOLOGICAL <u>RESPONSE MODIFICATION (CON'T)</u>		
28835-02	Cohen, S.	State Univ. of N.Y. at Buffalo "Cancer Chemotherapy and Murine Natural Killer Cells"
29769-01	Hersh, E.	University of Texas "Biological Response Modifier Therapy of Human Cancer"
32809-01	Butler, G.B.	University of Florida "Molecular Weight Effects on Polymeric Anti-Tumor Agents"
35340-01	Papahadjopoulos, D.	University of San Francisco "Liposome-Mediated Intracellular Delivery <u>In Vitro</u> "
COMPLICATIONS, ADVERSE EFFECTS AND RELATED PHENOMENA ATTENDING THE USE AND EVALUATION <u>OF BRM IN CANCER THERAPY</u>		
27598-03	Harris, J.	Rush University "Cancer Drug Effects on Patient Suppressor Cells"

TABLE II
 BIOLOGICAL RESPONSE MODIFIERS PROGRAM
 BIOLOGICAL RESOURCES BRANCH
 CONTRACTS PROJECTED FOR FUNDING IN FY 1983

CONTRACTS	Estimates (\$K)
Characterization and Analysis of Proteinaceous Materials	150
Phase I/II Clinical Trials of BRMs (Includes 7 Task Orders)	1,293
Collection, Storage and Quality Assurance of BRM Task A - Repository, Quality Assurance and Testing for IND	111
Task B - BRM Activity Testing	197
Technical Support for the Review and Evaluation of BRM	430
Chemical Coupling of Cytotoxic Agents to Tumor Reactive Monoclonal Antibody	300
Production of Monoclonal Antibody to Human Cytokines	150
Purchase of Monoclonal Antibody (Anti-Leu 2a)	20
Purchase of Recombinant Murine Alpha Interferon	50
Total - Extramural Contracts	\$2,701

TABLE III

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

INSTITUTIONS HOLDING BRMP CLINICAL TRIALS MASTER CONTRACTS

University of California/Los Angeles
University of California/San Diego
University of Cincinnati
Dartmouth University
Duke University
Fox Chase Cancer Center
Fred Hutchinson Cancer Research Center
Georgetown University
George Washington University
Hahnemann Medical School
Illinois Cancer Council
Institute de Cancerlogie, France
Mayo Clinic
University of Minnesota
Northern California Cancer Program
Ohio State University
Ontario Cancer Institute, Canada
University of Pittsburgh
Roswell Park Memorial Institute
Sidney Farber Cancer Institute
Sloan-Kettering Institute
University of Southern California
Temple University (Southeastern Study Group)
University of Texas (M.D. Anderson)
Vanderbilt University
Wayne State University
Wisconsin University

TABLE IV

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

CLINICAL TASK ORDERS FUNDED UNDER THE CLINICAL MASTER AGREEMENTPHASE I TASK ORDERS

<u>Institution</u>	<u>Agent</u>
Georgetown University	Leukocyte Interferon*
Sidney Farber Institute	Leukocyte Interferon*
Northern California Cancer Program	Leukocyte Interferon*
University of California, Los Angeles	Lymphoblastoid Interferon*
Duke University	Lymphoblastoid Interferon*
University of California, San Diego	Thymosin*
Fred Hutchinson Cancer Research Center	Thymosin
George Washington University	Thymosin
Sloan-Kettering Institute	Thymosin*
Northern California Cancer Program	Thymosin*
Vanderbilt University	MVE-2*
Ohio State University	MVE-2*
University of California, Los Angeles	Anti-T Cell Monoclonal Antibody
University of California, San Diego	Anti-T Cell Monoclonal Antibody
University of Southern California	Anti-T Cell Monoclonal Antibody
Fox Chase Cancer Center	Anti-T Suppressor Cell Antibody

PHASE II TASK ORDERS

Georgetown University	Lymphoblastoid Interferon
University of California, Los Angeles	Lymphoblastoid Interferon
Duke University	Lymphoblastoid Interferon
University of Wisconsin	Lymphoblastoid Interferon
Sloan-Kettering Institute	Lymphoblastoid Interferon

*Completed Studies

TABLE V

BIOLOGICAL RESPONSE MODIFIERS PROGRAM DECISION NETWORK COMMITTEE

LISTING OF POTENTIAL AGENTS AND APPROACHES

A. Immunomodulator Agents

*Azimexon
*NED-137
Therafectin (SM1213)
DTC (Sodium diethyldithiocarbamate)
Bestatin
Cimetidine
Cis-retinoic Acid
Krestin
Isoprinosine
NPT 15392
Russian glucomannan

B. Immunostimulator Agents

*MDP
*Tuftsin
Lentinan
Glucan (soluble)
Mannozyme
Levan

C. Immunorestorative Agents

Levamisole
Thiobendazole

D. Miscellaneous Chemicals

6-Ayrlpyrimidinoles
Substituted pyrimidine (Upjohn)
CL-246,738 (Cyanamid)
Prostaglandin Inhibitors (Aspirin, Indomethacin)

E. Miscellaneous Bacterial Extracts or Bacteria

BCG, (P3, CWS)
Nocardia rubra CWS
Picibanil (OK432)
Pseudogen
Brucella abortus (Bru-pel)

TABLE V (Cont.)

BIOLOGICAL RESPONSE MODIFIERS PROGRAM DECISION NETWORK COMMITTEE

LISTING OF POTENTIAL AGENTS AND APPROACHES

F. Miscellaneous Biologicals

- *Tumor necrosis factor
- "Immune" RNAs

G. Interferons and Interferon Inducers

- *Interferons
- *MVE-2
- Poly IC/LC
- Bru Pel
- Tilorones
- Levan
- Mumps Virus

H. Thymic Factors

- *Thymosins - $\alpha 1$
- *Thymosins - F-5
- Other thymic factors

I. Lymphokines - Cytokines

- Macrophage activation factor/MAF
- T-cell growth factor (TCGF) IL-2
- IL-3
- Lymphocyte activation factor (LAF)
- Cytotoxic factor (lymphotoxin - LT)
- Transfer factor
- Colony stimulating factor
- T helper cell replacing factor (TRF)
- Macrophage chemotactic factor
- Thymocyte mitogenic factor (TMF)
- Macrophage inhibitory factor (MIF)

J. Antibody

- *Monoclonal antibodies against:
 - Anti-T-cell
 - Anti-T supressor cell
 - Anti-tumor Ab
 - Antibody to lymphokines, cytokines, etc.
- Cytophilic antibody

TABLE V (Cont.)

BIOLOGICAL RESPONSE MODIFIERS PROGRAM DECISION NETWORK COMMITTEE

LISTING OF POTENTIAL AGENTS AND APPROACHES

K. Antigens

Tumor Associated Antigens

L. Effector cells

Effector cells - T-cell clones
NK-cells or clones
Macrophages
T-helper cells

M. Growth/Maturation Factors

Chalones
Antigrowth Factors
Maturation Factors
Growth Factors

N. Miscellaneous Approaches

Bone Marrow Transplantation and Reconstitution
Plasmapheresis & ex vivo immunoabsorption
Allogenic Immunization
Virus Infection of Cells
Vaccinia

NOTE: Agents are listed in order of priority under each category as assessed by the BRMPOC and BRMPDNC in 1981. Categories are not in any order of priority.

* Identified agents currently approved (per BRMPDNC) to enter in the BRM screen.

SUMMARY REPORT

BIOLOGICAL THERAPEUTICS BRANCH

October 1, 1982 to September 30, 1983

INTRODUCTION

The Biological Therapeutics Branch (BTB) of the Biological Response Modifiers Program (BRMP) was formed as a result of a merger in December 1981, of the Biological Development Branch of the BRMP with the Laboratory of Immunodiagnosis, DCBD, NCI. At that time, the entire staff of the Laboratory of Immunodiagnosis moved to the NCI-Frederick Cancer Research Facility. The staffs of both branches were merged and a reorganization plan for all personnel was developed. During the first year of operation of the BTB, it became clear that additional senior investigators were needed to provide adequate leadership and direction for the intramural research efforts. After a prolonged recruiting effort, Dr. Joost J. Oppenheim from the NIDR was selected to assume responsibility for three of the sections of the BTB (Biochemistry, Lymphokines, and Basic Mechanisms). This portion of the intramural BRMP was separated from the BTB and became the Laboratory of Molecular Immunoregulation. Under this reorganization, the BTB remains with four sections: Clinical Investigations Section (Kenneth A. Foon, M.D., Proposed Acting Head), Monoclonal Antibody/Hybridoma Section (A. Charles Morgan, Ph.D., Deputy Head), Natural Immunity Section (Ronald B. Herberman, M.D., Acting Section Head), and Immunopharmacology Section (Michael A. Chirigos, Ph.D., Head). The following is a summary of the major research findings of the Branch during this fiscal year.

CLINICAL INVESTIGATIONS SECTION

The Clinical Investigations Section of the BTB is responsible for the investigation of the therapeutic efficacy of biologicals and BRMs and the analysis of biologic response modification and these agents and approaches. This Section of the BRMP was established to facilitate the early clinical trials of biologic products with potential as anticancer agents. Agents tested initially included interferons, lymphokines, immunomodulators, and monoclonal antibodies. The Clinical Investigations Section is particularly concerned with in-depth Phase I and II trials of biologicals and biological response modifiers involving small numbers of patients. Optimal immunomodulatory doses, as well as maximum tolerated doses, of these new agents are being determined.

During this fiscal year, Dr. Stephen Sherwin, who was Head of the section, transferred from the BRMP, and Dr. Kenneth A. Foon assumed responsibility for the section. The other main personnel change was the transfer of Dr. Henry C. Stevenson from the Basic Mechanisms Section to the Clinical Investigations Section, where he will continue to have primary responsibility for the operation of the Leukapheresis Unit as well as perform clinical and laboratory research.

The Clinical Investigations Section of the BRMP began clinical trials in early May 1981. During the past two years, the Clinical Investigations Section has continued to concentrate on Phase I trials of various types of interferon, an

intralesional interferon study, a protein A column study for breast cancer, a Phase I cis-retinoic acid study, Phase II studies of recombinant alpha interferon treatment of lymphoma and breast cancer, and Phase I monoclonal antibody studies for treating chronic lymphocytic leukemia, T-cell lymphoma and malignant melanoma. Within the area of interferon testing, the research program has limited its attention to an examination of representatives of each of the three classes of interferons: namely, alpha, beta and gamma. A major part of the work of this section during the first year involved early clinical trials of two representative alpha interferons: highly purified nonrecombinant lymphoblastoid interferon (provided to the National Cancer Institute by the Burroughs Wellcome Company) and highly purified recombinant leukocyte A interferon (provided to the National Cancer Institute by Hoffmann-La Roche, Inc.). These two interferon preparations are currently available in large quantities and could conceivably be tested on a long-term basis in cancer therapy. Other clinical trials of representative interferons initiated during the past two years included a Phase I trial of immune interferon in cancer patients and a Phase I trial of genetically engineered fibroblast interferon. In this manner, the Clinical Investigations Section will have had an opportunity to study, in at least Phase I testing, an interferon of each of the three major types.

Phase I trials of highly purified recombinant and nonrecombinant alpha interferons were completed during the first and second year. Recombinant leukocyte A interferon was tested in two multiple-dose Phase I trials, where dosages were escalated in groups of 5 or more patients. Patients were extensively monitored for toxicity, antitumor effect, immunologic function and serum interferon activity. Eighty-one patients were entered by the Clinical Investigations Section on a three times weekly multiple-dose Phase I trial of this agent. The BRMP also participated in a collaborative manner, with the Baltimore Cancer Research Center, in a similar multiple-dose trial of this agent on a daily schedule of administration. These trials showed that recombinant leukocyte A interferon could be administered safely in doses up to 54×10^6 units daily and 118×10^6 units three times weekly. Antitumor effect was seen in 9 out of 81 patients (partial remissions) with diagnoses of non-Hodgkin's and Hodgkin's lymphoma, chronic lymphocytic leukemia, breast cancer and melanoma. Dose-dependent pharmacokinetics of serum interferon activity were demonstrated and evidence for steady-state accumulation on the three times weekly schedule of administration was found in doses greater than 50×10^6 units. Analysis of immunologic monitoring of patients revealed that most patients had no change or a decrease in their natural killer cell mediated cytotoxic activity. Approximately 80% of the patients demonstrated increased monocyte function as measured in a growth inhibition assay. Lymphocyte blastogenesis was uniformly decreased in patients treated with this agent, as has been previously reported for other interferons.

Highly purified nonrecombinant lymphoblastoid interferon was tested in an escalating dose trial by both intravenous and intramuscular routes of administration. Twenty-nine patients were treated by the intravenous route and 10 patients by the intramuscular route. A maximum tolerated dose has been defined for the intravenously treated patients at 30×10^6 units daily x 5 days. Very high levels of serum interferon activity were seen with the intravenously treated patients. Antitumor effect (partial responses) was seen in 3 patients with non-Hodgkin's lymphoma, undifferentiated carcinoma and hypernephroma.

Phase I trials of beta and gamma interferon have also been initiated. Six patients were treated with a recombinant beta interferon and, because of the lack of toxicity and circulating interferon levels, it was determined that the beta interferon did not have any activity. When this problem is resolved, the study will resume. So far, 32 patients have been treated on the Phase I dose escalation trial of nonrecombinant gamma interferon, and the maximum tolerated dose appears to be in the range of 50 million units/m² given on a twice weekly basis intravenously. We have not witnessed any clinical responses.

We have completed a Phase II trial of recombinant alpha interferon for the treatment of breast cancer. Eighteen patients with breast cancer were treated at a dose of 50 million units/m² 3 times weekly intramuscularly. This was the maximum tolerated dose as determined from our Phase I trial of the same recombinant alpha interferon. Sixteen patients progressed during therapy and 2 remained stable.

We are also performing a Phase II trial for lymphoma and CLL using recombinant alpha interferon at 50 million units/m² three times per week intramuscularly. We have so far treated 30 patients with lymphoma: 23 with favorable histology and 7 with unfavorable histology; 7 patients with chronic lymphocytic leukemia; and 9 patients with cutaneous T-cell lymphoma. Sixty percent of the patients with favorable histology lymphoma have had an objective response. All of these patients had been heavily pretreated with chemotherapy and/or radiation therapy and were no longer responsive to these agents. Three of 7 patients with chronic lymphocytic leukemia have had a partial remission, and 6 of 9 patients with cutaneous T-cell lymphoma have had a partial remission. Only 2 of 7 patients with unfavorable histology lymphoma have had responses. Overall, recombinant alpha interferon appears to be a promising agent for the treatment of refractory favorable histology lymphoma and cutaneous T-cell lymphoma.

Sixteen patients have been treated with 13 cis-retinoic acid. Toxicities included pruritis, dryness of the mouth and skin, fatigue, fever and anorexia. We have not witnessed responses in any patients treated with 13 cis-retinoic acid at dosages of 5 mg/m², 50 mg/m² or 100 mg/m².

A trial of intralesional recombinant alpha interferon has also been instituted. So far, 20 patients have been treated, and we have witnessed 2 responses in patients with malignant melanoma. None of the 3 patients with breast cancer have had responses. Toxicities have resembled those associated with systemic interferon therapy but have been relatively mild.

We have instituted a trial of infusion of autologous plasma perfused over a protein A column for the treatment of breast cancer. Perfusion is performed twice weekly with 50 ml of plasma over 0.6 mg of protein A with eventual escalation to 100 ml of plasma over 1.25 mg of protein A. Three patients have completed this Phase I study and no toxicity or clinical responses have been witnessed.

In June of 1982 we began a Phase I trial of T101 monoclonal antibody. This antibody binds to a 65,000 molecular weight antigen found on the cell surface membrane of normal and malignant T lymphocytes and malignant B lymphocytes. Twelve patients with chronic lymphocytic leukemia have so far been treated. We have observed transient reductions in the circulating leukemia cell count

without reductions in lymph nodes or other involved organs. We have identified T101 staining of circulating cells and bone marrow cells. Toxicity has included fever, urticaria, and shortness of breath. The shortness of breath appeared secondary to rapid intravenous infusion of 50 mg or greater T101 antibody over 2 hours. This toxicity was completely eliminated when infusions were slowed to 1 to 2 mg per hour. We have also treated 8 patients with cutaneous T-cell lymphoma with T101 antibody and have seen 5 minor skin responses. One patient had the disappearance of what appeared to be a malignant lymph node. We have not seen a reduction of tumor in other involved organs. We have identified T101 localization in cutaneous skin lesions in vivo. Similar to chronic lymphocytic leukemia, we have seen fever, urticaria, and pulmonary toxicity. The latter was eliminated by slow infusion of antibody.

We have also treated 8 patients with malignant melanoma with the 9.2.27 monoclonal antibody, which is an IgG_{2a} antibody directed toward a 250,000 molecular weight antigen which is almost entirely restricted to melanoma cells. Toxicity has included urticaria, fever and serum sickness in one patient. Unlike the T101 antibody trial, we have not seen any pulmonary toxicity. All patients were treated with five escalating doses: 1 mg, 10 mg, 50 mg, 100 mg, and 200 mg. We have identified 9.2.27 antibody localization in melanoma skin lesions at all dosages of 50 mg or greater, but we have not seen objective clinical responses.

The future direction of the Clinical Investigations Section includes continuation of Phase I and Phase II studies of various interferons and the interferon inducer poly ICLC, monoclonal antibody trials and protein A column perfusion. We expect in the next year to begin trials with monoclonal antibodies conjugated to radionuclides, drugs, and/or toxins. In view of the demonstrated ability of both T101 and 9.2.27 to localize on tumor cells in vivo, it is hoped that selective delivery of toxic agents to the tumor site will result in clinical benefits.

MONOCLONAL ANTIBODY/HYBRIDOMA SECTION

During this fiscal year, this section has undergone a restructuring and some major changes in emphasis. Dr. Alton C. Morgan, Jr. has transferred from the Biochemistry Section to the Monoclonal Antibody Section and assumed the position of Deputy Section Head. Along with Dr. Morgan, Dr. C. S. Woodhouse, a Visiting Fellow, has also transferred. This change served to increase the focus in the section on production of murine monoclonals to tumor-associated antigens, primarily in colorectal cancer, and has increased both biochemical expertise and provided needed experience in immunohistochemical techniques to carry out elicitation and characterization of monoclonal antibodies. The section was restructured into four interest groups which cross section boundaries and provide an arena for information exchange within a working group: one involved in murine monoclonal antibody production to tumor-associated antigens, a second which emphasizes methods of elicitation of human-human or human-mouse heterohybrids, a third which deals with immunotherapy in model systems including immunoconjugates, and a fourth which coordinates a major effort on production and therapeutic use of murine monoclonals to idiotypes of surface immunoglobulin on B-cell lymphomas.

The research within the section represents a broad spectrum of research on both solid tumors, leukemias, and lymphomas, all of which is motivated by a need to improve current treatment of human cancer. During this year, two clinical trials with monoclonal antibody have been initiated within the Clinical Investigations Section with patient monitoring carried out by the Monoclonal Antibody/Hybridoma Section. In the context of the T101 trial, a major finding has been the definition of antigenic modulation on the patients' leukemic cells. This observation has led to a number of laboratory studies on the role of monocytes and cytokines in enhancement of modulation, and the potential utility of this finding for immunoconjugate treatment is being pursued in both in vitro and in vivo animal models. Within the context of the 9.2.27/melanoma trial, we have established that antibody traffics to and specifically binds to cutaneous melanoma cells and is retained in tumor sites for up to 10 days following infusion. We have also established that with the protocol utilized, antiglobulin reactivity is only transient and does not deter localization in tumor sites. The clinical trial is closely tied to a nude mouse-human melanoma xenograft model with spontaneous metastasis, in which we are exploring the optimization of serotherapy with conjugated and unconjugated antibody and effector cells. These in vivo studies are made possible by continuing more basic studies in the section on immunoconjugate production. Within this setting, we have been one of the first groups successful in scale up of immunoconjugate studies, from in vitro to animal model systems, where we have shown in the L10 guinea pig hepatoma model that established primary tumor and metastases can be successfully treated.

Our basic program for production of murine monoclonals to tumor associated antigens has been highly successful in producing highly specific monoclonals through the use of novel immunochemically defined immunogens. In addition, we have initiated a similar set of fusions in large cell carcinoma of the lung along with continuing efforts on human melanoma. These efforts are required for generation of future agents for diagnosis and treatment. The section, in this regard, has shown an excellent capability of eliciting, characterizing and scaling up to clinical trial status with monoclonal reagents. This is typified by the antibody 9.2.27, which has considerable potential for the diagnosis and treatment of human melanoma, and the monoclonal antibody-defined 100K dalton melanoma-associated antigen, which has demonstrated usefulness in monitoring tumor burden in melanoma patients. Despite the clinical orientation of the research in the section, more basic efforts have not been ignored. A good example is the study of occult leukocyte antigens which was made possible by the development of a technique for analysis of internal antigens by flow cytometry. These studies will necessitate a reassessment of leukocyte differentiation schemes and leukemic cell classification.

In summary, the section carries out both highly relevant clinical and more basic research efforts tied to the common goal of employing monoclonal antibodies for the improvement of cancer diagnosis, prognosis, and treatment.

NATURAL IMMUNITY SECTION

The research activities of the Natural Immunity Section are primarily focused on the role of natural effector cells in resistance against cancer. There have been extensive studies on natural killer (NK) cells with emphasis on the characteristics of these cells, the factors regulating their activity, and their in vivo role in resistance against tumor growth.

During this fiscal year, two senior investigators have joined the section: Dr. Eliezer Gorelik, whose extensive background in studies with tumor metastasis and carcinogenesis brings considerable additional strength to the efforts to determine the in vivo relevance of natural cell-mediated immunity, and Dr. Bonnie Mathieson, who adds much expertise related to phenotypic characterization of the effector cells and to studies of the ontogeny and differentiation of functionally active cells.

A major advance in the characterization of NK cells has come from the finding of their close association with a subpopulation of lymphoid cells, termed large granular lymphocytes (LGL). Human LGL have been isolated with high purity and extensively characterized. Most active NK cells were shown to react with the monoclonal antibodies, OKT10 and OKM1, and a lower proportion reacted with OKT8 or anti-Ia. Most recently, several antibodies which react with the Fc receptor on LGLs and granulocytes have been shown to be valuable reagents for enumeration and separation of LGL. In contrast, HNK1 has been shown to recognize only a portion of the LGL and also reacted with some T cells.

In studies with rat LGL, the OX-8 monoclonal antibody has been shown to be a highly selective reagent for this population in nude rats. This has permitted a comprehensive survey of the histological localization of LGL. The tissues with the highest density and percentages of LGL included the paracortex of lymph nodes, bronchial-associated lymphoid tissue, the medullary cords and sinuses of lymph nodes, and intestinal epithelium. Large numbers of LGL have also been found in inflamed and/or infected tissues and in the subcutaneous and pulmonary sites of tumor growth.

Aged F344 rats have been found to have a high incidence of LGL tumors, and this has provided very large numbers of LGLs for extensive characterization. Transplantable LGL tumor lines have been established and shown to have high levels of cytotoxic reactivity against NK-susceptible target cells. Transplantation of these tumors in hybrid rats has clearly demonstrated that the cytotoxic cells from leukemic animals represent the transplanted tumor cells and not the activation of normal host LGL.

It has also become possible to obtain enriched populations of mouse LGL from the blood or spleen. These cells have been shown to express asialo GM₁, the alloantigen NK1.2, low levels of Thy 1 and Lyt 1, but no detectable Lyt 2. The LGL also appear to have low expression of Ly 5 and of Fc receptors recognized by a monoclonal antibody, 2.4G2. They have not reacted with a monoclonal antibody, anti-GM₁, against a myelocytic differentiation antigen. By further studies along these lines, it is anticipated that a detailed phenotypic characterization of mouse NK cells can be obtained, which should be highly useful for isolation of these cells and for assessment of their relationship to other populations of lymphoid cells.

Using the available separation procedures, we have analyzed the possible relationship of mouse NK cells to another natural effector cell, the natural cytotoxic (NC) cell. The LGL-enriched population has been shown to contain most of the NC activity, but some NC activity has also been detected in high density fractions depleted of LGL.

It has been possible to maintain and expand highly purified populations of LGL in vitro, by culturing these cells in the presence of IL-2. Cultures of human LGL grew rapidly for a period of over 1 month, and the cells underwent a major change in phenotype with the loss of most reactivity with OKT10, OKM1, or antibodies to Fc receptors and an appearance of reactivity with OKT3 and anti-Ia. It has been possible to clone LGL, but only about 25% of the clones demonstrated cytotoxic reactivity. Among the cytotoxic clones, a majority showed reactivity against all the NK-susceptible targets tested, but some clones had selective reactivity against only some of the targets. The clones were also found to be quite heterogeneous in their expression of various markers associated with LGL.

The ability of human LGL to grow in the presence of IL-2 has also permitted an examination of the peripheral and bone marrow precursors for NK cells. In contrast to the phenotype of most NK cells, the predominant peripheral progenitor was only positive for OKT11, and the bone marrow progenitors were mainly positive for OKT10 and B73.1. A similar approach is being utilized to study the possible presence of precursors for NK cells in the mouse thymus, and particularly a subpopulation of thymocytes that has a close similarity to the phenotype of LGL.

Studies have continued to determine the nature of the specificity of NK cells. In experiments with human LGL, it has been possible to begin a detailed biochemical analysis of the target structures recognized by NK cells by measuring the ability of solubilized materials to inhibit the binding of LGL to target cells. The target structures from the K562 cell line have been purified more than 200-fold and have been demonstrated to be high molecular weight glycoproteins. In another aspect of our studies of the specificity of human NK cells, primary solid tumor cells, including autologous tumor cells, have been shown to be susceptible to cytotoxicity by purified LGL. Similarly, in studies with rat NK cells, LGL have been shown to account for reactivity against a wide variety of target cells including normal fibroblasts and bone marrow cells, embryo cell lines, and solid tumor cells.

There have been some recent advances in our understanding of the mechanism of lysis by NK cells. Highly purified populations of human LGL have been shown to produce high levels of soluble cytotoxic factors, which have the ability to lyse NK-susceptible target cells. In addition, the granules from rat LGL leukemias have been isolated and shown to have high levels of cytotoxic reactivity against a variety of tumor target cells. These studies are expected to rapidly lead to new insights into the mechanism of killing by NK cells.

Interferon has been shown to have a variety of effects on immune reactivity, including the ability to rapidly augment the reactivity of NK cells. To obtain better insight into the nature of the diversity of the biological effects of interferon, various preparations of human natural, recombinant, and hybrid recombinant alpha interferon were tested for their ability to augment the reactivity of NK cells. Although most of the species tested significantly

augmented cytotoxic reactivity, appreciable quantitative differences in potency were observed among the various species. One recombinant species of interferon, IFN- α J which has antiviral and antiproliferative activity, was unable to boost NK activity and interfered with boosting by other IFN- α species. These studies are providing considerable insight into the portions of the interferon molecule associated with each biologic activity and offer the potential for constructing interferon molecules with highly selective biologic effects.

In addition to interferon, IL-2 potently augments human NK activity. Using highly purified LGL and pure IL-2, we observed augmentation equal to or greater than that seen with any interferon species. This boosting by IL-2 appears to be mediated at least in part by stimulation of endogenous production of IFN- γ by the LGL. The studies of the effects of IL-2 on NK cells have revealed that, in addition to this cytokine being a growth factor, it can augment cytotoxic reactivity in the absence of growth promotion.

Regulation of NK activity has also been extensively studied in mice. In vivo administration of various biological response modifiers, including interferon, has been shown to induce an expansion in the number of LGL in the spleen and to cause the LGL to become larger and of lower density than those found in normal, untreated mice.

New insights have also been obtained regarding the mechanisms involved in spontaneous or treatment-induced depression of NK activity. The immunoadjuvant, C. parvum, has been shown to induce potent suppressor cells for NK activity, which are predominantly nonadherent, high density cells completely separable from LGL. Normal mice of a low NK-reactive strain, SJL, have been shown to have potent suppressor cells. One subpopulation of suppressor cells has been shown to be nonadherent cells within the LGL population, which appear to have the ability to bind to target cells but are inefficient in lytic ability.

A continuing issue of central importance is the in vivo relevance of NK cells and other natural effector cells, particularly in regard to resistance against tumor growth. There are increasing indications that NK cells play a central role in resistance against metastatic spread of tumors. The rat has provided a very useful model system for investigating this issue in detail, since it is possible to depress NK activity in vivo and selectively reconstitute the animals with highly enriched populations of LGL. Such studies have indicated the ability of LGL to partially or fully restore cytotoxic reactivity against NK-susceptible target cells in vitro, the ability of treated rats to eliminate radiolabeled tumor cells from the lungs, and the ability to inhibit the development of pulmonary metastases from a mammary adenocarcinoma. These results provide the first direct evidence that NK cells are involved in in vivo resistance to tumors, particularly in the elimination of potentially metastatic tumor cells from the circulation and capillary beds.

Since adoptive transfer of purified NK cells appears promising, detailed studies have been initiated to understand the circulation of such cells following intravenous or intraperitoneal transfer. Following intravenous inoculation of radiolabeled LGL into normal recipients, a substantial proportion of the cells were found to localize in the lungs. With time, this proportion gradually decreased and the number of LGL in the spleen and liver increased. A small but significant percentage of LGL was recovered from small subcutaneous tumors,

thus demonstrating that these adoptively transferred cells are capable of entering the site of tumor growth. Following intraperitoneal inoculation into normal or tumor-bearing recipients, there was only a slow clearance of the effector cells from the peritoneal cavity. Conversely, it has been shown in both rats and mice that virtually no intravenously inoculated LGL migrate into the peritoneal cavity. The accumulating results from these studies are providing a strong basis for further exploring the usefulness of adoptive immunotherapy with fresh or cultured LGL.

NK cells have also been found to play an important role in the antimetastatic effects of an anticoagulant, heparin. Inoculation of heparin shortly before intravenous tumor challenge had a substantial antimetastatic effect. However, in mice with NK activity selectively suppressed by treatment with anti-asialo GM₁, the antimetastatic effect of heparin was completely abrogated. Conversely, heparin pretreatment of mice plus stimulation of NK activity by poly I:C had additive and profound inhibitory effects on metastasis formation.

IMMUNOPHARMACOLOGY SECTION

During this year, efforts have focused on an examination of the immunological and pharmacological mechanisms by which biological response modifiers modulate host reactivity, and on developing protocols for optimal and sustained modulation of host resistance in attempts to develop more effective therapy of cancer.

Several biological response modifiers were shown to augment NK activity in various tissue sites, with appreciable differences depending on route of administration. These BRMs were also shown to induce a variety of cytokines, in vivo or in vitro, including interferon, colony stimulating factor, and prostaglandin E₂. After multiple treatments with some BRMs, substantial hyporesponsiveness for augmentation of NK activity was noted, whereas no such hyporesponsiveness for activation of macrophages was seen.

Protocols have been developed which provide substantial preventive or therapeutic effects against lung metastases. Combined treatment of chemotherapeutic agents plus BRMs was substantially more effective than therapy by either type of agent alone. Similarly, treatment with BRMs immediately following surgical removal of large mouse mammary carcinomas resulted in a decreased number of lung metastases and a prolongation of survival time. The data indicate that BRM treatment may be particularly effective when utilized to augment effector cell activity for elimination of tumor cells remaining after reduction of tumor burden by chemotherapy or surgical therapy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09200-03 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phase I Trials of Recombinant and Nonrecombinant Interferons in Cancer Patients

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert K. Oldham, Associate Director, BRMP, NCI

COOPERATING UNITS (if any)

Hoffmann-La Roche, Inc., Nutley, NJ; Burroughs-Wellcome Co., Research Triangle Park, NC; NCI-FCRF

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Various recombinant and nonrecombinant interferons have been tested in Phase I trials in cancer patients in order to study the toxicity, antitumor effects, immunomodulatory effects and pharmacokinetics of these preparations. The initial Phase I trials employed highly purified recombinant leukocyte A interferon and human Namalva cell lymphoblastoid interferon. These trials, which are now complete, demonstrated that both interferons could be administered safely to patients with disseminated cancer and that the toxicities encountered resembled those previously reported for less purified Cantell alpha interferon. Antitumor responses were seen in 9 of 76 evaluable patients treated with recombinant interferon. These 9 patients included 5 patients with non-Hodgkin's lymphoma, and 1 patient each with chronic lymphocytic leukemia, Hodgkin's disease, breast cancer, and malignant melanoma. Three of 39 evaluable patients responded to lymphoblastoid interferon, including patients with undifferentiated carcinoma of the pelvis, nodular lymphoma, and hypernephroma. Pharmacokinetic analysis revealed dose-dependent levels of serum interferon activity. Immunologic monitoring data indicate unchanged or decreased natural killer cell mediated cytotoxicity, increased monocyte function in a growth inhibition assay and decreased lymphocyte blastogenesis. Other Phase I interferon trials recently initiated include trials of recombinant leukocyte A interferon as an intralésional agent and escalating dose trials of nonrecombinant gamma (immune) interferon and recombinant beta (fibroblast) interferon.

PROJECT DESCRIPTION

PERSONNEL

Paul G. Abrams	Expert	CIS	BTB	NCI
Gino C. Bottino	Medical Staff Fellow	CIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Jeffrey J. Ochs	Medical Staff Fellow	CIS	BTB	NCI
Mehmet Fer	Visiting Scientist	CIS	BTB	NCI
Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Akio Hizuta	Visiting Fellow	NIS	BTB	NCI

OBJECTIVES

Phase I trials of various recombinant and nonrecombinant interferons were conducted in order to determine the maximum tolerated dose and optimal biological response modifying dose for each preparation, which could be employed in future Phase II efficacy trials of these agents. Antitumor effects were noted, thereby giving some indication of the susceptibility of various malignancies to these agents. These trials were also designed to study the in vivo immunologic effects of the various interferons being administered and also allowed for extensive pharmacokinetic monitoring of the patients being treated.

METHODS EMPLOYED

In these Phase I trials, patients with a variety of histologically proven malignancies, refractory to standard curative therapy, were treated with escalating dosages of recombinant or nonrecombinant interferons. Patients were extensively monitored for clinical and laboratory signs of toxicity, in order to determine and objectively define the maximum tolerated dose for the interferon preparation. Antitumor effect was determined by serial measurements of indicator lesions on physical exam or on appropriate radiologic studies or serial measurements of tumor markers. Patients were also monitored extensively for immunologic effects in natural killer cell mediated cytotoxicity assays, monocyte growth inhibition assays, lymphocyte blastogenesis assays and enumeration of T-cell subpopulations by flow cytometry. Serum interferon activity was determined over a 24-hour period (0, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr, 24 hr) and periodically for steady-state determination (0, 1 hr, 2 hr, 4 hr, 8 hr) in all patients treated with the agents and was measured by a bioassay consisting of inhibition of plaque formation by vesicular stomatitis virus. Two Phase I trials of two different highly purified alpha interferons were conducted. A multiple-dose trial of highly purified recombinant leukocyte A interferon (specific activity 2×10^8 U/mg protein), supplied by Hoffmann-La Roche, Inc., was conducted in which patients received a fixed dose on a 3 times weekly or twice daily schedule for a period of 28 days. Dosages were escalated after groups of 5 or more patients were treated at a given dose. Dosages ranged from $1-136 \times 10^6$ units intramuscularly 3 times weekly. In the other Phase I trial of an alpha interferon preparation, individual patients received escalating doses of highly purified nonrecombinant Namalva cell interferon (supplied by Burroughs-Wellcome Co.) by intravenous infusion 5 days weekly over a 5-week period of time. The

dosages employed ranged from 0.1 to 50×10^6 units i.v. daily. Other Phase I trials initiated during this year include an escalating single-dose trial of nonrecombinant gamma interferon (Meloy Laboratories, Inc.), a multiple-dose trial of genetically engineered fibroblast interferon (Hoffmann-La Roche, Inc.), and an escalating-dose trial of recombinant leukocyte A interferon as an intralésional agent.

MAJOR FINDINGS

I. Clinical Results

The fixed multiple-dose Phase I trial of recombinant leukocyte A interferon in patients with disseminated cancer involved a total of 81 patients. Of these 81 patients, 54 completed the trial and 12 patients were removed from study for reasons of drug toxicity, 8 for reasons of tumor progression, and 7 for other unrelated medical problems. The toxicities observed with this preparation included fever, chills, fatigue, anorexia, myalgia, headache, nausea and vomiting, and dose-dependent reversible leukopenia and hepatic transaminase elevations. A maximum tolerated dose for this agent on a 3 times weekly was defined as 118×10^6 units, based on the occurrence of unacceptable hepatic transaminase elevations at higher doses. Objective evidence of antitumor effect qualifying as a standardly defined partial remission, was seen in 9 of the 76 evaluable patients. These 9 patients included 5 patients with non-Hodgkin's lymphoma and 1 patient each with chronic lymphocytic leukemia, Hodgkin's disease, breast cancer and malignant melanoma. Most of these patients had been heavily pretreated with chemotherapy and/or radiation therapy. Responding patients were continued on a maintenance therapy regimen of recombinant leukocyte A interferon. Maintained partial remission lasted up to 7 months. The patients on this trial received full 24-hour pharmacokinetic profiles on days 1, 15 and 27, as well as frequent monitoring of serum interferon activity at other times, in order to determine if steady-state accumulation occurred. Dose-dependent pharmacokinetics were observed on this trial, with increased peak levels of serum interferon activity at increasing doses. The half-life of serum interferon was determined to be between 6 and 9 hours. Evidence for steady-state accumulation of interferon was apparent at doses in excess of 50×10^6 units i.m. 3 times weekly.

In the single escalating dose trial of human Namalva cell lymphoblastoid interferon, patients were treated by either intravenous infusion or intramuscular injection over a 5-week period, during which the dose increased on a weekly basis from 0.1 to 1.0, 10.0, 30.0 and 50.0×10^6 units i.v. daily for 5 days. This interferon is a highly purified nonrecombinant preparation, which is a mixture of eight or more molecular species of alpha interferon including that of the recombinant leukocyte A interferon discussed above. The specific activity of this preparation is $1-2 \times 10^8$ units/mg protein. Twenty-nine patients were treated by the intravenous route and 10 patients by the intramuscular route. The toxicities encountered were similar to those mentioned above for recombinant leukocyte interferon and previously reported for other, less purified interferon preparations. The maximum tolerated dose appeared to be 30×10^6 units i.v. daily x 5, based on unacceptable leukopenia and hepatic transaminase elevation.

Objective evidence of antitumor effect qualifying as a partial remission was observed in 3 patients and these lasted 2-4 months. The diagnoses of the responding patients were: undifferentiated carcinoma of the pelvis, nodular poorly differentiated lymphocytic lymphoma, and hypernephroma. Patients were extensively monitored for serum interferon activity on this trial with full 24-hour pharmacokinetics being determined on day 1 of each treatment cycle for each patient and subsequent monitoring for steady-state activity. High levels of serum interferon activity were seen with this particular preparation and evidence of steady-state accumulation was noted in doses in excess of 10×10^6 units i.v. daily for at least 24-36 hours.

Thirty-two patients have been treated with a nonrecombinant gamma interferon. Each patient has been treated intravenously twice weekly in a dose escalation fashion at dosages of 10×10^6 U/m², 20×10^6 U/m², 30×10^6 U/m², 40×10^6 U/m², 50×10^6 U/m², and 60×10^6 U/m². Side effects have included fever, fatigue, and hypotension. The MTD appears to be related to hypotension at $50-60 \times 10^6$ U/m². We have so far not witnessed clinical responses in any of the treated patients, including patients with breast cancer, melanoma, and lung cancer.

II. Immunological Monitoring

All patients entered onto the Phase I trials of recombinant leukocyte A interferon and highly purified nonrecombinant lymphoblastoid interferon were monitored extensively for immune function, both prior to starting therapy and at various times following the initiation of therapy. The studies were designed to determine not only a maximum tolerated dose but also an optimum biological response modifying dose. A number of immunological assays were performed including assays for natural killer cell activity, monocyte function as measured in a monocyte-mediated growth inhibition assay, and T lymphocyte function as measured by the proliferative response of lymphocytes to mitogens and mixed leukocyte culture. In addition to these functional assays, determinations of lymphoid subpopulations were made using monoclonal antibodies and flow microfluorometry. To be certain that any changes observed after interferon administration were due to the interferon and not to other non-treatment related fluctuations in function, all assays were standardized and day-to-day variability in activity was assessed for each individual by repeated testing over several days prior to the start of therapy to determine baseline values. This allowed analysis in terms of significant alteration from the pretreatment range for each function being measured. An unexpected finding of this study was that in vivo administration of both types of alpha interferon did not lead to an augmented natural killer cell response but instead to no significant change in the majority and to a diminished response in about 30% of the patients. The number of patients who showed this diminished NK response was greater on the twice daily protocol than on the three times weekly protocol. This was not due solely to the administration of more interferon, as when the data were recalculated using a total weekly dose of interferon, the depression that was seen was still greater on the twice daily protocol. However, the depression was greater at the higher doses of interferon, with almost all patients receiving greater than 80 million units per injection showing a diminished response. At the lower doses, although there was no augmentation of NK activity, the depression was not as great as at the higher doses. In contrast to the results

with the NK assay, monocyte-mediated cytostasis was found to increase significantly in over 80% of the patients. Patients showed an increase when treated on any of the three protocols, and this increase was seen at all dose levels, from 1 million units to 136 million units. The lymphocyte proliferation responses to mitogens and in mixed lymphocyte culture were found to decrease in over 70% of the patients. This decrease was consistent at all dose levels and with all schedules of administration.

Analysis of leukocyte subpopulations, using monoclonal antibodies and flow cytometry, revealed no change in the percentage of cells reacting with OKT3 (total T lymphocytes) or any shift in the ratio of OKT4 (including helper) to OKT8 (including suppressor) lymphocytes following interferon administration. The percentage of cells reacting with OKT10 was increased soon after starting interferon and remained elevated for the duration of treatment. This antibody is known to react with NK cells and the increase observed may be an indication that the interferon was having an effect on NK cells despite the depression in function which was observed. The reasons for this unexpected depression of NK activity have been further investigated. We had some evidence suggesting a role for circulating inhibitory factors which interfered with the NK boosting effects of interferon. Examination of sera from a larger number of patients revealed that this was not a consistent finding and suggested that this was not the only mechanism involved in the depression of NK activity. A detailed study of the various stages in the NK cytotoxic process was carried out. The number of NK cells binding to the target cells was similar in the patients both before and during interferon administration and in normal donors. When tested in a single cell cytotoxicity assay which measures the cytotoxic ability of the purified NK cells which bind to the target cells, the patients showed a significantly depressed response during treatment when compared to their own pretreatment values or those of normal donors. Prior to starting treatment with interferon, the patients' NK cells were capable of augmentation by both alpha and beta interferon to the same extent as normal donors. After starting interferon treatment, however, the ability to be augmented by alpha interferon was diminished while the ability to be boosted by beta interferon was retained. After several weeks of interferon treatment, the ability to be boosted by beta interferon was also diminished. Further studies in mice are being undertaken to evaluate this depressed response by using a human recombinant interferon with known activity on murine cells and in vitro studies using human cells.

SIGNIFICANCE

These Phase I trials of various recombinant and nonrecombinant interferons in cancer patients will allow the determination of maximum tolerated doses that can be used in Phase II efficacy trials in specific types of cancer. These Phase II efficacy trials will in turn help to define the ultimate therapeutic role for these interferon preparations in a given malignancy. Moreover, the Phase I trials should serve as a model for the future early clinical trials of a variety of other biological response modifiers, genetically engineered and otherwise. In these Phase I studies, we have determined the maximum tolerated dose and have demonstrated some degree of antitumor activity. We have used this information to design Phase II studies for lymphoma and breast cancer.

PROPOSED COURSE

Phase I trials of the various recombinant and nonrecombinant α interferons have been completed. The information generated in these trials, including the definition of maximum tolerated dose, has been used to design Phase II trials to be performed in larger clinical research facilities and cooperative group settings, as well as in our own unit. Phase I trials with recombinant and nonrecombinant beta (fibroblast) and gamma (immune) interferons in cancer patients are ongoing. If we demonstrate antitumor activity, the data generated from these trials will be used to design Phase II trials.

PUBLICATIONS

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Maluish, A. E., Conlon, J., Ortaldo, J. R., Sherwin, S. A., Leavitt, R., Fein, S., Weirnik, P., Oldham, R. K. and Herberman, R. B.: Modulation of NK and monocyte activity in advanced cancer patients receiving interferon. In Merigan, T. C. and Friedman, R. M. (Eds.): UCLA Symposium on Molecular and Cellular Biology. New York, Academic Press, 1982, vol. XXV, pp. 377-386.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09233-02 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trials Recomb. Leukocyte IF in Pts. w/Breast C. & Lymphoproliferative Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert K. Oldham, Associate Director, BRMP, NCI

COOPERATING UNITS (if any)

Hoffmann-La Roche, Inc., Nutley, NJ; NCI-FCRF; NCI-Navy MOB, NCI;
POB, NCI; MB, NCI

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year a Phase I trial of recombinant leukocyte interferon in patients with a variety of disseminated cancers revealed that this agent could be administered up to doses of 50×10^6 units/m² i.m. 3 times weekly without unacceptable toxicity. This trial also showed objective evidence of antitumor response (partial remissions) in some patients with non-Hodgkin's lymphoma, breast cancer, chronic lymphocytic leukemia and Hodgkin's disease. Immunologic monitoring of patients receiving this agent failed to reveal any dose-dependent immunologic effect which correlated with tumor response. It was therefore decided to initiate a Phase II efficacy trial of recombinant leukocyte interferon at a maximum tolerated dose, with dose reductions as necessary for unacceptable toxicity. Phase II efficacy trials were initiated in patients with various lymphoproliferative disorders, including non-Hodgkin's lymphoma, chronic lymphocytic leukemia and mycosis fungoides, as well as patients with refractory metastatic breast cancer. Eighteen patients with metastatic breast cancer have been treated with recombinant leukocyte A interferon, with 16 patients progressing while on therapy and 2 remaining stable. Forty-six patients with a variety of lymphomas have been treated, with a greater than 50% response rate in favorable histology lymphoma and mycosis fungoides.

PROJECT DESCRIPTION

PERSONNEL

Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Paul G. Abrams	Expert	CIS	BTB	NCI
Mehmet Fer	Visiting Scientist	CIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI

OBJECTIVES

Recombinant leukocyte interferon has been previously tested by the Clinical Investigations Section in multiple-dose Phase I trials. These trials have demonstrated that the maximum tolerated dose of this agent on a 3 times weekly schedule of administration is 50×10^6 units/m². At higher doses, unacceptable myelotoxicity and hepatic toxicity were encountered. No evidence is available at this time with regard to lower doses that might have an optimal biological response modifying effect. In the same Phase I trials, objective evidence of antitumor effect (partial remissions) was seen in several patients with non-Hodgkin's lymphoma as well as occasional patients with breast cancer, melanoma, chronic lymphocytic leukemia and Hodgkin's disease. It was therefore decided to initiate Phase II efficacy trials in lymphoproliferative disorders and refractory metastatic breast cancer, using the maximum tolerated dose of 50×10^6 units/m² i.m. 3 times weekly. These trials were intended to provide more precise information as to the antitumor efficacy of recombinant leukocyte interferon in these specific malignancies as well as to provide further information and insights into the immunologic effects of this agent and its longer term toxicities.

METHODS EMPLOYED

Two trials have been initiated. One involves patients with various lymphoproliferative disorders, including previously treated patients with non-Hodgkin's lymphoma (both favorable and unfavorable histologies), chronic lymphocytic leukemia, American Burkitt's lymphoma, and mycosis fungoides. The other trial concerns patients with refractory metastatic breast cancer who have failed standard therapy with hormonal therapy (if ER positive) and combination chemotherapy. In both of these trials, patients receive 50×10^6 units/m² i.m. 3 times weekly for a period of 3 months. Patients are monitored carefully for hematologic and hepatic toxicity as well as systemic toxicities known to be associated with interferon. These systemic toxicities include primarily fatigue and anorexia. Patients have dose reductions to 50% and then 10% of their initial dose if unacceptable toxicity in any of these categories occurs. Patients are monitored carefully for antitumor effect by periodic physical examinations and appropriate radiologic studies. Immunologic monitoring is being performed on these patients, with periodic assays for natural killer activity, monocyte function as measured in a growth inhibition assay, lymphocyte

blastogenesis assays, and enumeration of lymphoid cell subpopulations by fluorescence flow cytometry. Patients are also being monitored monthly for serum interferon levels following injections, in order to determine whether a drop in peak serum interferon activity might be predictive of interferon antibody formation.

MAJOR FINDINGS

Eighteen patients with metastatic breast cancer refractory to chemotherapy have been treated with recombinant leukocyte A interferon. Sixteen of these 18 patients demonstrated progression of their disease during the course of this therapy and two were stable. It was decided at this point to conclude the study. Twenty-three patients with favorable histology lymphoma have so far been treated on this protocol and 60% have demonstrated good partial responses. Nine patients with cutaneous T-cell lymphoma have been treated with 6 excellent partial responses. Seven patients with chronic lymphocytic leukemia have been treated with 3 partial responses. Only 2 of 7 patients with unfavorable histology lymphoma have demonstrated partial responses to the leukocyte A interferon. The results of immunologic monitoring are currently being analyzed.

SIGNIFICANCE

These two trials have demonstrated that metastatic breast cancer does not respond to recombinant α interferon, while >50% of patients with refractory non-Hodgkin's lymphoma and mycosis fungoides had excellent partial responses.

The dose chosen for these trials was the maximum tolerated dose, as previously determined in a Phase I trial, with appropriate dose reduction for unacceptable toxicity. Future Phase II efficacy trials or Phase III trials might employ doses below the maximum tolerated dose when a sufficient data base exists for determining which lower dose or schedule of administration might be optimal for a biological response modifying effect. In addition to further defining the antitumor efficacy of this type of alpha interferon, these trials will further define the toxicities and immunologic effects of this interferon as well as its propensity to induce antibody formation in the recipients. The two trials described here are among the first Phase II efficacy trials of a genetically engineered biological response modifier in patients with cancer.

PROPOSED COURSE

While we do not see a role for recombinant leukocyte A interferon for breast cancer, it appears that Phase III trials for favorable histology lymphoma and mycosis fungoides are warranted. Such trials could compare the relative efficacy of interferon alone or in combination with other agents to standard therapy for the diseases in question. In addition, it might be worthwhile to examine the role of interferon in the adjuvant setting. At this point in the natural history of a malignancy, when tumor burden is minimal and immunologic competence

may be at a relatively high level, interferon as well as other biological response modifiers may well prove to be most effective.

PUBLICATIONS

Sherwin, S. A., Mayer, D., Ochs, J. J., Abrams, P. G., Knost, J. A., Foon, K. A., Fein, S. and Oldham, R. K. Recombinant leukocyte A interferon in advanced breast cancer: Results of a phase II efficacy trial. Ann. Intern. Med. 98: 598-602, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09234-02 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phase I Trial with 13 Cis-Retinoic Acid

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Stephen A. Sherwin, Expert, NCI

COOPERATING UNITS (if any)

NCI-FCRF; Hoffman-La Roche Inc.

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

13 cis-retinoid acid (13-CRA), a compound previously demonstrated to have antitumor activity in limited clinical trials, is known to have a variety of effects on the immune system. The compound was investigated in a Phase I clinical trial at three dose levels to determine whether there were any biological response modifying effects when administered to cancer patients. No unexpected clinical toxicities were observed and there were no changes in immune function observed at any dose level despite intensive monitoring of natural killer cell activity, monocyte function, lymphoproliferative responses, and leukocyte populations. There were no tumor responses observed during the time of treatment with 13-CRA.

PROJECT DESCRIPTION

PERSONNEL

Gino C. Bottino	Medical Staff Fellow	CIS	BTB	NCI
Paul G. Abrams	Expert	CIS	BTB	NCI
Mehmet Fer	Visiting Scientist	CIS	BTB	NCI

OBJECTIVES

13 cis retinoic acid has been shown in limited studies to have some antitumor effects in man and to have a variety of effects on the immune system. This study was designed to evaluate the effects of this compound as a biological response modifier in cancer patients by intensive immunological monitoring.

MAJOR FINDINGS

13 cis-retinoic acid (13-CRA) is a stereoisomer of retinoic acid which has been shown in preliminary clinical trials to have antitumor activity in patients with cutaneous neoplasms, epidermoid malignancies of the head and neck, and occasional patients with melanoma and colorectal carcinoma. In addition to direct effects on tumor cells, 13-CRA and other retinoid compounds have been shown to have a variety of effects on the immune system, thus raising the possibility that these substances may have indirect antitumor effects by acting as biological response modifiers. In order to more fully explore the effect of 13-CRA on the immune system and to further test its potential as an antineoplastic agent, we initiated a limited phase I trial of 13-CRA as a biological response modifier. Fifteen patients received 13-CRA at one of three different dose levels and were extensively monitored for in vivo immunologic effects as well as evidence for antitumor activity. All patients had disseminated cancer refractory to standard therapy, were ambulatory, and had normal hematologic, renal and hepatic function. 13-CRA was obtained from Hoffmann-La Roche Inc. (Nutley, N.J.). Patients were assigned sequentially to one of three doses (5, 50, or 100 mg/m²), which they received daily by mouth for up to six weeks. All patients were monitored clinically with weekly examinations, and tumor measurements were performed at the end of six weeks. Patients were monitored immunologically at 24 hours, 48 hours, 72 hours after the start of therapy and thereafter on a weekly basis throughout the study. The following immunologic assays were performed: natural killer activity in a standard 4-hour cytotoxicity assay against K562 target cells was determined using fresh Ficoll-Hypaque separated cells. Monocyte function was measured in a cytostatic assay which measures the ability of monocytes in the preparation to inhibit the growth of an NK-resistant tumor target cell line. Lymphoproliferative responses to the mitogen Concanavalin A and mixed allogeneic leukocytes were also performed. Both the monocyte function and lymphoproliferative assays were performed on cryopreserved cells, to reduce assay variation in these assays. Determinations of changes in leukocyte populations and lymphocyte subpopulations were made using a panel of monoclonal antibodies directed against cell surface antigens

and detection using flow cytometry. All patients completed at least 3 weeks of therapy, with 6 of the 15 patients discontinuing therapy between weeks 3 and 6 for tumor progression; the remainder completed the proposed 6 weeks. The toxicities seen in the patients were similar to those previously reported for 13-CRA. No serious or life-threatening toxicities were observed and no patients discontinued treatment due to toxicity. The most frequent toxicities seen were mucocutaneous including dry mouth (12/15), dry skin (7/15), pruritus (4/15), and rash (3/15). Some patients complained of fatigue (7/15) and anorexia (4/15). No hematologic, hepatic, or other major abnormalities were seen in the patients treated on this trial. All patients had measurable tumor and were evaluated for evidence of antitumor responses. Of the 15 patients, 6 discontinued therapy between 3 and 6 weeks due to progressive disease. Of the remaining 9 who completed the course of therapy, 8 also had progressive disease. One patient was felt to have disease stabilization and a subjective improvement in his symptoms and was continued on therapy. However, after 18 weeks of treatment, he had clear progressive disease and therapy was discontinued. For the immunologic testing, two to three baseline values were obtained for each function measured prior to starting therapy and from these values the limits of variability for each individual were determined. Only changes outside these limits of variability were considered to represent an increase or decrease which was related to the 13-CRA. No consistent changes were seen in any of the functions measured at any time point after the initiation of treatment. The baseline natural killer cell activity, monocyte function, and percentage of cells reacting with the various monoclonal antibodies were within the normal range found in our laboratory. The lymphocyte proliferation responses to mitogen and mixed leukocyte culture were depressed from the normal range but were within the range usually observed for cancer patients.

SIGNIFICANCE

These results indicated that 13-CRA does not have a major effect on the immune system of cancer patients previously treated with chemotherapy and radiotherapy and that previous reports of limited effects on tumor cells may have been due to some direct effects on the tumor cells, perhaps by means of tumor cell differentiation. Whether the lack of effect of immune function seen in this study is applicable to untreated patients with pre-malignant or early stage disease, or to patients without cancer receiving 13-CRA for various dermatologic disorders, remains to be determined.

PROPOSED COURSE

This was a Phase I study which has been completed. There are no plans to continue work with this compound.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09235-02 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phase I Trials of Antitumor Monoclonal Antibodies in Patients with Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert K. Oldham, Associate Director, BRMP, NCI

COOPERATING UNITS (if any)

NCI-FCRF; NCI-Navy MOB, NCI; MB, NCI

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

5.0

PROFESSIONAL:

2.5

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monoclonal antibodies reactive with various human tumor cells have been prepared from murine hybridomas according to standard techniques. Phase I trials of antitumor monoclonal antibodies initiated by the Clinical Investigations Section include studies of anti-T cell monoclonal antibodies in patients with chronic lymphocytic leukemia (CLL) and cutaneous T cell lymphoma (CTCL), anti-melanoma monoclonal antibody in patients with disseminated melanoma, and anti-idiotypic monoclonal antibody in patients with malignant lymphoma and CLL. We have treated 12 patients with CLL and 8 patients with CTCL with the T101 monoclonal antibody. We have witnessed transient reductions in circulating leukemic cells but have not seen reductions in the size of enlarged organs or lymph nodes. Five of 8 patients with CTCL had minimal improvement in their skin lesions. Toxicity has included mild fever and minimal shortness of breath. Eight patients with metastatic melanoma have been treated with an antibody to a 250,000 m.w. melanoma associated antigen. While we have seen no reductions in the size of metastatic lesions, we have seen in vivo localization of antibody in cutaneous lesions. We have successfully developed anti-idiotypic antibodies for 2 patients with B-cell lymphomas and one with CLL and expect to treat these patients in the next 3-6 months.

PROJECT SUMMARY

PERSONNEL

Paul G. Abrams	Expert	CIS	BTB	NCI
Jeffrey J. Ochs	Medical Staff Fellow	CIS	BTB	NCI
Gino C. Bottino	Medical Staff Fellow	CIS	BTB	NCI
A. Charles Morgan	Expert	MAS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Mehmet Fer	Visiting Scientist	CIS	BTB	NCI

OBJECTIVES

Antitumor monoclonal antibodies with reactivity against human tumor cells may have significant potential as anticancer agents in man. These antibodies may be administered alone or as vehicles for toxins, chemotherapy agents, and radioisotopes. The Clinical Investigations Section is strongly committed to the early clinical testing of antitumor monoclonal antibodies. Phase I trials initiated during this year include studies of an anti-T cell monoclonal antibody (T101) in patients with chronic lymphocytic leukemia and cutaneous T cell lymphoma, an anti-melanoma antibody (9.2.27) in patients with disseminated malignant melanoma, and anti-idiotypic monoclonal antibodies in patients with malignant lymphoma. In these trials patients will be carefully monitored for antitumor effect and toxicity and in addition will be monitored for specific and nonspecific effects on the immune system.

METHODS EMPLOYED

We have initiated a Phase I trial with the T101 monoclonal antibody which is an IgG_{2a} antibody and is supplied by Hybritech Inc. This monoclonal antibody reacts with normal T lymphocytes and malignant T and B lymphocytes and immunoprecipitates a 65,000 molecular weight glycoprotein. Patients treated include chronic lymphocytic leukemia and cutaneous T-cell lymphoma patients. Groups of six patients have been treated at a fixed dosage on a twice weekly schedule over a period of 28 days. Patients were carefully monitored for antitumor effect and toxicity. Patients were also monitored for the presence of free antibody and for the formation of anti-murine immunoglobulin. Antigenic modulation was followed by fluorescent flow cytometry analysis of anti-T cell monoclonal antibody-positive cells. Patients were tested for reactivity with the antibody prior to its administration as well as during therapy. Immunologic monitoring of various other immunologic parameters was performed as well, including assays for natural killer cell activity, monocyte function as measured in a growth inhibition assay, and lymphocyte blastogenesis.

In another Phase I trial we used the 9.2.27 anti-melanoma monoclonal antibody (IgG_{2a}), which reacts with a 250,000 dalton glycoprotein primarily found on melanoma cells. Individual patients received escalating doses of this antibody

and were monitored for antitumor effect, toxicity, and immunomodulatory effect in a fashion similar to that for the anti-T cell monoclonal antibody trial. In addition patients were subjected to biopsy before therapy to demonstrate reactivity of their tumor cells with the 9.2.27 antibody, tested by immunofluorescence and immunoperoxidase techniques, and repeat biopsies during the course of therapy to identify in vivo labeling of tumor cells.

The anti-idiotypic monoclonal antibody trial for patients with malignant lymphoma will involve the administration of anti-idiotypic antibodies prepared against immunoglobulins specifically being secreted by the patient's malignant cells. This will therefore involve the preparation of specific hybridomas against the tumor idiotype of each patient treated. The first step in developing an anti-idiotypic antibody is to fuse the patient's lymphoma cells with a murine myeloma cell line. The subsequent "heterohybridoma" secretes the lymphoma immunoglobulin. This immunoglobulin is then concentrated and purified and used to immunize BALB/c mice. Standard murine hybridomas are then prepared and anti-idiotypic monoclonal antibodies are generated.

MAJOR FINDINGS

Twenty patients have been treated with the T101 monoclonal antibody. This includes 12 patients with chronic lymphocytic leukemia: 3 patients treated with 1 mg, 3 patients treated with 10 mg, 2 patients treated with 50 mg of antibody over 2 hours, 2 patients treated with 50 mg of antibody over 50 hours, and 2 patients treated with 100 mg of antibody over 50 hours. We have demonstrated transient reductions of the circulating leukemia cell count in all of the patients treated and two of the patients treated with 10 mg have demonstrated a sustained 50% reduction in the chronic lymphocytic leukemia cell counts during the course of therapy. There have been no demonstrable changes in enlarged lymph nodes or other organs. Toxicity has included fever, urticaria, and shortness of breath and chest tightness for patients treated with 50 mg of antibody over 2 hours. This toxicity was completely eliminated when infusions were prolonged to 25-50 hours. We have identified localization of T101 antibody on bone marrow cells as well as on circulating leukemia cells. None of the patients with chronic lymphocytic leukemia demonstrated anti-mouse antibody responses.

Eight patients with cutaneous T-cell lymphoma have been treated with T101 antibody. Two patients have been treated with 1 mg of antibody, 2 patients with 50 mg of antibody over 2 hours, 2 patients with 50 mg over 50 hours, and 2 patients with 100 mg of antibody over 50 hours. Two patients at 10 mg, 2 patients at 50 mg and 1 patient at 100 mg have demonstrated minor improvement in their cutaneous skin lesions. These have been less than partial responses, however. One patient at 10 mg demonstrated a disappearance of an enlarged peripheral lymph node. There were no detectable responses in involved organs. Toxicity included fever, urticaria, and pulmonary toxicity at 50 mg over 2 hours. Pulmonary toxicity was eliminated when infusions were prolonged to 25-50 hours. In vivo localization of T101 antibody in cutaneous lesions was demonstrated in all patients studied at 50 mg or greater and in one patient at 10 mg.

Eight patients have been treated with the 9.2.27 anti-melanoma monoclonal antibody directed to the 250,000 molecular weight melanoma-associated antigen. Patients were all treated with 1, 10, 50, 100, and 200 mg of antibody, twice weekly with dosages given intravenously over 2 hours. None of these patients had any detectable beneficial clinical responses; however, all the patients demonstrated *in vivo* localization of antibody in skin lesions at 50-mg dosages or greater. Toxicity included fever, urticaria, and in one patient serum sickness. While human anti-mouse antibodies were demonstrated in a number of patients, it did not appear to interfere with *in vivo* antibody localization on cutaneous tumors. We have thus far prepared 3 anti-idiotypic antibodies and expect to treat these patients within 3-6 months.

SIGNIFICANCE

The early clinical testing of monoclonal antibodies with antitumor reactivity is of key importance for understanding the potential toxicity, immunomodulatory effects, and antitumor activity of these agents. Monoclonal antibodies may well be a major new therapeutic modality for patients with disseminated malignancy. We have demonstrated that the unconjugated T101 antibody has only minor antitumor effects. However, the T101 antibody causes significant antigenic modulation suggesting that conjugates of this antibody with a toxin or drug may be internalized into the tumor cell, which might enhance cell death. With the 9.2.27 anti-melanoma antibody we have not witnessed responses or seen any modulation of surface antigens following therapy. However, we have identified excellent *in vivo* localization of the antibody on cutaneous tumor cells. This antibody might have its greatest therapeutic impact when used to localize a radionuclide in the tumor, which would not depend on internalization for the tumoricidal effect.

PROPOSED COURSE

These Phase I trials will be followed by further tests of the same antibodies coupled to various chemotherapy agents, radionuclides, and toxic agents. We would hope to be able to determine whether monoclonal antibodies might be effective vehicles for the delivery of toxic agents directly to tumor cells. In addition, we hope to be able to determine whether monoclonal antibodies coupled to radionuclides could be used diagnostically. Finally, we hope to treat patients with a variety of B cell lymphomas with anti-idiotypic monoclonal antibodies.

PUBLICATIONS

Foon, K. A., Bunn, P. A., Schroff, R. W., et al. Monoclonal antibody therapy of chronic lymphocytic leukemia and cutaneous T cell lymphoma: Preliminary observations. In Langman, R. E., Trowbridge, I. S. and Dulbecco R (Eds.): Monoclonal Antibodies in Cancer. New York, Academic Press, 1983. (In Press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1CM09248-02 BTB

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Human Monocytes in the Immune Response to Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Henry C. Stevenson, Medical Officer, BTB, NCI

COOPERATING UNITS (if any)

Ingene Corporation, Santa Monica, CA; Developmental Genetics Laboratory;
NCI-FCRF

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

5.5

PROFESSIONAL:

4.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human monocytes are normal cells with numerous immunologic functions which have potential roles for preventing new tumors or eradicating established malignancies. Studies have been performed which have allowed us to establish a group of highly immunologically characterized normal human donors, to be used for a longitudinal analysis of the function of normal human monocytes and other components of the immune response. Investigations have particularly focused on the potential antitumor functions of normal human monocytes. We have been able to isolate large numbers of sterile human monocytes by elutriation and subsequently cryopreserve them in suspension, retaining function in the MIF, tumor growth inhibition, and several other assays of monocyte function. We developed methodology for separating human monocytes into subsets by size and density and have detected a monocyte subset with enhanced cytotoxic function and another subset with enhanced interferon production capabilities. We have performed several in-depth analyses into the genetic basis for human monocyte function using state-of-the-art genetic engineering methodologies and have documented elevation of monocyte total and messenger RNA following the activation to fibroblast growth factor (FGF) synthesis, but not interferon (IFN) synthesis.

PROJECT DESCRIPTION

PERSONNEL

Paul J. Miller	Biologist	CIS	BTB	NCI
Joann A. Beman	Research Nurse Expert	CIS	BTB	NCI
Yukio Akiyama	I.P.A.	CIS	BTB	NCI

OBJECTIVES

The general objectives of this project are (a) to establish a pool of normal human donors to be used for in-depth research studies of their immune response; (b) to establish a leukapheresis facility in which peripheral blood, leukapheresis specimens, and bone marrow specimens can be obtained for subsequent in-depth research; (c) to establish procedures for separation of large amounts of sterile human leukocytes into various subpopulations (platelets, granulocytes, red cells, T cells, B cells, NK cells and monocytes) for subsequent in-depth study; (d) to establish a cryopreservation bank of the aforementioned cells; (e) to establish a computerized monitoring system for longitudinal analysis of the immune response of normal human donors; (f) to develop procedures for the preservation and proliferation of human monocytes in vitro; (g) to characterize functional subsets of human mononuclear phagocytes; (h) to develop monoclonal antibodies against human monocyte subsets; (i) to isolate gene segments from activated human monocytes that code for the tumoricidal function of monocytes and/or the ability of monocytes to release cytokines; (j) to develop hybridomas between monocyte tumors and normal monocytes, that secrete large amounts of cytokines.

METHODS EMPLOYED

Human volunteers undergo extensive testing to ensure their normality, which includes detailed immunologic studies and histocompatibility typing. Leukocytes are removed from normal donors by leukapheresis. Human leukocytes are subfractionated into granulocytes, platelets, monocytes, T cells, and NK cells; monocytes are subfractionated by albumin gradients. Functional assays of human monocytes include release of monokines such as interferon, colony stimulating factor and interleukin-1; the ability of monocytes to migrate randomly in vitro is measured as is the inhibition of this function by monocyte migratory inhibitory factors; the in vitro phagocytic capabilities of monocytes for latex beads and IgG-coated particles is measured; the ability of monocytes to perform accessory cell functions in mitogen- and antigen-stimulated T cells and mitogen- and antigen-stimulated B lymphocytes is determined; purity and character of human monocyte preparations is assessed by staining for esterase, peroxidase, acid phosphatase, and lysozyme. Monocytes are sized by a computer-monitored automated cell sizer. Specific antigenic determinants on monocyte membranes are detected by assessing the amount of Dr antigens on their surfaces, by analyzing the amount of monocyte-specific antigens on their surfaces, and by examining the ability of monocyte preparations to bind lectins such as pokeweed mitogen which preferentially bind to these cells.

Monocytes are fused to HAT-sensitive human monocyte tumors to produce monocyte hybridomas, using standard hybridoma technology. Gene segments that code for products of activated monocytes are obtained by isolating the messenger RNA produced by activated human monocytes, producing cDNA copies of these messages, incorporating this cDNA into highly specialized plasmid vectors, and transfecting these plasmids into bacterial cultures and then (following the appropriate amount of expansion) transfecting these messages into eukaryotic cells. The eukaryotic cells thus transfected are analyzed for their ability to secrete well known monocyte-derived biologic response modifiers. Cryopreservation technology has been applied to the long-term storage of large numbers of purified human lymphocytes and monocytes. These cells are frozen in a sterile fashion with autologous serum for subsequent use in laboratory assays. All research laboratory data are coordinated into a central computer bank under the name of the normal donor from whom the cells were obtained.

MAJOR FINDINGS

I. Longitudinal Analysis of the Function of Normal Human Monocytes and Other Cellular Components of the Immune Response

The technology for running a cancer immunology research cytopheresis unit is well in place. Large numbers (0.5 to 8.0×10^9 cells) of purified leukocyte subsets are removed from normal donors on a daily basis. These sterile purified leukocyte subpopulations are then cryopreserved for subsequent laboratory examinations. The results of all experimental laboratory experiments (such as macrophage cytotoxicity, NK cell function, B-cell function, interferon production, MIF responses, chemotactic responses, T-cell subpopulations and others) are placed on computer file indexed by the name of the donor. We are in the process of making correlations between the laboratory examinations performed by widely disparate laboratories using a single donor's white cells at a single point in time. Moreover we are analyzing the immunologic profile of each normal donor in a longitudinal fashion.

II. Characterization of Functional Subsets of Human Mononuclear Phagocytes

We are currently investigating whether all human monocytes have the same functional properties or whether certain monocytes have distinct functional capabilities vis-a-vis the others. We are particularly interested in isolating monocyte subpopulations with markedly enhanced abilities to produce monokines of interest to our program. To date, we have isolated human monocyte subsets on the basis of size and density. Two monocyte subsets (RM and IM) have been obtained thus far. RM has enhanced cytotoxic potential; IM has enhanced potential for the release of most BRMs examined thus far, including interferon. We have shown that IM constitute the most mobilizable pool of subset cells in the monocyte reservoirs of man. The ultimate objective of these studies is to assess cancer patients for any defects in monocyte subset function that they might have, and to develop technologies whereby any defective monocyte subsets in cancer patients could be reconstituted.

III. Genetic Engineering of Human Monocytes: Establishment of an Activated Monocyte cDNA Library and Characterization of Monocyte Hybridomas

We are investigating the macromolecular synthetic mechanisms which lead to altered functional activity in human monocytes following activation. We have demonstrated that total RNA synthesis is transiently elevated in human monocytes early after activation to fibroblast growth factor (FGF) secretion, as opposed to unactivated human monocytes or monocytes activated to interferon (IFN) secretion. We are focusing particularly on message-specific RNA messages for interferon (IFN) which are produced in activated monocytes. We have isolated the messenger RNA from these activated cells, converted this message into cDNA form, incorporated this cDNA into plasmids, and have established a gene library in bacteria. Following amplification of these messages in bacteria, we have transfected this cDNA into eukaryotic cell systems and are screening these eukaryotic cells for distinct monocyte functions such as BRM release. We are particularly interested in cloning genes for certain potent BRMs (monokines) such as colony stimulating factor (CSF) and interleukin 1 (IL-1).

We are also applying hybridoma technology to the problem of isolating gene segments from human monocytes. In collaboration with the Monoclonal Antibody/Hybridoma Section, we have successfully converted a human monocyte cell line (U937) into a HAT-sensitive cell line. We have now successfully performed several fusions of this cell line with normal human monocytes in an attempt to create a proliferating, immortal cell line with distinct functional capabilities of normal human monocytes. Such fusion cell products (monocyte hybridomas) are currently being screened for their ability to perform unique monocyte functions (monokine secretion and tumor cytotoxicity) that are not possessed by the parent U937 line. We are particularly focusing on the ability of these immortal cells to secrete monokines such as IL-1, FGF, and CSF.

SIGNIFICANCE

The monocyte and its tissue counterpart, the macrophage, have been documented to play pivotal roles in the immune system, particularly vis-a-vis host defense. It is known that these cells are critical for the phenomenon of antigen processing, that they are required as accessory cells for a variety of T-lymphocyte and B-lymphocyte functions, that they secrete a variety of biologic response modifiers (monokines) that have dramatic immunoregulatory functions, that these cells are capable of producing a wide range of complement components, that these cells are major participants in the phenomenon of antibody-dependent cellular cytotoxicity (ADCC), and it has been demonstrated that monocytes have the capability of destroying tumor targets in vitro. Applications of current understandings about this multifaceted cell to the setting of the patient with malignancy are still forthcoming. A great deal of work still remains to be performed in order to translate current in vitro systems into workable understandings of the in vivo functions of monocytes in humans. Our laboratory is committed to furthering our understanding of the role of human monocytes in the immune response to neoplasia in the human setting. Since monocytes are the predominant secretors of a wide variety of biologic response modifiers that are of particular interest to this program (such as IFN, FGF, and CSF), we are employing biochemical

strategies (such as genetic engineering and monocyte hybridoma technology) whereby we can obtain maximal amounts of purified monokines for subsequent clinical trials. Moreover, we are committed to understanding the molecular basis for the release and activities of these potent monocyte secretory products.

PROPOSED COURSE

During the next year, we will continue to examine the role of human monocytes in the normal immune response. Genetic engineering of human monocytes appears to be the most promising area of current monocyte laboratory investigation. We have mastered the biochemical techniques for analyzing specific monocyte mRNAs and will continue to gather in-depth information into the biochemical basis for human monocyte BRM release at the level of macromolecular synthesis. A gene library of activated human monocytes has been established and over the course of the next year detailed experimentation will focus on the exact messages that are contained in that library. We will attempt to establish functional monocyte genes for monokines that are of programmatic interest. Similarly, we will screen the function of monocyte hybridomas that are being established for their ability to secrete monokines such as IL-1 and CSF that could potentially be used in clinical trials with cancer patients. Similarly, we will continue to perform in-depth investigations to determine whether abnormalities of functional subsets of human monocytes exist in cancer patients.

PUBLICATIONS

- Akiyama, Y., Miller, P. J., Thurman, G. B., Neubauer, R. H., Oliver, C., Favilla, T., Beman J. A., Oldham, R. K. and Stevenson, H. C.: Characterization of a human blood monocyte subset with low peroxidase activity. J. Clin. Invest., 1983 (in press).
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Stevenson, H. C., Beman, J. and Oldham, R. K.: Design of a cancer immunology cytapheresis unit. Plasma Therapy 4: 57-63, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09258-01 BTB

PERIOD COVERED

December 21, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trial of Plasma Perfused Over Immobilized Protein A in Patients with Breast Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Mehmet F. Fer, M.D., Visiting Scientist, BTB, NCI

COOPERATING UNITS (if any)

NCI-FCRF; Baylor College of Medicine, Houston, Texas

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

3

PROFESSIONAL:

2.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to explore the therapeutic potential and biological effects of autologous plasma perfused over protein A in patients with breast cancer. Patients with recurrent measurable breast cancer and good performance status are treated with previously stored autologous plasma perfused over protein A immobilized on charcoal collodion. The dose of protein A and the quantities of plasma that is perfused are escalated in a stepwise fashion depending on the patient's tolerance. The patients are closely observed for evidence of objective tumor regression, inflammatory changes that may occur around the lesions, toxicity, and biological response modifying effects. Four patients have entered the trial to date and it is anticipated that 15 patients will be studied. Some patients have experienced pain around tumors following plasma therapy, but to date there have been no objective tumor regressions or toxicity. Animal studies performed by direct injections of protein A have not as yet shown any antitumor effects.

PROJECT DESCRIPTION

PERSONNEL

Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Stephen A. Sherwin	Expert	CIS	BTB	NCI
JoAnn S. Beman	Nurse Specialist	CIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Robert K. Oldham	Associate Director		BRMP	NCI
John W. Pearson	Senior Investigator	MAS	BTB	NCI

OBJECTIVES

This trial is designed to explore the clinical utility and biological effects of plasma perfusion over immobilized protein A in patients with breast cancer. Considerable interest has been generated in this area over the past few years after reports from several institutions of antitumor effects of such plasma manipulation. The scope of this trial will encompass the assessment of reproducibility of the reported results and the determination of the toxicity associated with this ex vivo plasma manipulation. The mechanisms of possible antitumor action and biological response modifying effects associated with this approach are poorly defined and this study will attempt to elucidate some of these biological changes. Since the most dramatic antitumor responses have been reported by investigators at Baylor College of Medicine, the trial is conducted in direct collaboration with this group.

Animal studies are conducted in parallel to the clinical study, in efforts to elucidate the biological effects of protein A in these model systems.

METHODS EMPLOYED

Patients with recurrent, measurable breast cancer who have failed standard therapy are eligible for the trial. Since the major toxicity of this approach appears to be on the cardiovascular system, patients need to be in good performance status, ambulatory, and have good cardiovascular function. Prior to entry into the trial, patients undergo a staging workup to assess the extent of disease and to obtain baseline tumor measurements, a thorough cardiovascular examination, two-dimensional echocardiogram, electrocardiography and other cardiovascular function tests as indicated. Patients are required to be off of any chemotherapy or hormonal therapy for at least one month prior to entry into the trial. Eligible patients then undergo two phlebotomy procedures with manual plasmapheresis performed within approximately one week. The cellular components of the blood are immediately returned to the patient. Plasma is stored in 50 cc aliquots and frozen at -70°C . Plasma aliquots are thawed as needed for therapy, which is given twice weekly for a total of six weeks.

For the initial four procedures, the dose of protein A immobilized on charcoal-collodion is limited to 0.62 mg and the amount of plasma to 50 cc. Depending on patient tolerance, the dose of protein A is increased to 1.25 mg for the next four perfusions, and the quantity of plasma to 100 cc for the final four doses, to a total of 12 treatments over six weeks. Patients are closely monitored for toxicity and inflammatory changes around tumors, and other biological response modifying effects. Extensive immunological testing is performed at three time points during the six-week trial, both before and after the administration of autologous plasma perfused over protein A. Objective tumor response is assessed after the end of six weeks. Immunologic testing includes monitoring of natural killer cell activity, antibody dependent cellular cytotoxicity, changes in serum complement components and alterations in immune complex levels and acute phase reactants. Material is also frozen for future studies which may be deemed appropriate.

Animal experiments in parallel to the clinical study are being conducted. These studies are primarily focused on the effects of protein A directly injected intravenously, rather than used in a perfusion system. The animal models chosen for these studies include the guinea pig transplantable mammary carcinoma, transmissible venereal tumors of dogs and murine transplantable K1735 M2 melanoma. In the guinea pig system, tumor-bearing animals are injected with 0.065 mg, 0.125 or 0.25 mg doses twice weekly intravenously for three weeks. Dogs bearing the transmissible venereal tumor are infused twice weekly with 100 mg/kg protein A for 5 weeks. Studies in mice involve multiple dose levels and schedules (ranging from 0.6 to 256 μ g for 3 weeks), since larger numbers of animals can be studied. Animals are observed for antitumor effects and other biological changes.

MAJOR FINDINGS

The trial has recently started and only four patients have been entered to date. The first three patients have completed the six-week course of therapy and the fourth patient is currently on study. In the first three patients there have been no toxicity and no objective tumor reductions. However, the patients have reported some subjective changes which might be subtle indicators of some biological activity. All patients have had intermittent pain over areas involved with tumor. One patient has had severe aggravation of bone pain consistently after each therapy. One patient may have developed some edema around the locally recurrent chest wall lesion, but this finding was equivocal and not consistent. The trial is currently ongoing and it is too early to make any far-reaching conclusions.

Animal studies to date have not demonstrated any antitumor effects of protein A given intravenously. No objective tumor responses have been seen, and no inflammatory changes have developed around the lesions. Survival of animals injected with protein A was no different than that of animals in the control group. Studies in the canine transmissible venereal tumor model, conducted by Dr. Dan Cohen NCI-FCRF, are currently in progress.

SIGNIFICANCE

The available experience to date with plasma perfusion over protein A has been limited and most studies reported have employed different techniques, different quantities of plasma or protein A, and different patient populations. Some of the patients in these trials have received other concomitant therapies. Since it is possible that plasma perfusion over protein A could offer a varied method of cancer therapy, it appears imperative that the biological and antitumor effects of this approach be examined in a systematic fashion, as planned in this study.

PROPOSED COURSE

At this point, the lack of any objective tumor regression in the absence of toxicity suggests that the doses used may be very low. Even though the efficacy of this approach is not proven yet on a large scale, the toxicity has been somewhat similar in all other studies that have been reported, and none of these toxicities have occurred in our initial four patients. Thus, it appears logical that dose escalations be continued to reach a point where either biological activity is seen or toxicity becomes unacceptable. If plasma perfusion over protein A conducted in this fashion demonstrates any clinical efficacy, additional studies would be considered to apply this technology to other tumors and different clinical settings. In that event, detailed studies would need to focus on the biological mechanisms that are involved, with careful evaluation of the complement-mediated changes, immune complexes and cellular immunity, in both patients and animal studies.

PUBLICATIONS

No publications have resulted as yet from these early observations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09261-01 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intralesional Interferon (IFL-rA) in Patients with Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Paul G. Abrams, Expert, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

3

PROFESSIONAL:

3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Patients with subcutaneous or lymph node metastases have been treated with alpha interferon (IFL-rA) at doses of 3, 9, 18, 36 and 50×10^6 units per lesion to determine local toxicity, efficacy and cellular response to a pure lymphokine prepared by recombinant DNA technology. Eighteen patients have been entered and 15 were evaluable, receiving at least 3 doses. Local erythema in the skin over a responding lesion has been the only local toxicity. One patient with a prior history of seizures had a grand mal seizure with the first dose. One patient receiving 3×10^6 units per lesion had a partial response. No other responses were seen until a dose of 36×10^6 units/lesion was administered: there has been one complete response and three partial responses in patients receiving this high dose level. No cellular infiltrate has been identified which can be attributed to the administration of interferon.

PROJECT DESCRIPTION

PERSONNEL

Robert K. Oldham	Director		BRMP	NCI
Jeffrey J. Ochs	Medical Staff Fellow	CIS	BTB	NCI
Mehmet Fer	Visiting Scientist	CIS	BTB	NCI
Gino C. Bottino	Medical Staff Fellow	CIS	BTB	NCI

OBJECTIVES

- (1) To determine the safety and local toxicity of intralesionally administered recombinant alpha interferon (IFL-rA).
- (2) To determine the local and systemic response to intralesionally administered IFL-rA.
- (3) To determine the nature of the cellular response evoked by IFL-rA when administered intralesionally.

METHODS EMPLOYED

- (1) Patients are selected for the study who have documented malignancy and easily accessible subcutaneous or lymph node metastases.
- (2) Alpha interferon (IFL-rA) is injected directly into a lesion and another lesion is injected with vehicle control. After 5 lesions in at least 3 patients have received 4 bi-weekly injections without toxicity, the dose is escalated in the next group of patients according to the following schedule: 3×10^6 , 9×10^6 , 18×10^6 , 36×10^6 , 50×10^6 units/lesion.
- (3) One injected and one control lesion are biopsied after four weeks and examined by light microscopy, T-cell subset and NK cell-specific monoclonal antibodies, and lysozyme stains for macrophages to determine if there are differences between the interferon and control lesions.

MAJOR FINDINGS

Fifteen of 18 patients who received at least three injections are evaluable. One patient exhibited an erythematous eruption over a responding interferon-injected lesion which resolved when the interferon was stopped. Another patient, who had a prior history of seizures, developed a grand mal seizure after receiving a total dose of 86 million units and when his diphenylhydantoin level was subtherapeutic. With the exception of one partial response in a patient with melanoma receiving 3×10^6 units/lesion, no responses were seen until a dose of 36×10^6 units/lesion was administered: one complete and three partial

responses were observed in patients receiving 36 or 50 x 10⁶ units/lesion. No cellular infiltrate has been identified which can be attributed to the administration of interferon.

SIGNIFICANCE

If the local activity of interferon observed in these patients with melanoma and breast cancer can be extended to other tumors, it may emerge as a therapy for early stage or pre-malignant diseases. Since there appears to be negligible local toxicity, it has the potential of being administered repeatedly to palliate local disease. In addition, this study suggests a dose-response relationship which has not previously been reported for systemic interferon.

PROPOSED COURSE

The entry to this study is completed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09210-03 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

MoAb to Human TAA: Biological, Biochemical & Therapeutic Studies

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Alton C. Morgan, Jr., Deputy Section Head, BTB, NCI

COOPERATING UNITS (if any)

Laboratory of Immunology, Veterans Administration Medical Center,
Houston, Texas; NCI-FCRF

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

3.5

PROFESSIONAL:

2.25

OTHER:

1.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major objective of the Monoclonal Antibody/Hybridoma Section is the production of murine monoclonal antibodies to human tumor-associated antigens. For this purpose, we have employed human colorectal cancer as a system for testing the efficacy of new and present methods of elicitation. We have found that membrane peripheral protein extracts of human colorectal cancer from heterotransplants combined with Sepharose-insolubilized lectins was far more efficient in eliciting antibodies to TAA. As a result, we are presently extensively evaluating six antibodies by histochemical and molecular techniques.

A second major emphasis in evaluation of monoclonals is directed towards methods and assays which allow for selection of diagnostic and therapeutically useful antibodies. This second level of evaluation is exemplified by two monoclonal antibody-defined human melanoma-associated antigens, one of 100K daltons, the other of 250K daltons. The 100K dalton antigen levels in melanoma patients' sera correlated with tumor burden and 9.2.27 antibody to the 250K glycoprotein was effective in treatment of established human melanoma in nude mice.

A third level of evaluation of monoclonal antibodies is related to their therapeutic efficacy in patients. Ongoing laboratory studies, in coordination with a clinical trial within the Clinical Investigations Section, are presently assessing humoral and cellular responses to administered monoclonal antibody, antibody localization in tumor and normal tissues and the histopathological examination of tumor nodules for evidence of antigenic heterogeneity.

These levels of evaluation indicate a continuing improvement in the ability to elicit, select, and evaluate monoclonal antibodies for clinical utility, and to use them as probes in basic studies on the nature of tumor-associated antigens.

PROJECT DESCRIPTION

PERSONNEL

Clive S. Woodhouse	Visiting Fellow	MAS, BTB, NCI
John W. Pearson	Microbiologist	MAS, BTB, NCI

OBJECTIVES

(1) Elicitation and characterization of murine monoclonals to tumor-associated antigens of human colorectal cancer for the eventual use in immunodiagnosis and therapy. Related goals are directed at specificity of antigen reactivity and subclass of elicited monoclonals.

(2) Further define the host humoral response to the 100K dalton human melanoma associated antigen, MAA; determine the role of the antigen in generation of naturally occurring immune complexes; and further assess its usefulness as a monitor of tumor burden in sera of melanoma patients.

(3) Develop solid phase assays for detection of 250K dalton MAA in serum and other body fluids; and define its therapeutic potential. Specific goals are related to the antibody's ability to localize in tumor tissue and to inhibit tumor growth in nude mice through effector cells or toxin conjugates.

(4) In conjunction with a Phase I clinical trial with the antibody 9.2.27 to the 250K dalton MAA: (a) determine the localization of antibody in tumor and normal tissues; (b) assess the class, amount, and specificity of anti-murine immunoglobulin; (c) establish the pharmacokinetics of administered antibody; (d) determine the interaction of murine antibody with patient lymphoid cells; (e) monitor serum antigen levels of both the 250K and 100K dalton MAA; and (f) assess the effect of antigenic heterogeneity on localization and saturation.

(5) To determine if melanoma cells produce molecules functionally and biochemically similar to components of normal human serum.

MAJOR FINDINGSI. Colorectal Murine Monoclonals

A large-scale project was initiated to determine the efficiency of production of antibodies to tumor-associated antigens on human colorectal cancer. The three arms of the study involved immunization of BALB/c mice i.p. with human colorectal cells, harvested from individual patients' tumors with collagenase. A total of 6 fusions and over 3000 wells were screened. Approximately 1% of the cells were reactive against the immunizing cell and not against an autologous B-lymphoblastoid cell line. The second arm of the study utilized a membrane preparation from three human colorectal cancers heterotransplanted into nude mice. Three fusions and over 1500 wells were screened and about 0.5% passed the first screen and were cloned. The third arm of the study, which involved

5 fusions and over 2000 wells, utilized lithium di-iodosalicylate membrane extracts of pooled, xenografted colorectal tumors. These extracts were adsorbed to five different lectins, wheat germ, lens culinaris, Dolichos biflorus, peanut, and Ulex europeus. Although there were differences among the various lectins, an average of 20% of the wells from these fusions reacted with the immunizing preparation and cultured colon carcinoma cells but not to a pool of human B- and T-lymphoblastoid cells. In the second phase of screening, over 70 cloned hybridomas were evaluated by indirect immunoperoxidase against paraffin and frozen sections of tumor and normal colon and by ELISA against purified CEA, blood group substances, and synthetic sugar haptens. Of the 45 antibodies reacting with paraffin sections of colon tumors, 6 did not react with normal or hyperplastic colon, CEA or blood group substances. These six have been extensively evaluated for reactivity against 30 different normal tissues. Five of the six were found to only react with the brush border of small intestine and the sixth does not react with any normal tissue thus far examined. At present these antibodies are being evaluated by immunochemical methods to determine the molecules they are reactive against and the hybridomas are being expanded for utilization in radiolocalization, toxin conjugation, and immunotherapy in nude mice. Antibodies found useful in this setting will eventually be translated into clinical trials within our Clinical Investigations Section. Over 25 antibodies in this series remain unevaluated as yet. In addition another series of fusions utilizing Lubrol extracts with lectins have been initiated.

II. 100K Dalton MAA

This MAA is found in high quantities in spent culture media of malignant melanoma and carcinoma cell lines, and fetal uveal melanocytes. When analyzed under both reducing and nonreducing conditions by SDS polyacrylamide gel electrophoresis (PAGE), this MAA is a 100K dalton glycoprotein with N-asparagine-linked carbohydrate. In vitro, glycosylation is required for the shedding of the protein from the tumor cells. It has also been demonstrated in the cytoplasm of malignant melanoma and carcinoma cells obtained by biopsy. Detectable levels of MAA were found in all normal adult sera. Five of 10 patients with stage III and 9 of 16 patients with stage IV disease had antigen levels that exceeded the 95th percentile of serum levels in the normal donors ($p < 0.001$). Elevated levels of the 100K MAA were associated with evidence of residual tumor ($p < 0.008$): only 3 of 13 patients with no evidence of tumor had abnormal values whereas serum levels were increased in 9 of 16 patients with evident tumor. These studies suggest that measurement of the 100K MAA may be useful for monitoring tumor burden in patients with malignant melanoma. Levels of the 100K MAA were unrelated to the levels of soluble immune complexes, as detected by the fluid phase C1q binding test. The 100K MAA in serum was found to be associated with albumin in a strong, noncovalent manner. Upon disassociation with SDS, the 100K MAA in serum co-migrated with the molecule from spent culture medium of human melanoma cells.

The 100K dalton MAA also was found to circulate as part of an immune complex of which a part is antigen-reactive antibody. Thus, we have thus shown, for the first time, that a human melanoma-associated antigen, defined by a xenomonoclonal antibody, elicits a naturally occurring humoral response.

III. 250K Dalton MAA

We have developed a double determinant assay for soluble 250K, utilizing an IgG₁ antibody to capture soluble antigen and a biotinylated IgG_{2a} antibody (9.2.27) to the same molecule for detection. The assay is presently being utilized to screen sera of melanoma patients treated with monoclonal antibody 9.2.27 and of tumor-bearing and normal donors.

We have found, both in nude mice and nude rats, that infused antibody 9.2.27 can inhibit tumor growth. Antibody-treated tumors are characterized by a large mononuclear cell infiltrate. Effectiveness of antibody treatment is inversely related to tumor burden. Further studies are underway to characterize the optimal dosage and regimen and the role of effector cells in the antibody-mediated regression of established tumors.

We have monitored the first 8 patients receiving escalating doses (1-200 mg) of antibody 9.2.27. Although not yet complete, our studies indicate that as the dose is increased, a peak level of 120 µg/ml of 9.2.27 in the serum is reached immediately after infusion and that by 48 hours detectable antibody is no longer circulating. At higher doses, low levels of antibody remain in the circulation for up to 72 hours. This circulating antibody, however, is not antigen-reactive. Significant antiglobulin responses were found in only 3 of the 8 patients and in these it was only transient during the course of therapy. Transient changes in the numbers of monocytes and the number of antibody molecules bound by monocytes was noted in several patients following antibody infusion. It is expected that as dose levels of the antibody are increased, some of the humoral and cellular responses will become magnified. Antiglobulin responses in patients do not seem to limit the delivery of antibody to cutaneous tumor nodules. This type of intensive monitoring of patients receiving a new class of BRM should enable us to evaluate and understand its mode of action.

IV. Biologically Active Molecules Synthesized by Melanoma Cells

The rationale for this project area is that tumor cells produce molecules which may exert an influence on both neighboring and distant normal cells. These effects may be important in immune surveillance, growth regulation, and metastatic spread. The approach that has been taken is that one well characterized source of biologically active molecules is normal serum. Thus, we have asked whether melanoma cells synthesize and secrete molecules immunologically and immunochemically similar to those in normal serum. We have identified in spent culture medium of melanoma cells, by solid phase ELISA and indirect immunoprecipitation and SDS-PAGE, a globin-like molecule, peptides corresponding to proteolytically derived fragments of the C4 component of complement, and α_2 macroglobulin (α_2M). The latter molecule was subsequently shown to be synthesized and secreted by 10/13 cultured melanoma cells but not neoplastic cells of other histologic origins (0/5 breast, 0/2 colon, 0/2 lung, 0/2 glioblastomas, 0/1 neuroblastoma, 0/4 lymphoid). α_2M was also produced by one line of fetal lung fibroblasts, as has been previously shown, but not by two lines of normal fetal uveal melanocytes or two fetal skin fibroblast lines.

Biochemical characterization of melanoma α_2M indicated that it has a similar tetrameric structure but is smaller in size than serum α_2M . Preliminary studies indicate that melanoma α_2M can bind to protease and has possible functional capability.

The globin and C4-like molecules are presently under investigation. These results indicate that this is a fruitful approach to identifying biologically active molecules produced by human melanoma cells.

SIGNIFICANCE

The studies carried out within the Section on the therapeutic potential of monoclonal antibodies, together with more basic efforts at understanding the role of biologically active molecules in the growth, environmental influence, and process of metastatic spread will enhance our capabilities in determining the utility of monoclonal antibodies in immunodiagnosis, prognosis, and therapy.

PROPOSED COURSE

(1) Monoclonals to colorectal cancer. Further evaluation by immunoperoxidase of histologic sections and indirect immunoprecipitation and SDS-PAGE will characterize these antibodies to a sufficient level to move the more promising ones into diagnostic and therapeutic evaluation.

(2) 100K MAA. The primary emphasis with this tumor-associated antigen will be on its possible value as a serum marker of malignancy and tumor burden. Sera from patients with benign skin diseases, serial samples from melanoma patients and samples from patients receiving therapy with monoclonal antibody 9.2.27 will be assessed for levels of this antigen.

(3) 250K MAA. The primary emphasis in this project area will be on continued evaluation of this antibody as a therapeutic agent in both nude mice models and patients. The two primary areas will be the role of effector cells in regression of antibody-treated tumors and the effect of toxin and drug conjugates of the antibody on primary and metastatic growth of tumor (see project description of R. K. Oldham, this Section).

(4) Biologically active molecules. We will continue a biochemical comparative assessment of melanoma α_2M with the molecules produced by macrophages and serum α_2M . The globin and C4-like molecules produced by melanoma cells will also be further characterized.

PUBLICATIONS

Morgan, A. C.: Monoclonal antibodies to human melanoma associated antigens: elicitation and evaluation with immunochemically defined antigens. In Reisfeld, R. A. and Ferrone, S. (Eds.): Melanoma Antigens and Antibodies. New York, Plenum Press, 1982, pp. 279-288.

Morgan, A. C. and McIntyre, R. F.: Monoclonal antibodies to human melanoma associated antigens--an amplified elisa assay for the detection of antigen, antibody, and immune complexes. Cancer Res. (In Press)

Morgan, A. C., Rossen, R. D., McCormick, K. J., Stehlin, J. S. and Giovanella, B. C.: Hidden cytotoxic antibodies that react with allogenic cultured fetal and tumor cells contained in soluble immune complexes from normal human sera. Cancer Res. 42: 881-888, 1982.

Reisfeld, R. A., Galloway, D. R., Micabe, R. P. and Morgan, A. C.: Molecular and immunological characterizatio of human melanoma associated antigens. In Reisfeld, R. A. and Ferrone (Eds.): Melanoma Antigens and Antibodies. New York, Plenum Press, 1982.

Rossen, R. D. and Morgan, A. C.: Immune complexes in cancer. In Waters, H. (Ed.): The Handbook of Cancer Immunology, Vol. IX. New York, Garland STPM Press. (In Press)

Rossen, R. D., Crane, M. M., Morgan, A. C., Giannini, E. H., Giovanella, B. C., Twomey, J. J. and Hersh, E. M.: Circulating immune complexes and tumor cell ctyotoxins as prognostic indicator in malignant melanoma. A prospective study of 53 patients. Cancer Res. (In Press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1CM09226-03 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Efficacy of MoAb Therapy against Established Tumors and Disseminated Metastases

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John W. Pearson, Microbiologist, BTB, NCI

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NCI; NCI-FCRF

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

2.2

PROFESSIONAL:

1.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several animal tumor models have been established to evaluate the in vivo efficacy of monoclonal antibodies: a) a model for metastatic human melanoma has been developed in nude mice with brachial and axillary lymph node metastases, but no gross visceral metastases, produced by s.c. or i.v. inoculation of 500,000 cells of the melanoma cell line, FeMX Met II. b) Strain 2 guinea pigs bearing the syngeneic line 10 hepatocarcinoma were treated with immunoconjugates of the D3 monoclonal antibody with the A chain of abrin or diphtheria toxins. The maximum tolerated doses for the diphtheria and abrin conjugates were 25 and 85 μ g's, respectively, with toxicity consisting of severe loss of body weight, ruffled coats, and lethargy. Some retardation of tumor growth was seen after administration of 100 μ g of abrin conjugate to animals with tumors greater than 1 cm in diameter. c) To select drugs for immunoconjugate studies, a variety of chemotherapeutic agents were evaluated for in vivo efficacy against a transplantable spontaneous mammary carcinoma in guinea pigs. Cyclophosphamide was found to be most effective against established tumors, with less effect seen with Alkeran and cis-platinum, and no effect observed with adriamycin or 5-fluorouracil.

PROJECT DESCRIPTION

PERSONNEL

A. Charles Morgan, Jr.	Deputy Section Head	MAS	BTB	NCI
Richard V. Smalley	Chief		BRB	NCI
Robert K. Oldham	Associate Director		BRMP	NCI

OBJECTIVES

The aim of this project is to develop effective protocols using monoclonal antibodies alone or in combination with toxins and/or drugs for the control of established and metastatic tumors. The models under study include a human melanoma (FeMEX Met II) in nude mice, a guinea pig line 10 hepatocellular carcinoma (L10) and a transplantable spontaneous mammary carcinoma in strain 2 guinea pigs. The specific objectives of this project are as follows:

- (1) To develop and/or utilize appropriate animal model systems to test the therapeutic effectiveness of monoclonal antibody (MoAb)-drug or toxin conjugates in vivo.
- (2) To develop treatment protocols, (i.e., dose, route and schedule) that will maximize the efficacy of 9.2.27 MoAb serotherapy directed against the human melanoma, FeMX Met II, tumor. To develop treatment protocols utilizing specific immunoconjugates for control of disseminated metastases, as well as against local growth of the primary tumor.
- (3) To study the therapeutic potential of toxins and/or drugs conjugated to the D3 MoAb against L10 tumors and against axillary metastases following removal of the primary tumor.
- (4) To select the appropriate chemotherapeutic agents for subsequent immunoconjugate studies with the transplantable guinea pig mammary carcinoma.

RATIONALE

Monoclonal antibodies directed against tumor-associated antigens offer considerable potential for therapy of human cancer. However, much information needs to be accumulated regarding the factors determining in vivo efficacy and the problems and limitations associated with such treatment. Prior to large scale clinical trials, particularly with immunoconjugates with toxic agents, considerable preclinical studies are needed. The monoclonal antibody 9.2.27 against a 250K human melanoma associated antigen is already being studied in a phase I clinical trial and a nude mouse model system will assist in the development of therapeutic protocols with this antibody. The transplantation of FeMX Met II has been a particularly valuable analogue of the human disease because of its unusual ability to produce a high incidence of spontaneous lymph node metastases.

An alternative approach has been to develop syngeneic animal tumor systems with some analogues to human tumors. As a model for MoAb therapy of carcinomas, the line 10 tumor has some particular advantages. It spontaneously metastasizes to draining lymph nodes and to visceral organs, there is a highly specific MoAb available and the use of guinea pigs allows the evaluation of the effects of a mouse MoAb in a heterologous species which, similar to man, is susceptible to anaphylactic reactions. The other guinea pig tumor, a mammary adenocarcinoma, also provides a good model for studies of immunoconjugates with drugs of known efficacy for human breast cancer and it resembles the human tumor situation by spontaneously metastasizing to the lungs following surgical resection of the primary tumor.

METHODS EMPLOYED

- (1) Monoclonal antibody. D3 and 9.2.27 are produced by the intraperitoneal inoculation of pristane-treated BALB/c mice with tissue culture grown hybridoma cells. The ascites fluid is harvested approximately 8-12 days after inoculation, supernatant fluid obtained via centrifugation and purified by double Na_2SO_4 precipitation. Purity is assessed by SDS-PAGE and HPLC gel sieving. Antigen reactivity is determined by flow cytometry and solid phase-biotin avidin ELISA.
- (2) Antibody conjugation. The D3 and 9.2.27 antibodies are conjugated to the A chain of abrin or diphtheria toxins by disulfide linkage.
- (3) Determination of immunoconjugate toxicity and/or therapeutic efficacy. The parameters employed to assess immunoconjugate toxicity are severe body weight loss, ruffled coats, lethargy and mortality. The therapeutic effect is evaluated by growth or regression of an established primary tumor, delay or absence of palpable disseminated metastases, and survival time.
- (4) Tissues are examined histologically for the presence of tumor as well as for retention of antigenicity, as assessed by the immunoperoxidase technique.
- (5) Surgical techniques are applied to remove the primary tumor at the initial site of inoculation.

MAJOR FINDINGS

I. Transplantable Human Melanoma (FeMX Met II) in Nude Mice

Investigations in this model have focused on establishing the conditions for consistent metastatic spread in nude mice. Female outbred nude mice, 3 to 4 weeks of age, were injected with approximately 5×10^5 viable tumor cells either subcutaneously (s.c.) in the inguinal area or intravenously (i.v.). All mice bearing 10 or 15 mm inguinal tumors exhibited enlarged brachial or axillary nodes and histological examination confirmed the presence of tumor. Such nodes were not enlarged in mice exhibiting 5 mm inguinal tumors. No gross metastatic involvement was observed in other organs, i.e., liver, spleen, kidney, lungs, but these are now being evaluated for possible micro-metastatic foci. Of mice

injected i.v., only brachial or axillary node enlargement was detected at 8 or 10 weeks post inoculation. On gross examination, all visceral organs appeared normal. A similar study is underway with the ascites form of FeMX Met II. In this case, 5×10^6 or 5×10^5 tumor cells were injected s.c. into the inguinal area or i.v. These mice are presently under observation and will be sacrificed at various time intervals.

II. Transplanatable L10 Hepatocarcinoma (Refer to Dr. R. K. Oldham's project description for conjugate preparations)

Preliminary toxicity studies were recently initiated to evaluate the maximum tolerated doses of D3 MoAb following conjugation to the A chain of either abrin or diphtheria toxins. Tumor-bearing animals (1 cm) tolerated sequential doses of 5 and 10 μg of the D3-diphtheria toxin conjugate, escalated at 4 day intervals. However, following the third dose of 25 μg of immunoconjugate, all animals expired within 48 hours after treatment. Toxicity in the animals was apparent from the severe loss of body weight, ruffled coats, and a general lethargic state. Even though there was no apparent effect on the primary tumor growth at the time of death, gross examination of the tumor tissue revealed some necrosis. A similar approach utilizing the D3 MoAb conjugated to abrin A chain revealed that tumor-bearing guinea pigs tolerated escalating doses of 10, 25, and 50 μg when administered i.v. every 4 days. However, after the fourth injection of 100 μg , all animals died within 48 hours. The abrin conjugate induced a toxic reaction similar to that observed with the diphtheria conjugate. Interestingly, retardation of the primary tumor growth began to occur before the administration of 100 μg , despite the fact that tumor size was greater than 1 cm at time of treatment. This retardation of tumor growth was further substantiated by the necrotic nature of the tumor tissue. Presently, studies are ongoing to evaluate the therapeutic effect of these conjugates against the L10 tumor in guinea pigs with smaller established tumors (see Dr. R. K. Oldham's report).

III. Transplantable Spontaneous Mammary Carcinoma

We have analyzed the antitumor effect of several clinically active drugs against both the established primary and metastatic mammary carcinoma in strain 2 guinea pigs. Maximum tolerated doses of each drug given i.v. were determined as a single dose or two doses, 10 days apart. Cytosan, 150 mg/kg, was most active against both the established primary and metastatic tumors, with complete tumor regressions within 7 days. All drug-treated animals had eventual tumor recurrence. Alkeran (2 mg/kg) and cis-platinum (5 mg/kg) caused 50% tumor regression. Adriamycin (2 mg/kg) and 5-FU (40 mg/kg) had no effect on the established primary or metastatic tumors.

SIGNIFICANCE

Studies in animal model systems with MoAbs alone and/or conjugated to specific toxins or drugs are needed for understanding potential toxicity and immunomodulatory effects, in order to develop therapeutically effective protocols against primary and metastatic human tumors.

PROPOSED COURSEI. Transplantable Human Melanoma (FeMX Met II)

(A) Serotherapy studies utilizing the MoAb 9.2.27 developed against this human tumor will be undertaken to determine the following parameters: (1) optimal dose, (2) localization of 9.2.27 in the primary tumor following different routes of administration, (3) effect of MoAb on antigen density, and (4) influence of tumor burden on responsiveness to therapy.

(B) Immunoconjugate studies involving toxins and/or drugs will be undertaken to evaluate their effectiveness against either the established primary tumor and/or elimination of spontaneous metastasis. Types of conjugates may include the A chains of ricin, abrin, and diphtheria toxins.

II. Transplantable L10 Hepatocarcinoma

Studies involving the D3 MoAb conjugated to either toxins or clinically active drugs will be undertaken to evaluate the therapeutic efficacy against: (1) small palpable tumors; (2) established tumors (1 cm) as well as development of subsequent axillary metastases, and (3) spontaneous axillary metastasis following surgical removal of the primary tumor.

III. Transplantable Spontaneous Mammary Carcinoma

A collaborative effort has been established with Dr. Warran Evans (Laboratory of Biochemistry, NCI) to develop a specific MoAb against either the primary or metastatic mammary carcinomas. If successful, studies will be undertaken to utilize the MoAb as a carrier molecule for an active drug directed against the metastatic pulmonary foci, following surgical removal of the primary breast tumor.

PUBLICATIONS

Pearson, J. W., Knutsen, G. L., Brandhorst, J. S., Bucana, C. D. and Key, M. E.: Biologic and morphologic characteristics of a spontaneous transplantable mammary carcinoma in the guinea pig. J. Biol. Resp. Modif. 1: 187-192, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09236-02 BTB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of Hu T-Cell Hybrid., Monocyte-Macrophage Hybrid. & LGL Hybridomas

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)
Robert W. Schroff, Staff Fellow, BTB, NCI

COOPERATING UNITS (if any)

NCI-FCRF

LAB/BRANCH
Biological Therapeutics Branch

SECTION
Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION
NCI-FCRF

TOTAL MANYEARS:	1	PROFESSIONAL:	.5	OTHER:	.5
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CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The human macrophage line U927 and the human T-lymphoblastoid line HSB2 have been rendered sensitive to hypoxanthine-aminopterin-thymidine (HAT) culture medium by treatment with 8-azaguanine. The HSB2 cell line was fused to T lymphocytes that were stimulated with concanavalin A for 48 hours. Hybridomas were generated that constitutively produced interleukin 2 and chemotactic factor. Eight of these hybridomas have been cloned and continue to constitutively secrete (>12 months) interleukin 2 in concentrations from 2 to 40 times that of an equal number of mitogen-stimulated peripheral blood lymphocytes. In another experiment, HSB2 was fused to human T lymphocytes stimulated with concanavalin A for 24 hours. Three T-cell hybridomas were generated and cloned that constitutively secreted macrophage activating factor. Fusion of the U937 line with purified human monocytes has recently generated six human monocyte hybridomas that have been successfully cloned. The characteristics of these hybridomas and their secretory products are currently being investigated.

PROJECT DESCRIPTION

PERSONNEL

Paul G. Abrams	Expert	CIS	BTB	NCI
John R. Ortaldo	Biologist	NIS	BTB	NCI
Robert K. Oldham	Associate Director		BRMP	NCI
Ronald B. Herberman	Chief		BTB	NCI
Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Eugenie S. Kleinerman	Senior Investigator	MPS	BTB	NCI

OBJECTIVES

1. To develop human natural killer hybridoma cell lines to further study the functional and antigenic characteristics of the natural killer cell. To utilize this cell line to isolate soluble cytotoxic factors, the receptors for target cells, and/or the target cell structures, and characterize them biochemically.
2. To develop human monocyte hybridoma cell lines to study the antigenic and functional characteristics of monocytes and macrophages. To screen the supernatants of these cell lines for the production of various monokines such as interferon, colony stimulating factor, and interleukin-1 (lymphocyte activating factor).
3. To develop T-lymphocyte hybridomas that secrete various lymphokines including macrophage activating factor, migration inhibition factor, interleukin 2, and chemotactic factor.

METHODS EMPLOYED AND MAJOR FINDINGSI. T-Cell Hybridomas

Human peripheral blood lymphocytes were stimulated with concanavalin A for 48 hours in culture and then fused to the HSB2 (HAT-sensitized) T-cell line. Hybridomas were generated that constitutively secreted interleukin 2 and chemotactic factor. Eight of these hybridomas were extensively cloned and continued to constitutively secrete interleukin 2 and chemotactic factor. One clone has secreted interleukin 2 for over 12 months, 40 U/10⁶/ml, which is 40 times the production of an equal number of human peripheral blood lymphocytes stimulated with phytohemagglutinin. None of these clones produced α or γ interferon, migration inhibition factor or macrophage activating factor. In another experiment, peripheral blood lymphocytes were stimulated for 24 hours with concanavalin A and three T-cell hybridomas were generated that constitutively produced macrophage activating factor. These hybridomas were cloned and have continued to constitutively secrete macrophage activating factor.

II. Monocyte Hybridomas

The U937 cell line (HAT-sensitized) was fused with purified human monocytes and six hybridomas were identified. These hybridomas have been cloned and will be tested for various monocyte functions, enzymes, surface antigens, and secretion of various monokines including interferon and colony stimulating factor. We have so far not identified secretion of interleukin 1. All of this testing will be done over the next few months.

III. LGL Hybridomas

We have fused purified human LGL to both the HSB2 and the U937 lines. We have so far been unable to develop stable hybridomas. We have attempted to inactivate the cytotoxic reactivity of the LGL prior to fusion with various agents (lysing buffer and emetine), but these efforts have so far not led to stable fusions.

SIGNIFICANCE

Monocytes, natural killer cells, and cytokines are all major research areas of the Biological Therapeutics Branch and the Laboratory of Molecular Immunoregulation of the Biological Response Modifiers Program. The proposed hybridomas will be widely used and studied by various investigators. Hybridomas producing human macrophage activating factor and interleukin 2 could be excellent sources of these lymphokines for purification, characterization, and future clinical trials.

PROPOSED COURSE

We are currently growing human T-cell hybridomas that are constitutively producing interleukin 2 so that we can purify this interleukin 2 for characterization and other studies. If the T-cell hybridomas producing macrophage activating factor continue to constitutively produce this product, they will be used for further purification and characterization of this lymphokine. T-cell hybridomas also could be a rich source of macrophage activating factor and interleukin 2 for clinical trials. Monocyte hybridomas are currently being studied for their enzymes, surface markers, DNA content, and production of monokines. While we have so far been unable to identify any interleukin 1 production, we are also testing for interferon and colony stimulating factor.

PUBLICATIONS

No publications as yet have resulted from this project.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09237-02 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Murine Monoclonal Antibodies to Human Leukocytes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert W. Schroff, Staff Fellow, BTB, NCI

COOPERATING UNITS (if any)

NCI-FCRF; LCI, NIAID

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

1

PROFESSIONAL:

.5

OTHER:

.5

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed a series of monoclonal antibodies to human granulocytes, monocytes, platelets, and eosinophils. An IgG1 monoclonal antibody produced against human granulocytes reacted specifically with mature granulocytes and did not react with any other normal circulating or bone marrow cells. No reactivity was found when this antibody was tested with a series of fresh leukemia cells and leukemia cell lines. Immunoprecipitation of the antigen identified by this antibody demonstrated a 65,000 dalton protein. Another monoclonal antibody, also an IgG1, produced against human monocytes, reacted with human granulocytes, monocytes, eosinophils, and large granular lymphocytes. This antibody reacted with myeloid precursor cells in the bone marrow and reacted with a small proportion (20%) of the acute myelogenous leukemia cells tested. The molecular weight of the antigen identified by this antibody was 95,000 daltons. A series of monoclonal antibodies produced against human platelets were isotypic as IgG1, IgM and IgG2a. One of these antibodies appeared to react with platelets and granulocytes while the other two reacted exclusively with platelets and no other circulating normal cells. Further characterization of these antibodies, including the molecular characterization of the antigens they react with and their effect on platelet function, is ongoing. An IgG1 monoclonal antibody has been produced against human eosinophils. This antibody also reacted with monocytes, granulocytes, and myeloid precursor cells in the bone marrow. This antibody immunoprecipitated a 95,000 dalton protein.

PROJECT DESCRIPTION

PERSONNEL

Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Edward S. Kimball	Senior Staff Fellow	BS	LMI	NCI

OBJECTIVES

1. To develop monoclonal antibody reagents to identify differentiation antigens on human monocytes, granulocytes, eosinophils and platelets.
2. To utilize these monoclonal antibodies for the classification, diagnosis and possible therapy of leukemia.
3. To study the effects of these monoclonal antibodies on various immune functions including natural killer activity, monocyte cytotoxic activity in vitro against tumor target cells, chemotaxis, phagocytosis of latex particles, and mitogen and antigen responses.
4. To study the effect of platelet monoclonal antibodies on platelet aggregation, adherence and serotonin release.

METHODS EMPLOYED

Purified populations of human leukocytes and platelets were separated by the cell elutriator. Eosinophils were sent from the LCI, NIAID, from patients with the "hypereosinophilic syndrome." 10^7 purified cells of each cell type were injected 3 to 5 times, approximately one week apart, intraperitoneally into BALB/c mice. Four days after the last immunization the animals were sacrificed. The spleens were removed, prepared into a single-cell suspension and fused to a HAT-sensitive murine myeloma cell line. After approximately 3 weeks, hybridoma supernatants were screened by radioimmunoassay against the immunizing cell and either autologous B cells, allogeneic B cells, or B cell lines. Hybridomas producing monoclonal antibodies reacting specifically with the immunizing cell and not with B cells were then cloned. Clones were then screened against large panels of human leukocytes and leukemia cells and cell lines. Hybridomas producing antibodies with specificity for specific cell types were isotyped and molecular characterization of the antigen identified by the monoclonal antibody was performed by SDS-PAGE. Antibodies were also incubated with human bone marrow cells and stained by indirect immunofluorescence, using a fluorescent-tagged rabbit anti-mouse immunoglobulin reagent. Positive cells were separated on the cell sorter in order to specifically identify the level of maturation of the cells that the monoclonal antibodies reacted with. This was performed by preparing cell preparations in the CytoSpin and staining with Wright's stain.

In vitro experiments to study the effect of monoclonal antibodies on various immune functions including monocyte phagocytosis of latex particles, chemotaxis, granulocyte aggregation, granulocyte bactericidal activity, and lysozyme release have been carried out. In addition, the platelet antibodies have been studied for their effect on platelet aggregation, adherence, and serotonin release.

MAJOR FINDINGS

I. Monoclonal Antibody

An IgG₁ monoclonal antibody produced against monocytes has been shown to react with human monocytes, granulocytes, and eosinophils. Interestingly, these antibodies also reacted with large granular lymphocytes isolated from human peripheral blood cells. This antibody has been demonstrated to react with granulocyte and monocyte precursor cells in human bone marrow but not with erythroid precursors. This antibody also reacted with 20% of myeloid leukemia cells and no lymphoid leukemia cells. The molecular weight of the antigen identified by this antibody is 95,000 daltons.

II. Monoclonal Antibody to Human Granulocytes

An IgG₁ monoclonal antibody produced against human granulocytes has been identified. This antibody reacted with granulocytes and not with any other circulating blood cells. There was no reactivity demonstrated with granulocyte or erythroid precursor cells in normal human bone marrow or any of the fresh myeloid or lymphoid leukemia cells or cell lines tested. The antigen identified by this antibody has a molecular weight of 65,000 daltons.

III. Monoclonal Antibody

An IgG₁ monoclonal antibody to human eosinophils has been generated. This antibody reacted with granulocytes and monocytes but not with any other peripheral blood leukocytes, including large granular lymphocytes. Bone marrow studies demonstrated reactivity with eosinophil and granulocyte precursor cells in the bone marrow and no reactivity with erythroid precursors or lymphocytes. This hybridoma clone initially produced an IgM antibody and later switched to an IgG₁. The IgM antibody reacted with the sugar sequence of lacto-N-fucopentaose III and after 3 months in culture the clone switched to an IgG₁ which did not react with the sugar sequence but immunoprecipitated a 95,000 dalton glycoprotein. We are currently investigating this unusual event.

IV. Monoclonal Antibodies to Human Platelets

Three monoclonal antibodies have been generated against human platelets. The isotypes include an IgG₁, an IgG_{2a}, and an IgM. We are currently investigating the other cells these antibodies react with and will immunoprecipitate the antigens identified by these antibodies. We have also undertaken a series of investigations to see what affect these antibodies have on platelet function, including platelet adherence, platelet aggregation, and serotonin release from platelets.

SIGNIFICANCE

The identification of differentiation antigens on human leukocytes is a powerful tool to dissect the immune system and to better understand the mechanisms of cell-cell interactions. In addition, differentiation antigens are generally expressed on leukemia and lymphoma cells and may be useful for the diagnosis, prognosis, classification, and treatment of leukemia and lymphoma. Therapeutic approaches to leukemia and lymphoma have included monoclonal antibody serotherapy and monoclonal antibody treatment of leukemic bone marrows prior to bone marrow transplantation.

One of the major directions of this program is investigation of the human cellular immune system. The effect of cell-cell interactions and how they may be compromised or magnified by monoclonal antibodies that specifically react with different human hematopoietic cell populations could be a valuable avenue of investigation. In vitro assays that study monocyte, granulocyte and natural killer activity are extensively studied throughout the Biological Therapeutics Branch and the effect of monoclonal antibodies on such functions can be readily evaluated. The possible in vitro role of such antibodies could conceivably be extended to in vivo trials utilizing these various monoclonal antibodies either by themselves or mixed with effector cells.

PROPOSED COURSE

Further investigations of the antibodies developed against human monocytes and granulocytes are ongoing, to compare them to other antibodies that are currently available either commercially or from other investigators. Most of the reports in the literature have not described immunoprecipitation of the antigens identified by their antibodies and it has therefore been difficult to compare our antibodies with most of these other antibodies. It is apparent that neither the monocyte or granulocyte antibodies will be useful in the classification or treatment of myeloid leukemias but may serve a more useful purpose in understanding the differentiation of human myeloid cells. For instance, it is rather unusual that the mature granulocyte will express an antigen while none of the precursor cells or other myeloid-type cells such as monocytes express the antigen. Similar to the OKM1 monoclonal antibody, our monocyte monoclonal antibody reacts with human monocytes, granulocytes, and large granular lymphocytes. It will be interesting to directly compare our antibody with the OKM1 antibody. The human eosinophil monoclonal antibody, also an IgG₁, reacts with granulocytes, monocytes, and eosinophils. The occurrence of an isotype switch from an IgM to an IgG₁ and the apparent change in antigen specificity are most unusual and warrant further investigation. Some of our future experiments will include attempts to absorb out the activity of the IgG₁ antibody with purified lacto-N-fucopentaose III. The monoclonal antibodies developed against human platelets in preliminary experiments appear to have a wide range of inhibitory effects on human platelets including inhibition of platelet aggregation, serotonin release, and platelet adherence.

PUBLICATIONS

Fitchen, J.H., Foon, K.A., and Cline, M.J.: The antigenic characteristics of hematopoietic stem cells. N. Eng. J. Med. 305: 17-25, 1981.

Foon, K.A., Schroff, R.W., and Gale, R.P.: Surface markers on leukemia and lymphoma cells: Recent advances. Blood 60: 1-19, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09238-02 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibodies Against Human Lung Carcinomas

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Paul G. Abrams, Expert, BTB, NCI

COOPERATING UNITS (if any)

NCI-FCRF; University of Southern California

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nine murine monoclonal antibodies with specificity for human non-small cell bronchogenic carcinomas, as determined by screening against human tumor cell lines and by immunohistochemistry on pathology slides of human tumors, have been developed. These antibodies react with squamous cell lung carcinomas and with some bronchogenic adenocarcinomas, but do not react with adjacent normal lung in immunoperoxidase studies of fixed human tissues. One antibody, 353H10, has been shown to precipitate a protein of 143,000 daltons from cell lysates and cell membranes of positively reacting cells and nothing from cells which do not react by radioimmunoassay. Preliminary data indicates a lethal effect on cells bearing the 143,000 dalton antigen when incubated in vitro with 353M10 conjugated to the A-chain of abrin. A nude mouse model system with animals bearing human squamous cell carcinomas has been developed to test the efficacy of the 353H10/abrin conjugate in vivo. Further characterization of the antibodies against a panel of normal tissues is also now in progress.

PROJECT DESCRIPTION

PERSONNEL

A. Charles Morgan	Deputy Section Head	MAS	BTB	NCI
Clive Woodhouse	Visiting Fellow	MAS	BTB	NCI
Mehmet Fer	Visiting Scientist	CIS	BTB	NCI

OBJECTIVES

- (1) Development and characterization of murine monoclonal antibodies against the major histologic subtypes of bronchogenic carcinoma.
- (2) Development of in vitro and in vivo experimental systems to determine the efficacy of these antibodies in killing tumor cells when conjugated to toxins, and for localization of tumors in vivo when the antibodies are conjugated with radioisotopes.
- (3) Determination of the specificity of these antibodies by immunohistochemistry on human organ specimens obtained at surgery.
- (4) Selection of those antibodies demonstrating the best specificity for human tumors and the greatest in vitro cytotoxic activity as toxin-conjugates for therapy trials in animals and patients.

METHODS EMPLOYED

Murine monoclonal antibodies have been developed by immunization of BALB/c mice with whole cells, cell membranes, or cell extracts of human bronchogenic carcinoma cell lines and/or nude mouse xenografts. Splenocytes are harvested and fused with murine myeloma lines 653 or NS-1. Hybridoma supernatants are tested against the immunizing preparation, B cells and T cells, and a cell line of the tumor type used for immunization. Hybridomas exhibiting selectivity are cloned, screened against a large battery of cell lines, and then against cryostat sections of human tumors and normal tissues. Cytofluorograph analysis is employed to determine the percentage and intensity of stained cells with the antibodies. Cell surfaces are labeled by the iodogen method, and cells are metabolically labeled with S-35 methionine in methionine-deficient medium. Identification of the antigen detected by monoclonal antibodies is accomplished by indirect immune precipitation coupled with SDS-PAGE. Immunoperoxidase staining of paraffin-embedded, formalin-fixed tissues and cryostat sections is performed by the biotin-avidin technique as described by Hsu.

After purification from ascites fluid, monoclonal antibodies with specificity for human bronchogenic carcinomas are conjugated to the A-chains of plant toxins. In vitro sensitivity of cultured cell lines to the A-chain alone, the unconjugated antibody, and to the A-chain/antibody is determined by colony formation and thymidine uptake. When antigen positive cells are killed by the

A-chain/antibody conjugate, correlation with antigenic modulation in the presence of antibody is sought. Nude mouse xenografts are developed and used to demonstrate regression of xenografts.

MAJOR FINDINGS

A panel of murine monoclonal antibodies exhibiting varying specificities for squamous cell carcinoma of the lung has been developed, screened and partially evaluated. MA 353H10 reacted with squamous cell carcinoma of the lung cell lines, and some adeno- and large cell lines but not with small cell carcinomas. Although there was some reactivity with a T-cell lymphoma cell line and peripheral blood monocytes, immunohistochemical analysis revealed selective binding to cell membrane components on virtually all the tumor cells of 7/7 squamous carcinomas of the lung and 2/7 adenocarcinomas of the lung. Stromal cells, invading mononuclear cells, and normal lung tissues in the same sections did not stain. MA 353H10 precipitates a protein of 143,000 daltons from cell lysates and labeled cell membranes of positively reacting cells. Preliminary evidence indicates the ability of 353H10/abrin A-chain conjugate to kill the squamous cell carcinoma line SK-MES-1 in vitro. MA 353C7 reacted only with squamous cell carcinoma cell lines and is currently being grown in ascites form for evaluation by immunoprecipitation and immunohistochemical techniques.

SIGNIFICANCE

Murine monoclonal antibodies specific for bronchogenic carcinomas promise to be important reagents for the early diagnosis of this disease, for which surgical therapy with curative potential is available for less than 15% of the 120,000 new cases occurring annually in the United States. Detection of antigen in the blood or urine of asymptomatic individuals, or imaging with injected antibody in high-risk groups may significantly increase the percentage of those who may be cured surgically. Additionally, we are exploring the use of monoclonal antibody/toxin conjugates to effect regression of established tumors with minimal toxicity. Accordingly, monoclonal antibodies have a high priority at the Biological Response Modifiers Program.

PROPOSED COURSE

- (1) Murine monoclonal antibodies (MoAbs) against bronchogenic carcinomas shall be further evaluated with immunohistochemistry against a wider panel of normal tissues. These MoAbs shall also continue to be developed for use in diagnostic imaging and therapy, initially with tumor xenografts in nude mice. In addition, MoAbs 353H10 and/or 353C7 shall be conjugated to known chemotherapeutic agents to parallel our work with potent plant toxin/antibody conjugates.
- (2) Use of membranes and membrane extracts to immunize mice with human large cell and mixed large cell/small cell tumors of the lung, derived from xenografts established at the BRMP, to produce additional monoclonal antibodies against bronchogenic carcinomas, is underway.

(3) Development of an ELISA system to determine the presence of the antigens detected by these MoAbs in the sera and urines of patients and normals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09239-02 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Monoclonal Antibodies Against Human Carcinoma

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Paul G. Abrams, Expert, BTB, NCI

COOPERATING UNITS (if any)

LMI, BRMP, NCI; NCI-FCRF

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Monoclonal Antibody Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human monoclonal antibodies with specificity for human malignancies will be important biological agents, both to investigate the nature of the human immune response to neoplastic disease and for diagnostic and therapeutic purposes. The two key elements necessary for success in this field are: 1) development of sensitized lymphocytes antibodies to tumor-associated antigens; and 2) development of adequate human myeloma cell lines for fusion. In collaboration with the Lymphokines Section, LMI, this laboratory has developed a model for in vitro sensitization of human peripheral blood lymphocytes and subsequent fusion to a human myeloma cell line to produce antigen-specific human monoclonal antibodies. This laboratory has also determined the optimal human myeloma cell lines for construction of human hybridomas secreting antibodies. Current projects involve sensitization of lymphocytes with melanoma-associated antigen (250K) which has proved successful and then fusion of these lymphocytes with a human myeloma cell line.

PROJECT DESCRIPTION

PERSONNEL

A. Charles Morgan Deputy Section Head MAS BTB NCI

OBJECTIVES

- (1) Development of in vitro sensitization system, in collaboration with the Lymphokines Section, LMI, to induce lymphocytes to secrete antibodies specific for human tumor-associated antigens.
- (2) Determination of the optimal human cell lines for human monoclonal antibody production.
- (3) Development of human hybridomas secreting monoclonal antibodies specific for human melanoma, lung carcinoma and colon carcinoma.

METHODS EMPLOYED

Human lymphocytes, obtained from peripheral blood of patients treated with murine monoclonal antibody 9.2.27 (anti-250K melanoma-associated antigen), are sensitized in vitro and these cells are fused with human myeloma line HF-2, supernatants from both in vitro sensitization cultures and subsequent hybridoma cultures are tested for reactivity with preparations rich in the 250K antigen and a known negative control, by an enzyme-lined immunosorbent assay. Cloning and retesting of selective wells is carried out. Lymph nodes from patients with bronchogenic carcinoma and colon carcinoma are fused directly with HF-2 and selectively positive hybridomas are cloned and retested. Screening and evaluation is similar to that for murine monoclonal antibodies: determination of the antigen recognized by indirect immune precipitation and determination of its expression in normal and malignant tissues by immunohistochemistry.

MAJOR FINDINGS

Human myeloma cell lines deficient in HGPRT and exhibiting death in HAT medium have been developed by the principal investigator or obtained from other sources. These cell lines have been compared for fusion efficiency, death in HAT, growth rates and antibody secretion. One line has been selected on the basis of these characteristics for use in human monoclonal antibody production (HF-2). In collaboration with the Lymphokines Section, LMI, an in vitro sensitization system has been worked out to produce lymphocytes secreting antigen-specific antibodies, which have been successfully fused with the human myeloma line U-266 to produce human monoclonal antibodies specific for the sensitizing antigen (tetanus toxoid).

Following in vitro sensitization of melanoma patients' cells with a preparation rich in the 250K melanoma-associated antigen, human hybridomas have been produced which secrete antibody which appear, from preliminary evaluation, to exhibit specificity for the immunizing preparation. These hybrids are being expanded and cloned to try to establish stable antibody-producing hybridomas with specificity for the 250K antigen or other melanoma-associated antigens.

SIGNIFICANCE

Human monoclonal antibodies have two potential advantages over their murine counterparts: (1) they may detect more narrow tumor-associated specificities, which are immunogenic to the cancer patient, than those recognized by a heterologous species; and (2) the antibodies may be less immunogenic, thereby making repetitive administration less toxic. In addition, from the standpoint of tumor biology, human monoclonal antibodies may provide some insight into the repertoire of responses that patients are capable of making against their own tumors. Therefore, human monoclonal antibodies as well as murine monoclonals have a high priority in the Biological Response Modifiers Program.

PROPOSED COURSE

Fusions of human myeloma lines with human lymphocytes obtained from melanoma patients treated with murine monoclonal 9.2.27 and then sensitized in vitro with the 250K antigen by the Lymphokines Section is currently in progress and will be pursued. In addition, lymph nodes from patients with bronchogenic carcinoma and colon carcinoma shall be fused with human myeloma cells. The human hybridoma supernatants shall be screened for specificity as described above.

PUBLICATIONS

Abrams, P. G., Knost, J. A., Clarke, G., Wilburn, S., Oldham, R. K. and Foon, K. A.: Determination of the optimal human cell lines for human monoclonal antibody production. J. Immunol. (In Press)

Rossio, J., Knost, J., Pickeral, S. and Abrams, P. Human-human hybridomas producing antigen-specific IgG using in vitro immunized peripheral blood cells as fusion partners. J. Clin. Invest. (In Press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09253-01 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Immunoconjugates for Cancer Therapy and Diagnosis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert K. Oldham, Associate Director, BRMP, NCI

COOPERATING UNITS (if any)

Clinical Center, NIH; NCI-FCRF; LPP, NCI

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are evaluating the use of monoclonal antibodies (MoAbs) as carriers for cytotoxic agents and radioisotopes, to increase both the selectivity of the agents for cancer therapy and the sensitivity of radiodetection of occult tumor. Several immunoconjugates, composed of A chain of abrin toxin or diphtheria toxin and monoclonal antibodies, (i) D3, to the guinea pig L10 hepatocarcinoma, (ii) 9.2.27 to human melanoma, and (iii) human squamous carcinoma, were shown to retain antigen specificity. The therapeutic effect of D3-A/abrin conjugate was assayed and was capable of inhibiting the tumor growth of primary tumor and delaying or abolishing tumor metastases to lymph nodes. Long-term remission was induced in some tumor-bearers. We have also established two human tumor xenografts in nude mice which will provide models for testing the therapeutic activity of immunoconjugate against human tumor cells. In contrast to the D3 system, the 9.2.27 antimelanoma antibody could inhibit tumor growth in nude mice in unconjugated form, presumably through interaction with effector cells. To study antibody distribution in tumor-bearing animals, MoAb (9.2.27 and D3) were labeled with I-125. Both radioactive MoAb were selectively localized in primary tumors. In addition, I-125-D3 was capable of reaching metastatic tumor in lymph nodes. These studies indicated these MoAb have promising potential for early detection of occult tumor and for selective targeting of therapeutic agents.

PROJECT DESCRIPTION

PERSONNEL

A. Charles Morgan, Jr.	Deputy Section Head	MAS	BTB	NCI
John W. Pearson	Microbiologist	MAS	BTB	NCI
Paul G. Abrams	Expert	MAS	BTB	NCI
Gowsala Pavanasisivam	Visiting Fellow	MAS	BTB	NCI

OBJECTIVES

The overall objective of this project is to develop new immunoconjugates with potential for the diagnosis and therapy of cancer. In general this will be achieved by conjugating isotopes for radioimaging or cytotoxic agents with various monoclonal antibodies directed toward tumor-associated antigens. The immunoconjugates are tested first in vitro and then in an animal model, and the information generated may provide the basis for similar approaches in man. The specific aims are: (1) To produce several immunoconjugates consisting of the A chain of abrin toxin and different monoclonal antibodies: D3 (reactive with L10 hepatocarcinoma), 9.2.27 (reactive with human melanoma), and H10 (directed toward human squamous carcinoma of lung). (2) To produce drug-conjugates with the above antibodies. (3) To label monoclonal antibodies with radionuclides. (4) To evaluate both the therapeutic and diagnostic potentials of new conjugates in animal models. (5) To obtain information for the design of clinical studies with immunoconjugates.

METHODS EMPLOYEDI. Immunochemical

Affinity column chromatography, gel permeation, SDS-slab gel electrophoresis, reverse-phase high-performance liquid chromatography (HPLC), UV-visible scanning spectroscopy, flow cytometry (Ortho, Model 2150) using indirect immunofluorescent detector.

II. Radiolocalization

Monoclonal antibodies were labeled by either ^{125}I or ^{111}In and injected i.v. The uptake of MoAb by tumors and tissues was determined either by scintigraphic imaging using a gamma camera or by direct counting of radioactivity.

III. In Vitro Cytotoxicity

The cytotoxicity of conjugate or toxin subunits was assayed by (A) inhibition of in vitro translation; (B) inhibition of cell proliferation as measured by protein synthesis and DNA replication in intact cells; (C) viability by trypan blue exclusion. Colony formation assays were also employed for monolayer cells.

IV. Toxicity and Therapeutic Activity of Immunoconjugates In Vivo

Syngeneic guinea pigs bearing established L10 hepatocarcinoma and nu/nu mice with xenografted human melanoma or human squamous carcinoma of the lung were employed. The toxicity of the immunoconjugate was assayed by the loss of body weight or death of the animal. The therapeutic efficacy was evaluated by measuring tumor volume and survival.

MAJOR FINDINGS

I. In Vitro and In Vivo Studies on the Immunoconjugate (D3-A/abrin and D3-A/diphtheria toxin) Against L10 Hepatocarcinoma

The toxic A chain of abrin was isolated by affinity chromatography and was demonstrated to be a potent inhibitor of protein synthesis ($ID_{100} = 1 \times 10^{-9} \text{ M}$). A chain was coupled to a purified MoAb (D3) directed against L10 hepatoma. The immunoconjugate was functionally active, i.e., with binding to tumor cells in vitro and inhibition of their protein synthesis and was cytotoxic at $5 \times 10^{-9} \text{ M}$ toward antigen-bearing cells in vitro. Several antigen-negative cells were less susceptible to its cytotoxic effect. The cytotoxicity of conjugate appeared to be antibody-mediated, since pretreatment of cells with an excess of free antibody diminished the cytotoxicity of the immunoconjugate. The therapeutic efficacy of the conjugate was assayed by injecting a single dose s.c. or i.v. into syngeneic guinea pigs bearing L10 tumors. The in vivo studies showed that (1) conjugate at a dosage of 60-1120 μg per guinea pig was not toxic; (2) conjugate decreased or abolished the growth of established solid tumor; (3) conjugate delayed or inhibited tumor metastasis to lymph nodes and 20-40% of animals in some treated groups had a long-term complete regression.

The A chain of diphtheria toxin was coupled to D3. This conjugate was cytotoxic to L-10 hepatoma at $5 \times 10^{-8} \text{ M} - 5 \times 10^{-10} \text{ M}$, depending on the preparations. One less toxic preparation was found to have therapeutic effects against L-10 hepatoma in vivo. The therapeutic effect of a more toxic preparation is under investigation.

II. Studies on Immunoconjugates Against Human Melanoma and Against Squamous Carcinoma of the Lung

We are currently using a similar approach with two monoclonal antibodies against human tumors: 9.2.27, reactive with human melanoma; and H10, a recently developed antibody reactive with human squamous carcinoma of the lung. Both monoclonal antibodies were coupled to the A chain of abrin. Preliminary data indicate that H10 conjugate is quite potent in inhibiting colony formation of human squamous cell carcinoma. The ID_{100} was 0.5 $\mu\text{g}/\text{ml}$ and the conjugate was unreactive against another line of cultured human tumor cells (melanoma) up to 10 $\mu\text{g}/\text{ml}$. 9.2.27-A/abrin conjugates were tested against 3 human melanoma cell lines: FeMX Met was susceptible (ID_{100} , 0.1 $\mu\text{g}/\text{ml}$) but another antigen-positive cell line, A875 and an antigen-negative line, Lox, were not. Thus, the presence of target antigen is not sufficient for killing.

III. Establishment of Human Melanoma and Squamous Carcinoma in Xenografts (BALB/c nude/nude) for Evaluation of Therapeutic Activity of Immunoconjugates

To establish an animal model for testing the therapeutic effectiveness of the 9.2.27 antibody and its conjugates, a malignant human melanoma (FeMX Met) was inoculated s.c. into BALB/c nude mice (3 to 8 weeks old). The minimum dose to obtain 100% takes was 5×10^5 cells/mouse. 9.2.27 selectively localized in tumor and in unconjugated form delayed or completely suppressed tumor growth, depending on tumor burden.

Most recently, a human squamous carcinoma of the lung xenograft was also established for evaluating the H10 conjugate.

IV. Radiolocalization Studies with D3 in Guinea Pigs and 92.27 in Nude Mice

To determine the distribution in tumor and normal tissues, 9.2.27, labeled with ^{125}I , was inoculated i.v. It selectively localized in tumors, with a ratio of specific activity tumor/liver = 2.2, tumor/kidney = 3.3, and tumor/muscle = 5.5 (at 54 hours). Similarly, ^{125}I -D3 selectively localized in a dermal tumor, with tumor/blood = 2.0, tumor/kidney = 3.3, and tumor/liver = 17 (at 120 hours). In collaboration with Dr. John Weinstein, LPP, NCI, it was found that ^{125}I -D3 administered s.c. can also be selectively localized in metastatic tumor in lymph node and distal axillary node with ratios of tumor/blood = 172 and tumor/liver = 600.

PROPOSED COURSE

This project will continue to produce and evaluate new types of conjugates. We will continue to emphasize use of D3, 9.2.27, and H10 antibodies. Cytotoxic agents under evaluation include abrin and diphtheria toxin, and the drugs adriamycin, CC1065 (NSC298223) and anguidine. Cytotoxic agents will be tested in vitro against antigen positive and negative cells before in vivo assessment. Results have shown that the cytotoxicity to the tumor cells (L10, human melanoma, and squamous carcinoma) is apparent in the range of 100 pg to 100 ng/ml (>99% cytotoxic dose).

I. Expand the Investigation on Immunoconjugates Against Human Melanoma and Squamous Cell Carcinoma In Vitro and In Vivo

The human tumor cell lines which are sensitive to immunoconjugates, FeMX Met and SK Mes, will be heterotransplanted into nude mice. The therapeutic activity of specific and nonspecific conjugates will be defined by varying the dose of conjugate, route of administration, and tumor burden.

II. Continue Investigation on D3 Conjugate in L10/Guinea Pig Model

It is established that D3-A/abrin has therapeutic effects against established primary L10 hepatoma and metastatic tumor. We will investigate the optimal therapeutic regimen for this tumor. The main emphasis will be on dose and

frequency of administration, tumor burden, (primary vs. metastatic tumor), and route of administration. Attempts will also be made to minimize non-specific toxicity of immunoconjugate by administering an unrelated antibody in an attempt to block the uptake of immunoconjugate by RES or vital organs; by preventing the release of abrin A chain from the immunoconjugate by agents which will stabilize the disulfide linkage; and by RES blockade with agents such as carrageenan.

III. Investigate Mechanisms by which Immunoconjugates Kill Tumor Cells

We will emphasize (1) internalization of immunoconjugates determined by the immunoperoxidase technique or flow cytometric analysis; (2) antigen-variants of tumor cells; (3) the nature of target cells which undergo endocytosis of immunoconjugate. These studies will make use of the two antigen-positive melanoma lines, one sensitive and the other insensitive to immunoconjugate.

SIGNIFICANCE

This project represents a systematic approach to the design of therapeutic and diagnostic agents for solid tumors. Immunoconjugates, composed of the A chain of abrin and some monoclonal antibodies at nontoxic doses, have been shown to induce tumor regression and inhibit tumor metastasis. A more basic understanding of the cellular mechanisms involved in antigen-specific and nonspecific toxicity should enable us to enhance the therapeutic value of immunoconjugates.

PUBLICATIONS

Bernhard, M. I., Hwang, K. M., Foon, K. A., Keenan, S., Kessler, R. M., Frincke, J. M., Tallan, D. J., Hanna, M. G., Peters, L. and Oldham, R. K.: Localization of ¹¹¹indium and ¹³¹iodine labeled monoclonal antibody in guinea pigs bearing L10 hepatocarcinoma. Cancer Res. (In Press)

Key, M. E., Bernhard, M. I., Hoyer, L. C., Foon, K. A., Oldham, R. K. and Hanna, M. G. Guinea pig L-10 hepatocarcinoma model for monoclonal antibody serotherapy: In vivo localization of a monoclonal antibody in normal and malignant tissues. J. Immunol. 130: 1451-1456, 1983.

Oldham, R.K. Monoclonal antibodies in cancer therapy. J. Clinical Oncology. (In Press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09228-03 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Further Characterization of Natural Cell-Mediated Immunity in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Craig W. Reynolds, Staff Fellow, BTB, NCI

COOPERATING UNITS (if any)

LCC, NCI-FCRF; IB, DCBD, NCI; Pfizer Central Research Labs; Uppsala Univ., Sweden; UCLA; McMaster Univ., Canada; REP-Inst. of the Organ. for Health Res. TNO, Netherlands

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present studies in rats have further characterized the natural killer (NK) cell system in rats. Results using a wide variety of target cells have shown that the naturally cytotoxic effector cells for normal fibroblasts and bone marrow targets, lymphomas/leukemias, embryonic cell lines, and solid tumor targets are all included in the large granular lymphocyte (LGL) subpopulation, further emphasizing the importance of these cells as an antitumor effector mechanism. In addition, immunocytochemical localization of LGL in incidental and pathological lesions, and in lymphoid and nonlymphoid tissues of nude rats suggests that these cells play a role in the first lines of defense against not only tumors but also infectious agents. Other studies with the transplantable LGL leukemias in F344 rats have demonstrated a great deal of similarity with normal LGL, including similar cell surface antigens, histochemistry, and functional activity against NK-susceptible/resistant target cells. A number of similarities were noted between these LGL tumors and some previously reported cases of human Ty-CLL. Further biochemical analysis of the rat LGL leukemias has resulted in the isolation of cytoplasmic granules which contain highly cytolytically active material(s). Further analysis of these molecules should help to elucidate the lytic mechanism of NK cells.

PROJECT DESCRIPTION

PERSONNEL

Ronald B. Herberman

Chief

BTB NCI

OBJECTIVES

The objectives of this project are: (1) to investigate, in detail, the range of specificity for NK cells using normal bone marrow and adherent and nonadherent tumor targets; (2) to evaluate the histological distribution of NK cells in healthy, tumor-bearing, and cachectic nude rats; and (3) to further characterize the transplantable spontaneous LGL leukemias in F344 rats and to use these highly cytotoxic cells for the isolation of specific intracellular molecules responsible for target cell lysis.

MAJOR FINDINGSI. Use of Normal Bone Marrow and Adherent Tumor Cells for In Vitro NK Targets

Natural cytotoxicity in the rat was assessed against solid tumors and normal and embryonic rat monolayer culture lines, and the results compared with rat NK activity toward syngeneic, allogeneic, and xenogeneic lymphoma and bone marrow targets. The targets tested showed a very wide range of susceptibility to lysis by peripheral blood and spleen effector cells, in both 4- and 18-hour ⁵¹Cr cytotoxicity assays. The cytotoxicity against all of the targets tested was not age restricted and was potentiated by rat interferon. Natural cytotoxic reactivity was seen with effector cells from the blood, spleen, and peritoneal cavity of both W/Fu (euthymic) and rnu/rnu (congenitally athymic) rats. Cytotoxic effector cells from the blood were present in the low density fractions recovered from discontinuous Percoll density gradients and, as previously shown for lymphoma target cells, a strong correlation was observed between the killing of embryonic, normal and solid tumor targets, and the presence of LGL. These results indicate that the naturally cytotoxic rat effector cells for normal fibroblast and bone marrow targets, lymphomas/leukemias, embryonic cell lines, and solid tumor targets are all included in the LGL subpopulation.

II. Immunoperoxidase Localization of LGL in Normal Tissues and Lesions of Athymic Nude Rats

Although LGL have been shown to be responsible for NK cell and antibody-dependent cell-mediated cytotoxic (ADCC) activities in human, rats, and mice, a comprehensive survey of the histological localization of LGL in the lymphoid and non-lymphoid organs has not been previously reported. In collaboration with Dr. Jerrold Ward (LCC, NCI), the immunocytochemical localization of cells reacting with an LGL-specific monoclonal antibody (OX-8) was studied in athymic nude rats by the avidin-biotin-peroxidase complex immunoperoxidase technique. The tissues with the greatest density and percentages of LGL included the paracortex

of lymph nodes, bronchial-associated lymphoid tissue, the medullary cords and sinuses of lymph nodes, and intestinal epithelium. Ultrastructural analysis of the paracortical areas revealed granular lymphocytes in close association with interdigitating cells. Tissues with the fewest LGL included bone marrow, B-cell areas of lymphoid tissues, and parenchymal epithelial organs. In healthy and cachectic nude rats, various incidental and pathological lesions contained a significant number of LGL. Large numbers of LGL were also seen in suppurative pneumonic lesions, enteritis, focal ulcerative epithelial lesions, papovaviral sialoadenitis, and also subcutaneous and pulmonary sites of MADB106 tumor growth. The observation of a large number of OX-8 positive LGL in nonlymphoid organs provides strong support for the hypothesis that these cells play a major role in the first line of defense against not only tumors but also infectious agents.

III. Characterization of Transplantable Spontaneous LGL Leukemias in the F344 Rat

A. Morphologic, antigenic and functional characterization

Six transplantable LGL tumor lines in F344 rats were examined for NK and ADCC activity and found to be highly cytotoxic, even at low effector to target ratios, for NK-susceptible targets, but were unreactive against an NK-resistant target [(C58NT)D] and a macrophage-susceptible target (P815). Three lines showed significant levels of lysis against antibody-coated tumor cells. Following in vivo transplantation, the levels of cytotoxicity steadily increased in three lines, remained high in one, and decreased in one. Tumor cells injected intraperitoneally spread via the lymphatics to regional lymph nodes, mediastinal nodes, blood and eventually the bone marrow. Leukemia occurred concurrently with organ enlargement and increased levels of NK. Studies in (F344 x W/Fu)_{F1} rats clearly demonstrated that the cytotoxic cells from leukemic animals were the transplanted tumor cells themselves and not merely the activation of normal host LGL. These results demonstrate that naturally occurring transplantable LGL leukemias are an easily obtainable and excellent source of materials for those studies requiring a large number of functionally active LGL.

B. Isolation of cell surface receptors and intracellular molecules responsible for target cell lysis.

In collaboration with Drs. Pierre and Maryanne Henkart (Z01CB05018-13 I), we have examined a number of LGL leukemias for detailed biochemical, enzymatic, ultrastructural, and histochemical characteristics. Since these studies require a large amount of material they can be done only with cytolytically active LGL tumors. The present studies have clearly shown that cytolytic molecules could be isolated from the cytoplasmic granules of tumors but not from other tumor lines or normal rat liver or T cells. Presently, a number of studies are underway to examine the exact biochemical nature of these molecules in an attempt to ascertain the mechanism of NK cell killing. In addition, we are studying the cellular specificity of these molecules using normal cells and NK-susceptible or resistant tumor targets.

SIGNIFICANCE

Recent data in experimental animal systems has suggested that natural cell-mediated immunity may play a significant role not only in immune surveillance against tumors but also in resistance to viral infection and rejection of bone marrow grafts. The further understanding of this system in experimental animal systems should greatly facilitate the understanding of the significance of natural cell-mediated immunity in man. Our results clearly demonstrate that LGL can kill both leukemia and solid tumors as well as normal embryo fibroblasts and bone marrow cells. Our observation that large numbers of LGL can be found in the epithelium of normal and virally infected rats is further evidence that these cells play an important role in the first line of defense against viral infections. Our finding that LGL tumors can be identified in aged rats provides us with a large source of highly active cells for the isolation and analysis of target receptors, cytoplasmic granules, and lytic machinery. These cell lines also provide us with enough material to examine mitogen and/or antigen stimulation and lymphokine production by NK cells. These results should greatly facilitate studies to examine the overall function, lytic mechanism, and relevance of NK cells in human tumor systems.

PROPOSED COURSE

Further studies on the natural killer cell system in rats will be continued. These studies are carried out in the rat since the OX-8 monoclonal antibody is a highly specific marker for NK cells in nude rats and large numbers of active LGL can easily be obtained from the spontaneous LGL leukemias. Specifically, our efforts will be directed toward a further examination of LGL leukemias for the biochemical composition and molecular mechanisms involved in the binding and lysis of NK-susceptible tumor cells.

The further examination of LGL tumors will include a number of morphological, biochemical and functional studies. Specifically, we will continue to examine these cell lines for enzyme markers, ultrastructural morphology and histochemistry. We have also begun studies to disrupt these tumor cells and to isolate cytoplasmic granules, membrane target receptors and cytolytic molecules. Since these biochemical studies require a large number of cells, they can only be done with cytolytically active LGL tumors. A further examination of the functional nature of these cells will include experiments to determine if they proliferate in response to mitogens, antigens, or other BRMs and to investigate whether these tumor lines produce significant quantities of lymphokines produced by normal LGL (IFN, IL-1, IL-2, CSF).

PUBLICATIONS

Rees, R. C., Reynolds, C. W. and Herberman, R. B.: Rat natural killer cell activity against lymphoid and non-lymphoid tumor cells, and normal rat cell lines. Cancer Res. (In Press)

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- Reynolds, C. W., Ortaldo, J. R., Denn, A. C., III, Barlozzari, T., Herberman, R. B., Sharrow, S. O., Ramsey, K. M., Okumura, K. and Habu, S.: Cell surface antigenic characteristics of rat large granular lymphocytes. In Herberman, R. B. (Ed.): NK Cells and Other Natural Effector Cells. Academic Press, New York, 1982, pp. 139-143.
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- Ward, J. M., Argilan, F. and Reynolds, C. W.: Immunoperoxidase localization of large granular lymphocytes in normal tissues and lesions of athymic nude rats. J. Immunol. (In Press)
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09246-15 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characteristics, Regulation and In Vivo Relevance of NK Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ronald B. Herberman, Chief, BTB, NCI

COOPERATING UNITS (if any)

NCI-FCRF; University of Perugia, Italy; University of Rome, Italy; DV, Office of Biologics; V. Babes Institute, Bucharest; MB, NCI

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mouse natural killer (NK) and natural cytotoxic (NC) cells have been shown to be closely associated with large granular lymphocytes (LGL), as has been found previously for man and rats. Procedures have been developed for highly purifying these natural effector cells, by centrifugation on Percoll density gradients and by elimination of contaminating T cells by treatment with monoclonal antibodies to Lyt 1 and 2 plus complement. Fluorescence flow cytometry studies have indicated that the mouse LGL express low amounts of Lyt 1 and no detectable Lyt 2. LGL have also been shown to account for the natural cytotoxic activity of human leukocytes against freshly harvested human tumors and it has been possible to augment such reactivity by culturing these LGL in the presence of interleukin-2. Detailed studies have been performed on the regulation of the development and reactivity of mouse NK cells. It has been shown that various biological response modifiers, including interferon, cause an in vivo increase in LGL in the spleen, along with a change in their physical characteristics. The characteristics of suppressor cells for mouse NK activity have been studied in detail and the relationship between these cells and the effector cells has been clarified. Mouse model systems for induction of hyporesponsiveness to augmentation of NK activity, after multiple inoculations of interferon, have been developed.

PROJECT DESCRIPTION

PERSONNEL

John R. Ortaldo	Deputy Section Head	NIS	BTB	NCI
Angela Santoni	Guest Worker	NIS	BTB	NCI
Isaac Blanca	Guest Worker	NIS	BTB	NCI
Antonio Procopio	Visiting Fellow	NIS	BTB	NCI
Llewellyn Mason	Microbiologist	NIS	BTB	NCI

OBJECTIVES

The objectives of this project are: (1) to determine the characteristics of natural effector cells in mice and in man; (2) to determine the factors regulating the levels of NK activity in mice; (3) to evaluate the role of natural cell-mediated immunity in resistance against tumor growth.

MAJOR FINDINGSI. Characterization of Natural Effector Cells in Mice

It has been clearly shown that cells with a characteristic morphology, termed large granular lymphocytes (LGL) are responsible for human and rat NK activity. In contrast, characterization of mouse NK cells has been considerably more difficult. However, by modification of the conditions used for Percoll density gradient centrifugation, it has been possible to enrich LGL in low density fractions and to obtain high density fractions of lymphocytes that are virtually devoid of LGL. The LGL-enriched subpopulation has been shown to be enriched for NK activity and the LGL-depleted subpopulations have had no NK activity. The LGL-enriched fractions still have substantial contamination with typical T lymphocytes and efforts have been made to eliminate these cells and to study the effects of such treatment on NK activity. It has been possible to effectively eliminate T cells by pretreatment of spleen cell preparations with monoclonal antibodies to Thy 1, Lyl 1 and 2 plus complement and the low density Percoll fractions of the residual cells have been shown to retain high levels of NK activity, are enriched in LGL, and consist primarily of cells with no detectable expression of Thy 1 or Lyl 2 and low expression of Lyl 1. As expected from previous studies, the NK-enriched populations have been shown to express asialo GM1 and the alloantigen NK1.2. With the development of this technology, we have examined the possible relationship between NK cells and natural cytotoxic (NC) cells. The LGL-enriched population has been shown to contain most of the NC activity but some NC activity has also been detected in high density fractions.

II. Characteristics of Natural Effector Cells in Man

Studies have continued to characterize the natural effector cells reactive against primary, freshly harvested human carcinomas. LGL have been shown to

account for the detectable cytotoxic reactivity against autologous as well as allogeneic tumor cells. Recent emphasis has been placed on studies of reactivity against ovarian carcinoma cells, as a prelude to planned collaborative studies on therapy of ovarian ascites tumors with cultured NK cells. It has been possible to culture the LGL from ovarian cancer patients, in the presence of interleukin-2, and these cultured cells have been shown to have appreciable cytotoxic reactivity against autologous and allogeneic ovarian carcinoma cells as well as against typical NK target cells.

III. Regulation of Development and Reactivity of NK Cells

A. Activating factors

Studies have been performed to determine whether biological response modifiers (BRMs) that augment NK activity in vivo can affect the size or physical characteristics of the NK population, as well as activate the cytotoxic reactivity of the preexisting effector cells. Spleen cells from mice treated with various BRMs, including interferon, have been separated on Percoll density gradients and we have found that the NK activity is associated with LGL of larger size and lower density than found in normal, untreated mice. In addition, the total number of LGL in the spleens is substantially increased. Studies to date indicate that this increase is due to a proliferation of LGL rather than to a shift in the circulation patterns of NK cells.

As part of our evaluation of the immunomodulatory effects of various species of interferon, recombinant mouse gamma interferon and a human hybrid recombinant alpha interferon, A/D bgl, have been shown to augment mouse NK activity after administration in vivo. The ability of the human species of interferon to augment mouse NK activity provides a novel model for detailed immunopharmacologic studies of human interferon in an animal model system.

B. Negative regulatory factors

We have continued our studies to determine the mechanisms involved in the spontaneous or treatment-induced depression of NK activity. The immunoadjuvant, C. parvum, has been studied as a model agent for induction of suppressor cells for NK activity. Although some of the suppressor activity is due to adherent cells, nonadherent spleen cells from C. parvum-treated mice have been shown to account for much of the inhibition of NK activity. These suppressor cells were shown to be high density cells, completely separable from LGL and they also lacked markers characteristic of NK cells. Natural suppressor cells, in a low NK-reactive strain of mice, SJL, have continued to be studied in detail. Some clones of SJL NK cells have been developed and these have been shown to have substantial levels of NK reactivity, similar to that of other strains. However, even highly purified preparations of LGL from SJL mice have been shown to have rather low NK activity, which is boosted poorly by interferon. We have now obtained evidence for nonadherent suppressor cells within the LGL population, which appear to have the ability to bind to target cells but are inefficient in lytic ability. Thus, it appears that NK cells in SJL mice have a maturational or other defect in development of full lytic capability and that the poorly cytotoxic NK cells can competitively

inhibit cytotoxicity by other NK cells. A paradoxical finding in the clinical studies of therapy with interferon has been the failure of frequent, high doses of interferon to maintain augmentation of NK activity. Attempts have therefore been made to establish a mouse model system to examine such hyporesponsiveness to augmentation of NK activity, since understanding of this phenomenon might lead to more effective therapy. In collaboration with the BRMP Preclinical Screening Program, daily administration of mouse gamma interferon, and to a lesser extent A/D bgl human interferon, has been shown to result in a failure to sustain augmentation of NK activity. Studies are now in progress to understand in detail the mechanisms responsible for this hyporesponsiveness.

C. Circulation of NK cells

The peritoneal cavity of normal mice has negligible levels of NK activity, but after intraperitoneal inoculation of various BRMs, high levels of NK activity rapidly develop. It has been unclear whether this augmentation of peritoneal NK activity is due to influx of NK cells from other sites or to the activation and expansion of resident NK cells. To address this problem, radiolabeled LGL have been injected intravenously after intraperitoneal inoculation of a BRM. Virtually no efflux of the circulating LGL into the peritoneal cavity has been detected, indicating that the augmentation at this site is not due to migration of fully developed LGL. Studies are now in progress to determine whether the augmentation is due to resident cells or to the possible influx of NK-precursors from the bone marrow.

SIGNIFICANCE

For adequate understanding of the role of natural effector cells in resistance to tumor growth and how these effector cells may be involved in the therapy of cancer, there is a need to develop better insights into the characteristics of the effector cells, how they develop, how their activity is regulated, both naturally and in response to BRMs, and how they circulate in vivo. This information should lead to the development of rational protocols for optimal and sustained augmentation of NK activity, which may result in more effective therapy of cancer patients.

PROPOSED COURSE

Studies will continue on the possible relationships between NK cells and NC cells. Particular emphasis will be placed on determining whether NK cells represent a particular stage of differentiation in the same pathway of development as NK cells, or whether the two types of effector cells are derived from separate cell lineages.

Studies of NK activity of cancer patients against autologous tumor cells will be continued, with an emphasis on studies of patients with ovarian carcinoma. NK cells will be obtained from patients prior to chemotherapy or radiotherapy, and these cells will be expanded in culture for possible therapeutic attempts

by intraperitoneal infusion. Studies on the mechanisms for the hyporesponsiveness to augmentation of NK activity after repeated, frequent administration of interferon will be continued. The possible role for induction of macrophage suppressor cells will be examined as well as the possible development of lytically inactivated NK cells.

Detailed studies will be performed to determine the mechanisms involved in in vivo augmentation of NK activity by various BRMs, with particular emphasis on the contribution by proliferation of NK cells from precursors and by alteration in circulation patterns of NK cells or their precursors.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09247-03 BTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Natural Cell-Mediated Immunity in Man: Studies of Fresh LGL

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John R. Ortaldo, Biologist, BTB, NCI

COOPERATING UNITS (if any)

NCI-FCRF, Naval Medical Ctr.; Uniformed Services Univ. Health Sci.; MET, NCI; I, NCI; Bureau of Biologics; PO, NCI-FCRF

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human natural killer (NK) cells and K cells mediating antibody-dependent cellular cytotoxicity have been shown to be large granular lymphocytes (LGL). The majority of LGL form lytic conjugates with a wide variety of NK-susceptible target cells. The nature of the target cell structures involved in NK recognition are being studied and purified. This structure, isolated and partially purified from K562, has been shown to be a glycoprotein of 30-150,000 M.W. Maximal activity in a binding inhibition assay was seen when target structures were associated with lipids. NK cytotoxic factors (NKCF) are being examined for specificity and their mechanism of action. These NKCF are produced by LGL and have a general specificity pattern similar to intact killer cells. Fresh LGL subpopulations, cultures, and clones of LGL are being tested for reactivity against a variety of NK targets, to identify subsets that demonstrate functional selectivity. In addition, LGL have been shown to produce $IFN\alpha$ and γ in response to target cells or lectin. Additional lymphokines (interleukin 1 and 2, B-cell growth factor) and the LGL subset producing them, are being examined.

PROJECT DESCRIPTION

PERSONNEL

Ronald B. Herberman	Chief	BTB	NCI	
Issac Blanca	Guest Worker	NIS	BTB	NCI

OBJECTIVES

The objectives of this project are:

- (1) To study natural cell-mediated immunity to tumors in man and analyze the phenotypic, biochemical, and functional characteristics of the effector cells;
- (2) To study the nature and mechanism of cytotoxicity by LGL and to attempt to isolate and characterize soluble cytotoxic factors;
- (3) To analyze the interaction of natural effector cells with other components of the immune system;
- (4) To evaluate immune parameters in cancer patients, with regard to possible correlation with clinical course of disease

MAJOR FINDINGSI. Characteristics of Human NK Cells

A major advance in the characterization of human NK cells has come from the finding of their close association with a subpopulation of lymphoid cells, termed large granular lymphocytes (LGL). By Percoll density centrifugation and removal of cells forming rosettes with sheep erythrocytes at 29°, we have been able to reproducibly obtain fractions containing >90% LGL.

In studies with monoclonal antibodies, most, if not all, of the active NK cells were shown to be OKT10 positive and the majority were also OKM1 positive. A low proportion of the functional NK cells possessed the OKT8 or Ia antigens. Recently we have examined several new antibodies, B73.1, 3G8, and HNK1. B73.1 and 3G8 recognize the Fc receptor on LGLs and granulocytes, and react with >90% of LGL, and almost no other mononuclear leukocytes in the peripheral blood. Thus, the B73.1 and 3G8 antibodies appear to be very useful reagents for enumerating and separating LGL. In contrast, the HNK1 reagent (Leu 7) has been shown to recognize only about 50% of the LGL and to also react with an appreciable proportion of T cells.

We have recently utilized a limiting dilution assay with IL-2 to examine the nature of the peripheral and bone marrow precursors for NK cells. In contrast to the phenotype of most NK cells (OKT10, OKM1, B73.1 positive), the predominant peripheral progenitor was only positive for OKT11. Initial results indicate that

the bone marrow progenitors of NK-like activity are mainly positive for OKT10 and B73.1.

II. The Specificity of Human NK Cells

To directly study the nature of the target structures recognized by NK cells, we have begun a detailed biochemical analysis of materials inhibiting the binding of purified LGL to NK-susceptible targets. Solubilized membranes of K562 target cells, inserted into lipid vesicles, efficiently inhibited binding, but did not block lysis. The target structure(s) from K562 have been demonstrated to be glyco-protein(s) (sensitive to 65°C, sensitive to trypsin, adherent to Con A-Sepharose columns), 30-150,000 MW. The specific activity (based on 50% inhibition of binding) could be increased 200-fold by sequential lectin and sizing column chromatography. In addition, for several NK-sensitive targets, these NK target structures have been shown to be specific, not inhibiting ADCC or NK by non-human effectors (rat LGL).

III. Mechanism of Human NK Activity

Studies have been initiated to study cytotoxic factors produced by NK cells (NKCF). These factors have been shown to be produced in high quantity by NK cells after incubation with lectin or NK-susceptible targets. NKCF has a restricted pattern of lysis, similar to that of LGL. In addition, spontaneously transformed primate cell lines, which exhibit NK activity, have been shown to produce NKCF and should provide a good source for large scale production.

SIGNIFICANCE

Natural cell-mediated immunity may play an important role in immune surveillance against tumors. Understanding the in vitro role of natural immunity in human tumor systems should be very useful for assessing the significance of human natural cell-mediated immunity in vivo. The recent findings of the morphologic counterpart of NK cells greatly facilitates the studies of the mechanisms and relevance of NK cells in vivo and in vitro. The further characterization of phenotype of NK cells offers the ability to directly enumerate NK cells in clinical situations.

PROPOSED COURSE

Extensive studies on natural cell-mediated immunity against tumors will be continued. Much of our efforts will be focused around the recent finding that human NK cells are LGL: (1) A more extensive 2 parameter phenotyping of human LGL, especially attempts to find functional subsets of LGL. Particular focus will be on reagents which provide insight into the lineage of these cells, especially their possible relationship to either T cells or monocytes; (2) We are particularly interested in determining whether LGL have functions which have been associated with mature T cells and whether these cells produce interferon or other lymphokines in response to stimuli including tumor cells, tumor antigens and polynucleotides (such as poly I:C); (3) a detailed examination of the

biochemical mechanism involved in cytolysis by NK and ADCC. Studies of NKCF are planned to examine: (a) the specificity and regulation of production of NKCF, (b) the biochemical nature of NKCF, (c) the nature of the target structure that the NKCF binds to on the surface of NK-susceptible targets, (d) the relationship of NKCF to the lytic mechanism by LGL; (4) Studies are planned on a limited basis to examine the in vivo circulation of lymphocyte subpopulations as well as the possible in vivo differentiation of isotopically or fluorescein-labeled cells; and (5) the cytolytic activity of NK cells against autologous primary tumor cells and the reactivity of expanded and/or interferon-activated NK cells on autologous and allogeneic primary tumor cells.

Another area of continuing interest in our studies of NK cells is the characterization of the specificity of their interaction with target cells. Experiments will be continued to fractionate cell membranes of NK-susceptible target cells and characterize the nature of the structures involved in the conjugate formation between highly purified NK cells and target cells. Further separation and biochemical identification of soluble materials will be performed and reagents prepared (monoclonal antibodies) to purify, identify, and enumerate target cell structures.

PUBLICATIONS

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NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1CM09255-01 BTB

PERIOD COVERED

November 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of NK Cells in the Control of Tumor Growth and Metastasis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Eliezer Gorelik, Expert, BTB, NCI

COOPERATING UNITS (if any)

Laboratory of Molecular Immunoregulation, BRMP, NCI; NCI-FCRF

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2.1

PROFESSIONAL:

1.3

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Influence of syngeneic peritoneal macrophages ($M\phi$) on NK reactivity of mice and metastasis formation in the lungs was studied. Thioglycollate-elicited macrophages (TG- $M\phi$) inoculated i.v. caused the inhibition of the ability of mice to eliminate tumor cells from the lungs and a dramatic augmentation of metastasis formation. In parallel, inoculated TG- $M\phi$ suppressed the cytotoxic activity of spleen cells of the recipients and abrogated the stimulatory effects of poly I:C on NK activity as well as on resistance to metastasis. These effects were mediated only by TG- $M\phi$ but not resident $M\phi$ or $M\phi$ elicited by proteoseptone or *C. parvum*. Adoptively transferred, activated tumoricidal TG- $M\phi$ had no antimetastatic effect and were able to augment metastasis formation as well as nontumoricidal TG- $M\phi$. Both viable and disrupted TG- $M\phi$ induced neutrophil aggregation in the capillary beds of the lungs and probably released some substances with anaphylactic activity which may influence the NK reactivity of mice and permeability of the vessels for tumor cells. The involvement of NK cells in the antimetastatic effect of anticoagulant drugs was also investigated. Heparin had substantial inhibitory effects on the formation of metastases by B16 melanoma cells. However, in mice with NK activity depressed by pretreatment with anti-asialo GM1, the antimetastatic effect of heparin was completely abrogated. In contrast, stimulation of NK cell activity by poly I:C resulted in augmentation of the antimetastatic effect of heparin.

These data provide better understanding of some of the factors influencing the metastatic process and particularly add further evidence for the important role of NK cells in the control of the metastatic spread of tumors in mice.

PROJECT DESCRIPTION

PERSONNEL

Samuele Peppoloni

Visiting Fellow

NIS BTB NCI

OBJECTIVES

Our previous data demonstrated that NK cells may play an important role in the elimination of tumor cells and prevention of metastatic growth. $M\phi$ are also considered important elements in antimetastatic defense. Several attempts were made to explore the use of tumoricidal $M\phi$ in the adoptive immunotherapy of metastases. Adoptively transferred $M\phi$ may interact with NK cells and influence their contribution to antimetastatic defense. The main objectives of this project are (1) to determine in vivo interaction between $M\phi$ and NK cells by examining the influence of i.v. inoculated tumoricidal and nontumoricidal peritoneal $M\phi$ on NK cell activity and the formation of metastases in the lungs; and (2) to evaluate the possible role of NK cells in the antimetastatic effects of antiplatelet and anticoagulant drugs.

MAJOR FINDINGS

I. Influence of Adoptively Transferred Macrophages on NK Reactivity and Metastasis Formation in Mice

Several experimental data clearly indicate that $M\phi$ may regulate in vitro the activity of NK cells. $M\phi$ are important accessory cells in poly I:C-induced augmentation of NK cell activity in vitro. On the other hand, $M\phi$ can have profound inhibitory effects on in vitro NK cell function. Studies have now been performed to evaluate the possible in vivo interactions between $M\phi$ and NK cells, particularly in regard to the ability of NK cells to eliminate tumor cells from the blood stream and prevent metastasis formation. Mice were inoculated i.v. with peritoneal $M\phi$ and at various intervals inoculated i.v. with radiolabeled or nonlabeled tumor cells (YAC-1 lymphoma, B16 melanoma, or Lewis lung carcinoma, 3LL); then rapid elimination of tumor cells from the lungs and the number of metastatic foci developing in the lungs were evaluated. Thioglycollate-elicited macrophages (TG- $M\phi$) had potent inhibitory effects on the clearance of the radiolabeled tumor cells and also caused an increase in the number of metastatic tumors which developed in the lungs. The augmentation of metastasis formation was only observed when TG- $M\phi$ were inoculated before or shortly after tumor challenge, suggesting that they affected early steps in the metastatic process, before the tumor cells start to extravasate into the lung parenchyma. The augmentation of metastasis formation was mediated by killed or disrupted TG- $M\phi$ as well as by viable cells. In contrast, resident $M\phi$, $M\phi$ elicited by proteose-peptone or C. parvum, or $M\phi$ tumor lines (IC-21, PU5-1.8) had no effect on the survival of i.v. inoculated tumor cells or on the number of metastatic foci developing in the lungs. We have also found considerable differences among $M\phi$ elicited by various types of TG media, with Brewer's TG medium most effective and NIH TG broth having only

minimal effect. The augmentation of TG-M ϕ was dependent on i.v. transfer of the cells, since i.p. inoculated TG-M ϕ had no effect on lung metastases. Differences in the influence of TG-M ϕ or proteose-peptone elicited M ϕ (PM ϕ) on metastatic growth could be attributed to the differences in the pattern of migration after i.v. inoculation. ¹¹¹In-labeled TG-M ϕ were shown to arrest and remain preferentially in the lungs. In contrast, 1 to 2 days after i.v. inoculation, less than 2% of PM ϕ could be found in the lungs and more than 25% of the PM ϕ settled in the spleen.

Since tumoricidal M ϕ have been thought to have in vivo antimetastatic properties, it was of interest to evaluate the effect of tumoricidal C. parvum-stimulated peritoneal M ϕ (CPM ϕ) or MVE-2-stimulated TG-M ϕ on metastasis formation of B16 melanoma cells. Pretreatment with CPM ϕ had no antimetastatic effect, which could be explained by the observation that CPM ϕ preferentially migrated into the spleen. Although tumoricidal TG-M ϕ stayed in the lungs, they failed to prevent metastatic growth of i.v. inoculated B16 melanoma cells. On the contrary, tumoricidal and nontumoricidal TG-M ϕ had a similar augmenting effect on metastasis formation. Histological analysis of the lungs of mice inoculated with TG-M ϕ showed multiple early vascular foci containing mainly neutrophils, which were replaced 1 to 2 days later by emboli in the lung capillaries, containing a mixture of neutrophils and monocytes. A similar histological pattern was observed when disrupted TG-M ϕ were inoculated. In contrast, mice inoculated with other types of M ϕ showed no such lesions. Embolization induced by TG-M ϕ did not involve intravascular coagulation, since pretreatment of mice with heparin did not influence the histological picture in the lungs or the augmented growth of metastases. Since live and disrupted TG-M ϕ induced similar changes in the vascular bed of the lungs, it seems unlikely that the plugging of the capillaries could be a result of the mechanical obstruction by TG-M ϕ , but mostly by triggering some mechanisms which caused the aggregation of the polynuclear leukocytes. A similar picture in the lungs has been observed during anaphylactic reactions. Indeed, several substances (C5a complement fraction, chemoattractant, leukotriene B₄) with anaphylactic activity induced neutrophil aggregation and increased the permeability of the vessels. These reactions followed i.v. inoculation of TG-M ϕ and may influence NK cell function and metastasis formation in the lungs.

Our study of NK cell activity in mice with adoptively transferred M ϕ revealed that TG-M ϕ had depressed NK activity of spleen cells and abrogated the augmentation of NK cytotoxic activity following poly I:C stimulation. In contrast, other types of peritoneal M ϕ did not influence spontaneous or poly I:C-augmented NK activity. In parallel, it was shown that the antimetastatic effect of poly I:C was completely abrogated in mice receiving TG-M ϕ . Furthermore, TG-M ϕ inoculated into intact or poly I:C-treated mice had a similar augmenting effect on the growth of B16 melanoma in the lungs. In mice with low NK reactivity (after treatment with anti-asialo GM₁ serum or in beige mice), transfer of TG-M ϕ induced a further increase in the number of metastatic foci in the lungs. Thus, the augmentation of lung metastases by transfer of TG-M ϕ appeared to be due at least in part to suppression of NK activity. However, other mechanisms may also be involved, e.g., induction of increased permeability of vessels with promotion of extravasation of tumor cells into the parenchyma.

II. Role of NK Cells in the Antimetastatic Effect of Antiplatelet and Anticoagulant Drugs

Numerous experimental studies demonstrated a profound antimetastatic effect by the various drugs which prevent platelet aggregation and/or thrombus formation but the mechanism for this effect has not been clearly determined. We hypothesized that platelet aggregation or fibrin coagulation on the surface of tumor cells might prevent binding and killing of tumor cells by NK cells in the blood, and therefore, antiplatelet or anticoagulant drugs might facilitate destruction of metastatic cells. To assess this hypothesis, the effect of such drugs was evaluated in mice with depressed NK reactivity and in mice with NK augmented by an IFN inducer. In contrast to reports that prostacycline (PGI₂), which inhibits platelet aggregation, had antimetastatic effects, we found no effect of PGI₂ on the formation of the artificial metastases by B16 melanoma or the Madison lung carcinoma, M109. In contrast, multiple or single injections of heparin (40 units) 1 hour before tumor cell inoculation had a substantial antimetastatic effect. However, in mice with NK activity suppressed by anti-asialo GM₁ pretreatment, the antimetastatic effect of heparin was completely abrogated. Conversely, heparin pretreatment of mice plus stimulation of NK reactivity by poly I:C had additive and profound inhibitory effects on metastasis formation by B16 melanoma. These results indicate that NK cells are of central importance for the induction of the antimetastatic effect by anticoagulant drugs.

SIGNIFICANCE

The studies with TG-M ϕ are important for understanding the true role of M ϕ in the regulation of NK cell function in vivo. Our experimental model clearly indicates major differences in the properties of the various peritoneal M ϕ . These data demonstrate that the shock-like syndrome induced in mice inoculated i.v. with more than 3×10^6 TG-M ϕ can be a result of neutrophil aggregation and the associated release of substances with anaphylactic activities. These reactions may be responsible for the suppression of NK cell function and augmentation of metastasis formation. Furthermore, the data obtained clarify the mechanisms responsible for the failure of adoptive immunotherapy using tumoricidal M ϕ .

Our data also provide a new insight into the mechanism of the antimetastatic effect of an anticoagulant drug (heparin) and indicate the important role of NK cells in its in vivo activity. These results demonstrate that fibrin coagulation (or platelet aggregation) on the membrane surface of tumor cells can be one of the mechanisms which helps tumor cells to escape destruction by NK (or other cytotoxic) cells. Furthermore, this may provide a way to screen for the combination of treatments which may have additive antimetastatic effects.

PROPOSED COURSE

During the next year we will continue to study the processes involved in the augmentation of metastatic growth in mice following adoptive transfer of TG-M ϕ . Using complement-deficient strains of mice (AKR, A/J), we will investigate the role of C5a in the elicitation of M ϕ with the ability to augment metastasis formation. Special attention will be devoted to studies of the role of neutrophil aggregation and suppression of NK activity, especially in poly I:C-treated mice. The role of NK cells in the antimetastatic effect of antiplatelet and anticoagulant drugs will be further investigated. The antimetastatic effects of PGI₂ or nafazatrom (a PGI₂ inducer) will be screened against other experimental tumors. The influence of antiplatelet and anticoagulant drugs on the development of spontaneous metastases in NK-suppressed or NK-stimulated conditions will be tested. The combined treatment of chemotherapeutic and anticoagulant drugs may abrogate the antimetastatic effect of anticoagulant drugs, by inhibition of NK cell function. This possibility will be analyzed in order to evaluate the proper conditions for combined chemotherapeutic and anticoagulant treatment.

We plan to initiate investigations designed to use combined specific and nonspecific immunostimulation for experimental immunotherapy of malignant disease in mice. Specific immunization will be based on the approach developed by Boon et al. (1979). Following mutagen treatment of tumor cells, highly immunogenic tumor clones can be obtained. In addition, treatment of tumor cells with 1% cholesterol hemisuccinate (CHS), which increases the rigidity of the cell plasma membrane, may also increase the immunogenicity of tumor cells. Lethally irradiated clones or whole populations of tumor cells can be used for specific immunization and this immunization can be potentiated by using C. parvum as an adjuvant, which can also stimulate M ϕ and NK cells. Endotoxin or lipid A treatment was shown to result in tumor necrosis. However, tumor regression usually was observed only when immunogenic tumors were tested, since T-cell mediated immunity is considered an important part of tumor regression. Using immunogenic cloned populations for stimulation of specific T cell-mediated immunity and endotoxin or lipid A treatment, we expect to induce not only necrosis but also regression of the parental nonimmunogenic or weakly immunogenic experimental tumors. A similar approach will be used for the immunotherapy of postoperative pulmonary metastases in mice.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09256-01 BTB

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Natural Cell-Mediated Immunity in Man: In Vitro Activated and Cultured LGL

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John R. Ortaldo, Biologist, BTB, NCI

COOPERATING UNITS (if any)

Uniformed Services Univ. Health Sci.; Bureau of Biologics; Roche Inst. of Molecular Biology, Nutley, NJ

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2

PROFESSIONAL:

2

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human natural killer (NK) cells and K cells mediating antibody-dependent cellular cytotoxicity have been shown to be large granular lymphocytes (LGL). The majority of LGL form lytic conjugates with a wide variety of NK-susceptible target cells. Interferon caused augmentation of NK and K cell activities of LGL and only LGL demonstrated either spontaneous or interferon-activated NK activity. Natural, recombinant and hybrid recombinant alpha, beta, and gamma interferon molecules have been shown to augment NK activity but vary widely in their potency relative to antiviral activity. A recombinant J species of IFN- α has recently been shown to be unable to augment NK at a dose of 10,000 antiviral units and this finding may lend to insight into the structure-function relationship of IFN and NK boosting. IL-2, (T-cell growth factor), in addition to IFN, has demonstrated a potent ability to augment NK activity. Fresh LGL have been shown to show selective killing patterns by adsorption experiments on selected tumor monolayers. In an attempt to examine this apparent heterogeneity, cultures and clones of highly purified LGL, grown in the presence of IL-2 have demonstrated morphology and cytotoxic patterns similar to fresh LGL.

PROJECT DESCRIPTION

PERSONNEL

Paola Allavena	Visiting Fellow	NIS	BTB	NCI
Antonio Procopio	Visiting Fellow	NIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI

OBJECTIVES

The objectives of this project are:

- (1) To study the factors regulating the activation and development of natural killer (NK) and related natural effector cells;
- (2) To analyze the interaction of natural effector cells with other components of the immune system;
- (3) To study cultures and clones of LGL for their cytolytic activity and their production of soluble products in regulation of the immune system.

MAJOR FINDINGSI. Regulation of Human NK Activity

To obtain better insight into the nature of the diversity of the biologic effects of interferon (IFN), various preparations of human natural, recombinant, and hybrid recombinant alpha IFN were tested for their ability to augment the reactivity of NK cells and monocytes. At higher doses of interferon (i.e., >500 units) most IFN species significantly augmented antiviral NK activity and monocyte-mediated cytotoxicity and cytostasis. However, at low levels of IFN (10-50 units), appreciable differences among the various species were seen. Pure recombinant A and D IFN have been shown to vary in their antiviral activity and their effects on NK cells. In an attempt to determine a structure-function relationship, restriction enzyme-derived hybrid recombinant molecules of A and D were studied. D and the A/D hybrid were able to augment the activity of murine NK cells, whereas A and D/A or A/D/A hybrids had no effect. In addition, recombinant IFN- α J, which has antiviral and antiproliferative activity, was unable to boost NK activity and interfered with boosting by other IFN- α species. These findings are providing considerable insight into the portions of the IFN molecule associated with each biologic activity and offer the potential for constructing IFN molecules with highly selective biologic effects.

In addition to IFN, IL-2 potently augments human NK activity. Using highly purified LGL and pure IL-2 from the gibbon MLA-144 cell line, the dose and kinetics of augmentation have been studied. Unlike the rapid activation by

IFN- α , IL-2 required 6 to 10 hours of treatment to activate NK cells. The degree of augmentation by IL-2 was equal to or greater than that seen with any IFN- α species. The IL-2 boosting appears to be mediated at least in part by stimulation of endogenous production of IFN- γ by the LGL. Of considerable interest has been the ability of IL-2 to augment NK activity in the absence of growth promotion, indicating an additional function of this growth factor.

II. Cytotoxicity by Cultured and Cloned Cells

Cultures of highly enriched LGL cultures, as well as of T-cells, grew rapidly in the presence of IL-2 for a period of ~ 30-45 days. The phenotype of the T cells maintained relative stability in culture, while the LGL cultures demonstrated major changes in phenotype by 10 days, with a dramatic loss of OKT10, OKM1, and Fc receptors and the appearance of Ia and OKT3 antigens as well as the ability of a subpopulation of cultured LGL to form rosettes with sheep erythrocytes at 29°C. The cultured LGL demonstrated killing against NK-susceptible targets and primary tumors, and their activity was boosted by interferon.

Clones of LGL and T cells were obtained and these were studied for their cytotoxicity and phenotype. The majority of cytolytic LGL clones demonstrated a broad pattern of killing, similar to fresh LGL populations. However, only about 25% of the clones demonstrated cytotoxicity against the panel of targets tested. However, about 25% of the cytotoxic clones demonstrated selective killing of only some of the panel of NK targets. None of the T-cell clones demonstrated killing of NK-susceptible targets. The clones were found to vary widely in their expression of the following markers (OKT3, OKT4, OKT8, OKT10, OKM1, B73.1), with no correlation seen between the expression of a particular marker and the degree of cytotoxic activity or the pattern of killing.

SIGNIFICANCE

IFN(α , β , and γ) have all been shown to be potent modulators of NK activity. In addition, the finding that both natural and recombinant interferons are potent boosters of NK activity provides important information regarding potential mechanism of therapeutic effects in clinical trials. In addition to IFN, interleukin 2 (IL-2) also seems to be a potent enhancer of NK activity and can also support the growth of LGL for long periods (months) in vitro.

Cultures and clones of LGL (maintained on IL-2) have been shown to exhibit apparent clonal restriction of specificity. In addition, clones of LGL expressed T-associated markers (OKT3) not present on fresh LGL. In general, cultures of LGL exhibit enhanced lysis of fresh primary tumors of the colon, breast, and ovary, whereas fresh LGL demonstrate significant but low levels of lytic activity against these fresh tumors. The development of methodologies to obtain highly active and large quantities of LGL offers the possibility for therapeutic trials with highly purified and activated NK cells.

PROPOSED COURSE

Extensive studies on natural cell-mediated immunity will be continued using in vitro activated and cultured LGL. Future studies will attempt to determine (1) whether the specificity of the cytotoxicity by cloned LGL is stable, indicating true clonality for the heterogeneous patterns observed; (2) whether specificity of cloned LGL can be altered by maturational agents; (3) whether LGL clones produce cytokines and if so, to enumerate the frequency of secreting clones and the diversity of the secreted factors; (4) the biochemical mechanism involved in augmentation of NK and ADCC by interferon and IL-2. The biochemical mechanisms of interferon induction of antiviral resistance have been determined. We will attempt to determine if the same pathways are involved in interferon-induced augmentation of NK cells. In addition, the mechanisms of augmentation by other agents (e.g. IL-2) will be examined.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

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PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vivo Antitumor Activity of Natural Killer Cells in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Craig W. Reynolds, Staff Fellow, BTB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

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PROFESSIONAL:

1.5

OTHER:

1.0

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present studies in rats have demonstrated an important in vivo antitumor role for natural killer (NK) cells. A number of biological response modifiers (BRM) have been shown to augment the activity of these cells and thereby inhibit the establishment and growth of tumor cells in the lungs. To obtain additional evidence on the in vivo behavior of NK cells and to obtain data needed for the investigation of the possible immunotherapeutic use for T cells and large granular lymphocytes (LGL, the population known to mediate NK activity), adoptive transfer studies with chromium-51 and indium-111 labeled LGL and T cells were performed. The results demonstrate a distribution pattern which resembles the organ distribution for these cells, with a significant percentage of both LGL and T cells recovered from subcutaneous mammary tumors. In addition, the adoptive transfer of LGL into recipients with depressed NK activity was shown to restore in vitro tumor cell cytotoxicity, in vivo clearance of tumor cells from the lungs, and to inhibit the development of artificially induced lung metastases. These results provide the first direct evidence for an important in vivo role for NK cells and suggest that the adoptive transfer of highly enriched LGL populations should be further considered as one potential immunotherapeutic regimen in cancer patients.

PROJECT DESCRIPTION

PERSONNEL

Ronald B. Herberman	Chief		BTB	NCI
Teresa Barlozzari	Visiting Fellow	NIS	BTB	NCI
Hiroyasu Fukui	Guest Worker	NIS	BTB	NCI

OBJECTIVES

The objectives of this project are: (1) to investigate, in detail, the ability of various biological response modifiers (BRM) to augment and/or depress in vivo levels of natural cytotoxicity; (2) to evaluate the tissue distribution of adoptively transferred NK cells in normal and tumor-bearing rats; and (3) to further study the role of natural effector mechanisms in the in vivo resistance to tumor growth and metastasis.

MAJOR FINDINGSI. Effect of BRM on In Vivo Levels of Natural Cytotoxicity

In recent years, a number of BRM have been used in various animal studies to investigate the ability of these agents to augment NK activity and enhance tumor resistance. Many of these same agents have also been employed in certain treatment protocols involving cancer patients. However, the mechanism of action for these BRM is still not known. Our present studies have been designed to investigate the ability of various BRM (MVE-2, poly IC:LC, C. parvum, and OK432) to inhibit the growth and metastasis of the rat mammary tumor, MADB106, and to study the mechanism by which these agents work. Our results have demonstrated that all of the BRM tested significantly augmented NK activity in both the spleen and blood following i.v. or i.p. injection. With the exception of MVE-2, these BRM also augmented the cytotoxicity of peritoneal macrophages. Pretreatment of rats with these agents had only minimal effect on the s.c. growth of tumors. However, the injection of these agents prior to, or immediately following, the i.v. injection of MADB106 tumor cells enhanced the clearance of tumor cells from the lungs and reduced the number of metastases. To ascertain the mechanism of action for these agents, we are currently studying these BRM in rats with a reduced number of NK cells or macrophages. These results suggest that both NK cells and macrophages can be activated in vivo and subsequently function as antitumor effector cells in inhibiting the development of metastases. We are also examining these BRM in combined treatment regimens with cytoreductive agents (e.g., surgery, cyclophosphamide) to investigate the potential usefulness of such protocols in cancer patients.

II. The Role of Natural Effector Mechanisms in In Vivo Resistance to Tumor Growth and Metastasis

The present studies were performed to further evaluate the possible in vivo involvement of NK cells in host resistance against tumors. Selective depression of NK activity in W/F rats was induced by the i.p. or i.v. injection of anti-asialo GM₁ antiserum. This antiserum has previously been shown to produce a decrease in NK activity and a parallel increase in tumor growth in mice. In the present study, rats treated with this antibody showed a parallel decrease in NK activity and in the frequency of LGL in spleen and blood, indicating that the antiserum-induced depression of NK activity in these sites was due to a reduction in the number of effector cells. To further determine the possible role of LGL in tumor rejection in vivo, we studied LGL involvement in the rapid clearance of radiolabeled tumor cells from the lungs. Rats treated with anti-asGM₁ antiserum had a decrease in the rate of clearance of tumor cells from the lungs and a subsequent increase in the number of lung metastases. Furthermore, the adoptive transfer of a highly enriched population of LGL into NK-depressed animals 2 hours before tumor challenge partially or fully restored: (1) cytotoxic activity against established cell lines in vitro, (2) the ability to eliminate radiolabeled tumor cells from the lungs, and (3) the ability of asialo GM₁-treated animals to inhibit the development of pulmonary metastases. These results provide the first direct evidence that NK cells are involved in in vivo resistance to tumors, particularly in the elimination of potentially metastatic tumor cells from the circulation and capillary beds.

III. Distribution of Radiolabeled LGL Following i.v. or i.p. Transfer into Normal and Tumor-Bearing Rats

The recent demonstration of an increased resistance to tumor growth following the adoptive transfer of NK cell-containing lymphocyte populations would indicate that the transfer of NK cells may be of therapeutic value when employed in adoptive immunotherapy. The clinical usefulness of such observations is still unclear, however, because previous studies have not determined the distribution of NK cells following transfer. As a model for adoptive immunotherapy using NK cells in cancer patients, highly enriched populations of rat LGL and T lymphocytes were labeled with either ⁵¹chromium or ¹¹¹indium-oxine and injected either i.v. or i.p. into normal or tumor-bearing syngeneic recipients. Following i.v. inoculation of labeled LGL or T cells into normal recipients, a large proportion of radioactivity (18-33%) was recovered within minutes in the lungs. By 2 to 4 hours following transfer, more LGL than T cells remained in the lungs. Decreasing levels of radioactivity in the lungs were accompanied by corresponding increases in the spleen and liver. At early time points, a significantly higher proportion of T cells was found to be distributed to the spleen, while labeled LGL persisted longer in the blood and lungs. A small but significant percentage of both LGL (2.5%) and T cells (1.5%) were recovered from small subcutaneous MADB106 mammary tumors, demonstrating that these adoptively transferred cells are capable of entering the site of tumor growth. Very similar results were obtained using either radiolabel, suggesting that the radiolabeling procedures used had little or no effect on the inherent distribution of these lymphocytes. This distribution pattern for LGL and T cells resembles our previously reported organ distribution for these cells. Following i.p. inoculation into normal or tumor-bearing

recipients, there was slow clearance of labeled LGL or T cells from the peritoneal cavity, with <20% of the radiolabel found in peripheral organs by 24 hours. The results from these studies provide a firm basis for further exploring the usefulness of adoptive immunotherapy with both fresh and cultured LGL or immune T cells.

SIGNIFICANCE

A large amount of data in experimental animal systems now supports the notion that natural cell-mediated immunity may play a significant role in immune surveillance against tumors. Our observations regarding the mechanisms of NK augmentation by BRM should lead to more effective protocols for augmenting NK activity in cancer patients. The results obtained regarding the distribution and persistence of passively transferred LGL are an important prerequisite to the development of clinical protocols involving the adoptive immunotherapy of cancer patients with activated or cultured LGL. In general, these results should greatly facilitate studies to examine the overall function and relevance of NK cells in human tumor systems.

PROPOSED COURSE

Extensive studies on natural cell-mediated immunity in rats will be continued. The rat provides an ideal system for the analysis of NK activity in experimental animals since sufficient blood can be obtained to test PBL, highly purified LGL can be isolated from discontinuous Percoll density gradients, and large numbers of active LGL can easily be obtained from the spontaneous LGL leukemias in F344 rats. Specifically, our efforts will be directed toward: (1) a further examination of the mechanism(s) by which in vivo administered BRM augment NK activity; and (2) a better understanding of the in vivo role of NK cells in reactivity toward transplanted and carcinogen-induced primary tumors.

Further studies with BRM will focus on both the mechanism(s) by which these agents augment NK activity and the ability of these agents, either alone or in combination with chemotherapy, to inhibit the growth or metastasis of transplanted mammary tumors. In particular, we hope to provide a more detailed understanding of how these agents might regulate the number of LGL and the activity of these cells in vivo. In an attempt to more closely correlate with the present means of cancer therapy, animals with existing tumors are being given cytoreductive treatment (surgery, cyclophosphamide, etc.) followed by BRM treatment. These results should indicate what type of BRM might be most effective in patients with alterations in their immune response due to prior treatment, and should suggest more effective means for treating cancer patients with a combination of cytoreductive agents and BRM.

To better understand the in vivo role of NK cells, we will continue to examine the effect of NK suppression and specific LGL reconstitution on the growth of transplanted mammary tumors, the development of spontaneous and artificially induced metastases, and on the production of carcinogen-induced tumors (NMU,

DMN-OAC). A particular focus will be on determining whether there are critical times at which LGL seem to play an important preventative role in the development of tumors in these systems.

Previous studies have also indicated an *in vivo* role for NK cells in the mediation of allogeneic bone marrow graft rejection. We will therefore use our previously described system of selective depression/reconstitution of NK activity, to examine the ability of adoptively transferred, highly enriched LGL populations to affect the *in vivo* or *in vitro* growth of syngeneic and allogeneic bone marrow cells.

PUBLICATIONS

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Wiltrot, R. H., Brunda, M. J., Gorelik, E., Peterson, E. S., Dunn, J. J., Leonhardt, J., Varesio, L., Reynolds, C. W. and Holden, H. T.: Distribution of peritoneal macrophage populations after intravenous injection in mice: differential effects of eliciting and activating agents. RES (In Press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09259-01 BTB

PERIOD COVERED

February 3, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Differentiation of NK Cells and Lymphocyte Subsets

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Bonnie J. Mathieson, Senior Staff Fellow, BTB, NCI

COOPERATING UNITS (if any)

Laboratory of Microbial Immunity, NIAID; NCI-FCRF; Memorial Sloan Kettering Cancer Center, New York; Yale University, New Haven, CT

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1.07

PROFESSIONAL:

0.67

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The phenotypic characterization of naturally occurring murine cytotoxic cells has been further developed to understand the origin, differentiation, and normal function of this population. Cells from spleen, thymus, blood, and bone marrow have been characterized. Effector cell activity has been monitored against appropriate targets for both natural killer (NK) activity and natural cytotoxic (NC) activity. Their phenotype has been monitored by immunofluorescence (IF) with flow cytometry and by complement (C)-mediated, antibody-dependent cytotoxicity. Splenic subpopulations enriched for large granular lymphocytes (LGL) from nylon wool nonadherent cells subjected to density separation techniques have been characterized with a series of monoclonal antibodies to T-cell antigens, to myelocytic antigens, and to other hematopoietic subsets. A subpopulation of low density splenic lymphocytes which is enriched both for cells with the morphology of LGL and NK function also contains a unique population of cells identified by two-parameter analysis for Ly 5 antigen expression and cell size. This population appears to be distinct from other nylon wool nonadherent lymphocytes in the spleen and will be further characterized for NK and NC activity. Thymic lymphocytes with a phenotype similar to that of some NK cells (i.e., low Ly 1, low Thy-1 antigen expression) are being further characterized for functional activity and for the ability to develop into NK cells.

PROJECT DESCRIPTION

PERSONNEL

John R. Ortaldo	Biologist	NIS	BTB	NCI
Llewlyn Mason	Microbiologist	NIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI

OBJECTIVES

The major objectives of this project are: (1) to identify and characterize the cell surface phenotype of cells that have NK and NC activity, and (2) to study the origin, differentiation and development of these cells.

Specific objectives:

- (1) To develop a panel of monoclonal antibodies and other reagents with selective or differential reactivity against mouse NK cells.
- (2) To develop enrichment and selection techniques to isolate highly-purified populations of NK cells, their subsets and their precursors.
- (3) Characterize and isolate precursors of NK cells from the thymus and bone marrow and compare with prothymocytes and pre-T cells.

METHODS EMPLOYED

NK activity was monitored with ^{51}Cr -labeled YAC target cells and NC activity was monitored with ^{51}Cr -labeled WEHI-164 target cells. The level of activity was determined by measuring the percent of radioactivity released into the culture supernatant in 4- or 18-hour assays.

Phenotypic characterization was done primarily by two methods: flow microfluorometry analysis was used to quantify antigen expression. Monoclonal antibodies, or in some cases immune sera, were incubated with aliquots of the test populations. Antibody binding was usually detected by appropriate fluorescent anti-immunoglobulin reagents. Cells were subjected to Percoll density gradient fractionation or other enrichment methods whenever possible. Cytotoxic elimination with antibody and C served two purposes: to directly assess phenotype or to eliminate unwanted or irrelevant cell populations in subset enrichment methods for spleen, bone marrow, and thymus. Monoclonal antibodies were obtained from commercial sources or through collaborative arrangements.

Morphology of LGL was assessed on Giemsa-stained cytocentrifuge preparations.

MAJOR FINDINGS

Initially we confirmed previously reported findings from this and other laboratories that have indicated that splenic cells enriched for LGL and/or cells exhibiting NK activity are also enriched for cells with low or negligible Ly 1 and Thy-1 expression. The proportion of Ly 2⁺ cells in the same fraction was reduced and could be eliminated by antibody plus C without affecting NK activity.

Preliminary analyses indicate that two reagents appear to identify a subset within the low density splenic population which expresses a lower level of Ly 5 (T200) antigen than that seen on most lymphocytes. The "lower" Ly 5⁺ cells were slightly larger by light scatter than the majority of cells in this fraction. Thus on dual parameter analysis for Ly 5 immunofluorescence and forward light scatter, these cells comprise a clearly distinguishable subset. The same (or a very similar) population of cells were weakly positive with antibody to Fc receptor (monoclonal 2.4G2, from Jay Unkeless, Rockefeller University, New York).

By elimination experiments a monoclonal antibody (anti-GM-1, from F.-W. Shen, Sloan-Kettering, Institute, New York) to a myelocytic differentiation antigen expressed on granulocytic cells did not react with most of the cells with either NK or NC activity in bone marrow or spleen. These data favor the concept that both NK and NC cells are more related to the T-lymphocyte lineage of cells than to the myelomonocytic lineage.

In collaborative work with B. J. Fowlkes, NIAID we have identified a minor subpopulation of thymocytes that may represent the thymic subcapsular cell or intrathymic precursor population. This population is remarkably similar in phenotype to the LGL, but these cells do not have detectable NK activity nor do they contain the granules evident in splenic LGL populations. However, the similar phenotype may indicate a cell population that has just begun to develop T lineage characteristics but which still has the capacity to differentiate into functional NK and/or NC cells if it is removed from the thymic environment and appropriately stimulated.

SIGNIFICANCE

Several theories have been proposed to explain the biological relevance of NK and NC cells. Natural immunity as exhibited by NK or NC cells may represent a primitive but unique lymphocyte subset that acts as a first line of defense against complex immunogens such as tumor cells and metastases or parasites. NK or NC cells may alternatively serve as feedback regulators on the lymphoid components or myelocytic components of hematopoietic differentiation. Finally, they may be "wayward" cells incapable of completing a normal T-cell differentiation pathway without the appropriate or sufficient interaction with a thymic environment, e.g., this would explain the high proportion of NK cells in nude mice.

The latter possibility is least likely because NK cells and LGL in particular display a combination of cell surface and morphological characteristics that distinguish them from the bulk of conventional T cells, and other hematopoietic

cells. However, NK and NC cells share a number of characteristics with each other beyond their occurrence in unprimed hosts. Currently the reproducible differences are limited to differential target sensitivity for NK and NC cells, to selective elimination of NK activity with monoclonal antibody to Qa5, and to the decreased NK activity with age in some strains of mice. In contrast, conventional cytotoxic T cells normally express Ly 2 antigen and require antigenic stimulation for activation to functional effector status. The precursors of all of these cells are virtually unknown and only limited experimental analysis has been attempted in bone marrow reconstituted mice. The availability of several monoclonals to allelically-determined bone marrow antigens now makes such experiments easier to approach. In addition, several antigens associated with NK cells that were initially defined by IF or C-dependent cytotoxicity were not quantitatively assessed for differential expression on lymphocyte subsets. Some of these differences may be useful for selectively enriching or eliminating appropriate populations. Thus the previous failure to find a unique marker for NK cells in mice or in humans may be overcome by combined selective enrichment and monoclonal antibody technology currently available. Finally, manipulation of precursor lymphoid populations from murine bone marrow can be very informative where precursor-product relationships can be assessed with genetic markers. Therefore the development of methodology to identify, isolate, monitor, and assess natural immunity populations is highly appropriate at this time.

PROPOSED COURSE

There are three major areas of this project which we intend to develop during the next year: (1) Phenotypic and functional comparison of splenic and bone marrow subpopulations obtained from Percoll density fractions enriched for NK and NC characteristics. (2) Assessment of the development of splenic NK functional activity from bone marrow precursors transferred to irradiated, bone marrow reconstituted mice in comparison with the kinetics for thymic repopulation using genetic Ly markers. (3) Continued and expanded studies of thymic subsets and their relationship to NK activity. In particular, we intend to explore the effect of biological response modifiers on the development of cells with NK activity from immature versus mature thymocyte subsets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM06146-06 BTB

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Regulation by Immune Modifiers and Chemotherapy in the Tumored Host

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Michael A. Chirigos, Head, Immunopharmacology Section, BTB, NCI

COOPERATING UNITS (if any)

Natural Immunity Section, BTB, NCI; Lymphokines Section, LMI, NCI; Immunobiology Section, LMI, NCI; Laboratory of Viral Diseases, NIAID; LVD, NIAID; LCHPH, NCI

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Immunopharmacology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2.8

PROFESSIONAL:

2.0

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Of five BRMs examined, three (poly ICLC, MVE-2, Picibanil) significantly augmented NK cell and macrophage (M ϕ) cytotoxic activity. In vitro and in vivo studies indicate that the tumor cell antiproliferative effects of these BRMs are not direct but mediated through effector cells. Augmented NK activity varied in different tissues (peritoneal greater than blood greater than spleen) and according to treatment route (i.p. greater than i.v. greater than s.c.). NK and M ϕ cytotoxic activity was maintained over 7 days following single injection. Multiple treatment with MVE-2 and poly ICLC resulted in hypo-responsiveness (HRP) of NK but not M ϕ . PGE2 induction by BRM-activated M ϕ was one reason for the development of HRP. In vitro incubation of normal M ϕ with the BRMs poly ICLC, MVE-2, or α IFN resulted in increased PGE2 secretion; poly ICLC and α IFN increased CSF secretion; and poly ICLC increased IFN secretion. Poly ICLC, MVE-2 and Picibanil reduced metastatic lung tumor development possibly by augmenting peripheral blood (PB) NK activity. These BRMs reconstituted PB NK activity in mice treated with a selective NK-depressing antibody. Treatment of established B16 melanoma and M109 lung carcinoma, by Cytoxan chemotherapy followed by either poly ICLC, MVE-2 or Picibanil resulted in a significant reduction of lung tumors and increase in survival time. The combined treatment was more effective than with Cytoxan or BRM alone. Surgical debulking of 16/C mammary adenocarcinoma followed by poly ICLC or MVE-2 treatment resulted in decreased metastatic lung tumors. Azimexon reconstituted nucleated bone marrow cells in irradiated mice through CSF-enhanced myeloid stem cell proliferation. Poly ICLC, BM 41.332, and murine α IFN also stimulate the production and secretion of CSF capable of forming myeloid colonies from stem cells in vitro.

PROJECT DESCRIPTION

PERSONNEL

Tohru Saito	Visiting Fellow	IS	BTB	NCI
Erich Schlick	Visiting Associate	IS	LMI	NCI
Mario Piccoli	Guest Worker	IS	BTB	NCI
Ralf Ruffman	Guest Worker	IS	BTB	NCI

OBJECTIVES

This project examines the immunological and pharmacological mechanisms by which biological response modifiers (BRM) regulate the various cellular components of the immune system and cause the production and release of soluble cellular components (e.g., IFN, CSF, interleukin 1 and 2, PGE). Protocols for optimal sustained modulation of these responses are developed and such protocols of active BRMs are combined with antitumor cytoreductive treatment to establish more effective therapy of cancer.

METHODS EMPLOYED

Established carcinogen, virus-induced, and spontaneous tumors (melanoma, mammary carcinoma, lung carcinoma, leukemia), are employed in vitro and in vivo. Specific humoral or cellular immune assays and radioimmune PGE assay are employed.

MAJOR FINDINGS

The immunopharmacokinetic studies of five BRMs (MVE-2, poly ICLC, Picibanil, azimexon, DTC) and their capacity to induce soluble factors showed that MVE-2, poly ICLC and Picibanil augmented NK and M ϕ tumoricidal activity for over 7 days. In contrast, diethyldithiocarbamate (DTC) augmented natural killer (NK) cells but not macrophages (M ϕ). NK activity varied in the different tissues (peritoneal exudate cells [PEC] > blood > spleen) and according to route of treatment (i.p. > i.v. > s.c.). In vitro antiproliferative tumor cell studies showed DTC alone was effective. In vivo, marked antiproliferative effects were observed with MVE-2, poly ICLC, Picibanil, and $\alpha\beta$ IFN but not DTC. The antiproliferative effect was shown to be associated with M ϕ and NK stimulation. Studies of IFN induction in vivo showed poly ICLC and Picibanil as high, MVE-2 as low, and azimexon and DTC as non-inducers of IFN. Normal M ϕ s incubated in vitro with BRMs released: PGE₂: high levels (poly ICLC), low levels (MVE-2, DTC, azimexon, $\alpha\beta$ IFN); CSF, high (poly ICLC, $\alpha\beta$ IFN), and low (azimexon); only poly ICLC-induced IFN secretion. Single versus multiple treatment studies showed the development of hyporesponsiveness (HRP) of NK, but not M ϕ , after multiple treatments with MVE-2 and poly ICLC. Injection of MVE-2 or poly ICLC with indomethacin resulted in a higher NK activity than attained with BRM alone. Inhibition of cyclooxygenase by indomethacin was considered to decrease or eliminate the production/release of PGE₂ by the activated M ϕ , leading to the higher NK activity. PGE₂, secreted by activated M ϕ , may be causing the HRP.

Comparative studies of total peritoneal exudate cells and non-adherent peritoneal exudate cells from poly ICLC or MVE-2 treated mice show a higher NK activity in the non-adherent cells. Addition of indomethacin to the total peritoneal exudate cells resulted in increased NK activity, indicating that the $M\phi$ in the total cell population may be decreasing NK activity through the secretion of PGE₂.

The establishment of lung tumors was inhibited by poly ICLC, MVE-2, or Picibanil treatment. Kinetic studies indicate a good correlation between augmentation of NK activity in the peripheral blood and the prevention of spread of the tumor cells to the lung. Treatment of established lung tumors (B16 or M109) with Cytosan followed by BRM (poly ICLC, MVE-2, or Picibanil) reduced lung tumors and increased survival time. Combined treatment was more effective than therapy by drug or BRM alone. Co-injection of B16 melanoma cells and asialo GM₁ antibody, which selectively depresses NK cell cytotoxic activity, leads to the establishment of a higher number of lung tumors than occurs with tumor cells alone. MVE-2, poly ICLC and Picibanil, which alone prevent the establishment of B16 melanoma lung tumors, were found to also decrease the number of lung tumors in asialo GM₁ antibody-injected mice. These results indicate that these BRMs interfere with the NK-depressive capacity of the antibody and/or cause recruitment and augmentation of a new population of NK cells, or that $M\phi$ or other effectors but not NK are responsible for the antitumor effects. Treatment with MVE-2 or poly ICLC immediately following surgical removal of large (>15 mm) mouse mammary adenocarcinomas resulted in a decreased number of lung metastases and a prolongation of survival time. Effective tumor debulking was a critical factor in demonstrating the efficacy of BRM treatment of MBL2 leukemia. Chemotherapy when tumor burden was low followed by MVE-2 or poly ICLC resulted in a high percentage of "cures." When chemotherapy was delayed until tumor burden was high (i.e., 10-12 days before death) and followed by MVE-2 or poly ICLC, a decreased response was achieved resulting in only an extension of survival time without "cures." The evidence suggests that the more effective response achieved with subsequent BRM treatment was due to the augmentation of a sufficient number of effector cells (NK and $M\phi$) capable of eliminating residual tumor cells remaining after initial chemotherapy.

CSF and PGE are involved in the positive and negative feedback control of myeloid stem cell proliferation. Azimexon, which in vitro induces $M\phi$ to secrete CSF but little PGE, was examined in vivo for its capacity to protect bone marrow cells against sublethal doses of X-irradiation. Azimexon treatment after total body irradiation resulted in a more rapid reconstitution of nucleated bone marrow cells and a significant level of CSF in serum. These results indicate that azimexon-induced CSF in vivo reconstituted nucleated bone marrow cells by enhancing myeloid stem cell proliferation. Supernatants from normal $M\phi$ s incubated with poly ICLC, BM 41.332, and $\alpha\beta$ IFN, were shown to contain high levels of GM-CSF myelopoietic growth factor. In vitro, these supernatants stimulated the growth and differentiation of bone marrow granulocyte-macrophage precursor cells.

SIGNIFICANCE

The Immunopharmacology Section investigates and develops potential therapeutic agents which may alter biological responses important in the resistance to cancer growth and metastasis. The multidisciplinary approach is entirely directed towards: increasing the host's antitumor response through augmentation and/or restoration of cellular effector mechanisms; increasing the host's cellular and humoral immune responses by the administration of natural or synthetic effectors or mediators; and, increasing the ability of the host to tolerate damage of normal cellular components resulting from cytotoxic modalities of cancer treatment. Experimental studies conducted with chemical and biological agents with respect to their: immunoadjuvant effects; most efficacious treatment regimens; and, usefulness in combined treatment modalities, has provided information leading to the inclusion of some of these agents for the treatment of human breast, colon and rectal carcinomas, head and neck tumors, and leukemias. The ability of several immune modulators to selectively and strongly augment host immunity when they are used alone or in concert with established cancer treatment modalities, is of practical value in preventing and/or controlling cancer. Basic research studies conducted with these immunoregulatory agents are defining the cellular components which are activated by these agents and the specificity of their tumoricidal activity.

PROPOSED COURSE

Each of the current objectives will continue to be pursued.

The major treatment modality for various cancers is chemotherapy. In many cases, this cytotoxic treatment results in hematopoietic damage, particularly bone marrow depression. BRMs capable of protecting or stimulating proliferation and differentiation of bone marrow (myelopoiesis) would be advantageous to the tumor-bearing host undergoing cytoreductive therapy. Studies will be continued to assess the capacity of selected BRMs to stimulate colony stimulating factor (CSF) activity in: normal and tumor-bearing individuals; and tumor-bearers undergoing cytoreductive therapy.

Pharmacokinetic studies will be conducted to determine the effects of BRMs on various immunologic effector mechanisms as related to such variables as: dosage; best route of administration; most effective regimen of treatment (particularly when used in combination with cytoreductive therapy); development of hyporesponsiveness of effector cells to BRM; and, the duration of effector cell response. This information will lead to the development of BRM treatment protocols leading to maximum and prolonged increases of effector cell activities. Since treatment with various chemotherapeutic agents may lead to a depression of hematopoietic cells, particularly effector cells, we propose to determine the sequence of treatment regimens in combined chemotherapy and BRM treatment which is best suited to reconstitute or augment effector cell responses.

Prostaglandins of the E series (PGE), produced by tumors and mononuclear phagocytes, have many biological activities and are involved in the regulation of myelopoiesis and of the cytotoxic activities of M ϕ and NK cells. M ϕ

activation leads to the secretion of two cell regulating factors, PGE and CSF. BRMs which stimulate $M\phi$ and NK cell activity, will be examined for their capacity to regulate secretion of PGE and/or CSF to assess whether the BRM induced PGE secretion from $M\phi$ s is exerting a negative feedback inhibition on effector cell responses in vitro and in vivo.

PUBLICATIONS

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SUMMARY REPORT

LABORATORY OF MOLECULAR IMMUNOREGULATION

October 1, 1982 to September 30, 1983

INTRODUCTION

The Laboratory of Molecular Immunoregulation (LMI) was established in the BRMP intramural program on Feb. 2, 1983. The laboratory comprises three of the seven sections that were formerly included in the Biological Research and Therapy Branch of the BRMP: namely, the Biochemistry, Lymphokine/Cytokine and Basic Mechanisms Sections. The latter has subsequently been renamed the Immunobiology Section. Dr. Joost J. Oppenheim was appointed to head the new laboratory. The reorganization is intended to make better use of the existing resources of the BRMP and strengthen the pursuit of basic investigator initiated intramural studies. It is the mission of the LMI to investigate, at a molecular level, the inter and intracellular processes that regulate host defense mechanisms to neoplastic disorders. Studies of the modulating effects of lymphokines and cytokines will be emphasized. In the pursuit of such information, new assays for evaluating the effects of biological response modifiers on the host immune response should be developed and agents that are active BRMs identified. The specific personal changes and major research activities of the staff during the past fiscal year can best be summarized on a section by section basis.

BIOCHEMISTRY SECTION

The Biochemistry Section of the LMI conducts research on the isolation, purification, biochemical and biological characterization of tumor-associated antigens, soluble mediators of the immune response and cellular growth factors. The section investigates the use of these substances as therapeutic agents, as stimulants in the production of monoclonal antibodies, and reagents in the development of clinically useful diagnostic immunoassays. Members of the section determine amino acid sequences of purified proteins to determine their nucleotide sequences and to obtain the CDNA coding for the protein for gene cloning purposes.

The Biochemistry Section underwent a major organizational change during the past fiscal year with the transfer of Dr. A. Charles Morgan and his support staff to the Monoclonal Antibody/Hybridoma Section. The Biochemistry Section retains Dr. James A. Braatz, who serves as Acting Head, and Dr. Edward S. Kimball. Support personnel include Dr. David T. Hua, Dr. Myung Kim, Mr. Gerald L. Princler, and Mr. Thomas Warren. A search for a third senior-level molecular biologist for the section has been initiated with the expectation that this new group will be on board and fully operational by the end of this fiscal year. An effort is being made to identify a scientific investigator with expertise in genetic engineering to complement the work and interests, not only of the Biochemistry Section but of the other members of the BRMP as well.

Studies by Dr. Braatz of a human lung tumor-associated antigen (LTA) have continued with emphasis on the improvement of immunoassays for quantitating the tumor antigen and the application of these assays in the evaluation of LTA as a marker for lung cancer. An enzyme-linked immunosorbent assay (ELISA) has

been developed which is based on an immobilized, highly purified form of LTA and a second generation anti-LTA serum. This antiserum was prepared by injection of a goat with highly purified LTA. The goat antiserum detects the same antigenic determinant with approximately the same binding affinity as a previously described rabbit antiserum. Although the titer of the goat antiserum is lower by about one-third, 15 liters were collected by plasmapheresis which compensates for this drawback. The ELISA is a more convenient and antigenically-economical assay than the former RIA and has proven effective in several aspects of the overall program. For example, the ELISA is being used as a screening assay for the detection of monoclonal antibody, for monitoring during antigen purification, and for assessing the clinical utility of LTA in patients specimens. With regard to this last item, LTA levels in the serum of three terminal lung cancer patients were monitored and LTA levels were found to be an effective indicator of disease recurrence and, in fact, more predictive than CEA. The presence of LTA in bronchial washings from lung cancer patients was also observed, and studies are in progress to evaluate this as a potentially useful diagnostic or monitoring aid.

Research efforts of Dr. Kimball, concerning human urinary transforming growth factors (TGF), have increased significantly during this fiscal year to the point that this area has now become a major project within the Biochemistry Section. Based on an observation made originally in Dr. Stephen Sherwin's laboratory, a form of TGF of characteristic size, which appears to be greatly elevated in cancer patients, is being studied. The factor(s) is being isolated and has already been highly purified in an attempt to compare its properties to those of known growth factors such as epidermal growth factor (EGF) and tissue culture-derived growth factors. To facilitate these studies, a rapid and sensitive assay has been developed which measures binding competition for labeled EGF to immobilized membranes containing EGF receptors. This assay offers many advantages over the previously used soft agar growth assay. Using this assay and reverse phase high performance liquid chromatography, 5 species of urinary TGF were identified. Although functionally similar, they are chemically distinct from growth factors produced by cultured cells. Both qualitative and quantitative differences have been observed in TGFs isolated from cancer patients relative to normal individuals. The diagnostic utility of these findings is being assessed. In the process of performing the above studies, another activity was detected in normal and patients' urines. This factor induced murine thymocyte and human fibroblast proliferation and in its biochemical characteristics resembles Interleukin 1. This observation may provide a ready source and reveal a physiological role for this macrophage and epidermal cell-derived mediator of inflammation.

LYMPHOKINES/CYTOKINES SECTION

The Lymphokines/Cytokines Section investigates "hormone-like" products of cells (cytokines), especially the products of lymphocytes (lymphokines) which may regulate host defense mechanisms capable of mediating tumor destruction in cancer patients. Investigators in the section are involved in the production and purification of lymphokines, the development and standardization of bioassays, and treatment protocols in animal models and cancer patients. During the past year, some significant advances in our understanding of these natural products have occurred, including the definition of the complete amino acid sequences of several polypeptide cytokines (thymosins, interleukin 2). The merging of technologies of cell biology, biochemistry, and genetic engineering promise to further

unravel the complex mechanisms by which lymphokines and cytokines regulate immunological responses.

This year has seen a major reorganization of the Lymphokines/Cytokines Section. The major change has been the incorporation of the section in the newly-formed Laboratory of Molecular Immunoregulation. There has been an almost complete change in the personnel of the section associated with this move, as follows:

Dr. Gary B. Thurman, formerly Acting Section Head, has been reassigned to the Extramural Branch of the BRMP, DCT, and is no longer a member of the section. In his new capacity, however, Dr. Thurman has assumed responsibility for obtaining lymphokines for study by the section and the Laboratory. Dr. Joost J. Oppenheim, Laboratory Chief of the LMI, is now the Acting Section Head.

Dr. Guy Bonnard has left the section and returned to Europe to continue his research.

Dr. Jose Alvarez completed his Fellowship and has gone to South America to pursue studies in immunoparasitology.

Dr. Luigi Varesio has been transferred to the Immunobiology Section, and his research will be summarized in that section's report.

Dr. Stephen A. Sherwin, whose research on tumor-promoting growth factors was supported by the Lymphokines/Cytokines Section, has left the BRMP and is currently affiliated with Genentech, Inc.

A number of new investigators have joined the section during the past year. Dr. William J. Farrar, who has published extensively on the interactions between cells and lymphokines, joined the section in March 1983. His report describes his plans for studying the relationships between the endocrine system and lymphokines. It is likely that both these systems interact and share some functional similarities in modes of action, cell receptors, etc.

Dr. Francis Ruscetti is an Expert who transferred to the program in May 1983, from Dr. Gallo's laboratory. Dr. Ruscetti has published some of the seminal papers on interleukin-2 and is interested in more closely investigating the cellular mechanisms by which lymphokines control immune reactivity and growth of neoplastic cells. He is joined in his endeavors by Dr. H. Abe, a Guest Scientist from Japan. Dr. Abe is trained as an industrial biochemist and is familiar with the latest technologies in protein isolation, characterization and sequencing. He will be pursuing studies of B cell growth factors.

Dr. Erich Schlick, a Visiting Associate, has transferred from the Immunopharmacology Section to the Lymphokines/Cytokines Section and is continuing his work on colony stimulating factors. He is initiating studies of autocrine cytokines.

An additional scientist with expertise in the interferon area is expected to join the section near the end of this Fiscal Year.

A continuing effort to identify sources of lymphokines and cytokines has yielded adequate amounts of some materials such as interleukin-2 in the form of crude material and highly purified material from the MLA-144 cell line; interferons alpha, beta and gamma, of both mouse and human origin, including recombinant

alpha interferon; and tumor necrosis factor. It is one of the section's goals to establish assays either in-house or through contractors program to enable us to investigate a variety of lymphokine/cytokine containing materials. At this point, in-house assays for interleukin-1, interleukin-2, alpha, beta and gamma interferons, colony stimulating factor, migration inhibitory factor and macrophage activating factor are performed routinely at this time.

Monoclonal antibodies have been obtained to human alpha interferon, gamma interferon, CSF and Il-1 from collaborators and are being used to study the modes of action of these lymphokines/cytokines.

In collaboration with the Monoclonal Antibody/Hybridoma Section, BTB, BRMP, we are developing human/human T cell hybridomas which constitutively secrete lymphokines such as human interleukin-2 and colony stimulating factor. These hybridomas are then adapted to growth in serum-free medium and hopefully will be a good source for isolating lymphokines for future studies.

Significant progress has been made in defining the relationship between migration inhibitory factor (MIF) and interferon. Using a micro-adaptation of the MIF assay which employs human monocytes purified by centrifugal elutriation as the indicators, it was shown that antibody to gamma interferon did not remove MIF activity from a lymphokine preparation. Gamma interferon was shown, however, to be a potent inhibitor of human monocyte migration. Work is continuing to define how many migration inhibitory factors exist and the physiological roles of each factor.

A model system for studying the control of T cell growth in normal and neoplastic T cells by the lymphokine IL-2 (TCGF) has been developed by Dr. Ruscetti. Cell lines have been established which are sensitive to glucocorticoid-mediated control to TCGF required for cell growth. These lines are being used to study the autocrine nature of TCGF action on normal and neoplastic T cells.

Also, cell lines, which respond to variants of TCGF with distinctly different biochemical properties, have been derived. These lines are being used to help define the mechanisms of TCGF action on T lymphoid cells.

In collaboration with the Immunopharmacology Section, Dr. Schlick is evaluating the effects of various biological response modifiers on the release of the lymphokine GM-CSF by human monocytes and bone marrow cells and by murine macrophages. Poly-ICLC, human and mouse interferons, BM41.332 and LPS could induce GM-CSF secretion. With the exception of human interferon, these BRMs also induced secretion of prostaglandin E, which acts as a feedback inhibitor of GM-CFU-C colony formation. The inhibitory pathway could be blocked by Indomethacin, resulting in the accumulation of larger amounts of GM-CSF. One research question is, Do the endocrine receptors and lymphokines operate through similar intracellular mechanisms, and are they related to one another at the molecular level? Published evidence that some subpopulation of human lymphoid cells, including the LGL population, analogous to the murine NK cells, exhibit receptors for the endocrine hormone beta-endorphin, opens new doors to a more unified theory of cellular regulation. If, as seems the case from preliminary data of Dr. Farrar, the endorphin receptor can be triggered to affect the arachidonic acid pathway within the cell, and thus prostaglandin production, it may be possible to apply endocrine antagonists and/or agonists to control lymphoid cell populations.

An application of lymphokines is the enhancement of in vitro human immune responses through potentiating lymphocyte sensitization to antigens in vitro. Progress was made during this year in obtaining in vitro human antimelanoma immune responses using lymphocytes from melanoma patients undergoing treatment with a monoclonal antimelanoma antibody of mouse origin. Cells from the in vitro immunization were then used to produce human/human fusions in collaboration with the Monoclonal Antibody/Hybridoma Section. These fusions show promise as continuous sources of human antitumor antibody. Preliminary evidence shows that crude preparation of human lymphokines can enhance the sensitization of human lymphocytes in vitro. Experiments are now concentrating on defining the optimum way in which these peptides may be used to produce strong responses and to provide large pools of antigen-specific B lymphocytes for use in hybridoma fusions.

IMMUNOBIOLOGY SECTION

The objectives of the Immunobiology Section of the Laboratory of Molecular Immunoregulation are to investigate cell-mediated immunity to tumors, to study the immunobiology of antitumor effector mechanisms, and to understand the molecular events which underlie the effects of biological response modifiers on the host response to tumor growth. Included in the areas that are under investigation are 1) antitumor effects of macrophages and natural killer cells in mice, 2) human monocyte-mediated cytotoxicity, 3) the role of lymphokine cascade and macrophages in lymphocyte activation, 4) the role of macrophage differentiation and activation, 5) biochemical aspects of monocyte differentiation, 6) mechanisms of response of macrophages-monocytes to biological response modifiers, and 7) changes in lipid composition during the process of macrophage activation.

There have been several major personnel changes this fiscal year that will have impact on the future direction of the research in this section. Dr. Joost J. Oppenheim, Chief, LMI, is now a member of the Immunobiology Section. Working with him are Drs. Giuseppe Scala, Kouji Matsushima, Yan-de Kuang, and Kikuo Onozaki. Dr. Howard T. Holden, who had been Acting Section Head, will leave the section in July to take a position in the Cancer Therapy Evaluation Program, NCI, and Dr. Luigi Varesio has been appointed Acting Section Head. Dr. Thomas Hoffman left the section in October of 1982 to take a position in the Office of Biologics, FDA. Dr. Henry C. Stevenson, who had been Acting Section Head, has transferred to the Clinical Investigation Section. Hence, at the moment, the senior personnel in the section are Dr. Luigi Varesio, Acting Section Head; Dr. Joost J. Oppenheim, Chief, LMI; Dr. Eugenie Kleinerman and Dr. Robert H. Wiltrott.

During the past year there have been a number of findings made by members of the section which help delineate the nature of the host-tumor interaction and which provide information about the molecular and cellular events involved in these responses. Drs. R. Wiltrott and Howard Holden have studied the relative role of macrophages and natural killer cells in the host response to tumor growth. It appears that natural killer (NK) cells may inhibit the formation of metastases during the blood borne phase, whereas macrophages ($M\phi$) only exert antitumor activity at local tissue sites after extravasation of tumor cells from the bloodstream. By utilizing antiserum to asialo GM1, which depletes natural killer activity when administered in vivo, an in vivo model has been established for selectivity studying $M\phi$ -mediated antitumor effects in normal or biological

In related *in vitro* experiments, we have shown that murine recombinant gamma IFN (γ IFN) supplies both the priming and triggering signals for M ϕ activation. This means that γ IFN may play an important role in the generation of M ϕ -mediated antitumor events *in vivo*.

Because of the major role that RNA plays in any process of cellular differentiation, the changes in RNA metabolism that occur during the transition from resting to tumoricidal (activated) macrophages have been analyzed by Dr. G. Varesio. There was a direct correlation between expression of tumoricidal activity and decrease in RNA synthesis. Molecular analysis of the species of RNA affected revealed a selective inhibition of the 28S ribosomal RNA in activated macrophages. This was observed in macrophages that had been activated *in vitro* by endotoxin, lymphokines or interferon, or *in vivo* by endotoxin or Corynebacterium parvum. Based on these observations, it was established that agents which inhibit RNA synthesis activate macrophages to a tumoricidal state. Furthermore, increasing evidence has been obtained on the positive effects of inhibitors of RNA synthesis on macrophage activation by demonstrating synergy between these agents and suboptimal doses of BRMs.

Human monocytes have been examined by Dr. Kleinerman and co-workers for their ability to be activated to a tumoricidal state. Macrophage activation factor (MAF), produced by human lymphocytes after activation with Sepharosebound Concanavalin A, can activate monocytes to become tumoricidal. MAF has been shown to be distinct from IFN γ . However, IFN in some experimental conditions can also be demonstrated to have the capacity to activate macrophages to be tumoricidal. Soluble MAF or MAF encapsulated into multilamellar liposomes will induce a tumoricidal state although the latter method is much more effective. Liposomes can also be used to activate macrophages by muramyl dipeptide or MTP-PE, a lipophilic derivative of muramyl dipeptide. Using rodent and human MAF it has been shown that species specific surface receptor binding can be bypassed using liposome encapsulated MAF. MAF may have at least two separate "functional" parts of the molecule: one for cell surface binding and a second for initiating the activation process.

Changes in lipid of composition of macrophage membrane and lipid metabolism have been examined by Drs. Philippe Bognoux and Ezio Bonvini with Thomas Hoffman. Mouse macrophages that have been activated *in vivo* by adjuvants express higher levels of the glycolipid, asialo GM1. Biochemical analysis indicated that there are other major shifts in neutral glycolipids and gangliosides when macrophages go from resting to activated states. In lymphocytes, α and β IFN caused an increase in arachidonic acid as well as in other polyunsaturated fatty acids in the surface phospholipids, resulting in a more unsaturated pattern in their fatty acid composition. These changes result in an alteration of the plasma membrane motional state which may promote certain membrane-dependent cell functions such as cytotoxicity. Similarly, there were changes in the lipids of the NK-susceptible tumor target cells, K562. These changes correlate with increased sensitivity of K562 to lysis by NK cells after treatment with IFN. IFN also inhibits phospholipid (PL) methylation in mononuclear cells, an effect that might contribute to alterations in the properties of the membrane of IFN-treated cells. Other studies of methylated nonpolar lipids in monocytes have revealed an inverse relationship between activation of the oxidative burst and the rate of phospholipid and nonpolar lipid methylation. The inhibitory effects on lipid methylations in stimulated monocytes suggests a relevant role of these pathways in the response of monocytes to stimuli. Functions of accessory cells, which are

required for the process of lymphocytes activation, have been examined by Dr. Oppenheim and co-workers. DR antigen expression can be increased on human monocytes by treatment with γ IFN, in vitro, whereas exposure to prostaglandin decreases DR expression. Since only DR⁺ monocytes can mediate lymphocyte activation, these agents regulate their capacity of monocytes to present activating agents to lymphocytes. Paraformaldehyde-fixed human monocytes can activate T lymphocytes only if they were preincubated with the stimulant for several hours at 37°C and subsequently supplemented with IL-1. Exposure of the monocytes to lysosomotropic agents during this preincubation period inhibits their ability to activate lymphocytes suggesting that stimulant processing at the lysosomal level is necessary for accessory cell function. Several nonmonocytic cell types such, as B lymphocytes and some NK cells that express DR antigen, were demonstrated to produce IL-1 and shown to function as accessory cells. Finally, the cascade of cell-cytokine interactions required to promote lymphocyte activation has been further delineated. Exposure to exogenous stimulants initiate a sequence of factor-cell interactions that amplify the immune response as follows: colony-stimulating factor stimulates macrophages or monocytes to produce IL-1. IL-1, in turn, enhances lymphocyte production of IL-2, which in turn promotes the production immune interferon (IFN γ). IFN γ has a multiplicity of immunoenhancing consequences through its differentiation affects on specific and natural killer lymphocytes and macrophages, and also exerts a positive feedback effect by enhancing monocyte DR/Ia antigen expression of augmenting lymphocyte activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09213-03 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Tumor Antigens and Transforming Growth Factors

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Edward S. Kimball, Senior Staff Fellow, LMI, NCI

COOPERATING UNITS (if any)

NCI-FCRF; BTB, BRMP, NCI-FCRF; LVC, NCI-FCRF

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed a screening procedure for transforming growth factor activity in the urine. Analysis of acid extracts of urine from normal donors and cancer patients by reverse phase HPLC revealed the presence of five EGF-related growth factors with soft agar colony promoting activity. In addition, factors were identified for the first time in urine which had colony stimulating activity only in the presence of EGF, but which were not EGF-related. Determination of EGF activity and quantitation of levels of EGF activity were accomplished using a solid phase radioreceptor assay developed in this laboratory this past year. Of the EGF-related activities observed using this assay, two were elevated in cancer patient urines, one of which correlated with a high molecular weight TGF previously shown by gel filtration to be unique to most cancer patients. Another TGF was found at high levels in normal control urines. Thus, using reverse phase HPLC, we were able to resolve five major species of EGF-related TGF. These are functionally similar, but chemically distinct from TGF isolated from tissue culture tumor cells. Distinct qualitative and quantitative differences are seen in TGF urinary moieties of cancer patients compared to normal controls. Purification methods are being developed to allow isolation of the tumor-associated urinary TGF in sufficient quantities to allow complete biochemical characterization and comparison to TGF produced by tumor cell lines in tissue culture.

PROJECT DESCRIPTION

PERSONNEL

Myung Kim	Visiting Fellow	BS	LMI	NCI
Paul D. Abrams	Expert	CIS	LMI	NCI
Daniel Twartzig	Senior Staff Fellow		LVC	NCI

OBJECTIVES

1) To purify and biochemically characterize TGF found in cancer patient's urine; 2) to study biochemical relationships which exist between tumor-associated TGF (30,000 m.w.) and TGF found in all normal controls (6000 m.w.) as well as in cancer patients; 3) to determine whether monoclonal antibody 503-D8 will be useful for immunodiagnosis and treatment of lung cancer; 4) to identify and biochemically characterize lung tumor antigens on human lung tumor cell lines and on fresh tumor explants detected by antibody 503-D8.

METHODS EMPLOYED

Ultrafiltration, reverse phase HPLC, gel filtration and ion-exchange chromatography to isolate and purify TGF. Soft agar growth assay and competitive radiobinding to EGF receptors to assay for TGF. Surface labeling and biosynthetic labeling cells and their secreted products, immunoprecipitation with 503-D8. The immunoprecipitated products are analyzed by SDS-PAGE and fluorography techniques. Standard biochemical techniques including ultraviolet and visible spectroscopy, affinity chromatography, ultracentrifugation, preparative gel electrophoresis, size exclusion, peptide mapping by HPLC, ELISA assay, immuno-histochemical techniques are also used in these studies.

MAJOR FINDINGSI. Studies on Transforming Growth Factors (TGF)

During the past year, studies have concentrated on establishing the biochemical identity of TGFs found in the urine of cancer patients. These TGFs promote colony formation of a normal rat kidney fibroblasts in soft agar. The TGFs are acid soluble and have been shown to fall into two molecular weight categories: 30,000 daltons and 6,000 daltons. We have recently developed a rapid two-step purification procedure in which urine is acidified, concentrated by ultrafiltration and the TGFs purified by reverse phase HPLC. We have also developed a solid phase EGF radioreceptor assay using cell membranes immobilized in 96-well plates as targets for EGF competition. This assay has allowed us to establish that there are five species of EGF-related TGF in the urine. HPLC revealed that there are significant quantitative and qualitative differences in this type of TGF activity in normal individuals and cancer

patients. When a panel of urine samples were screened, two of the TGF observed (TGF_A and TGF_D) were elevated only in cancer patient's urine up to tenfold over that seen in normals and correlated with high molecular weight (30,000) TGF (tumor-associated). A third TGF (TGF_E) was found at high levels in normal urine and appeared to be absent from in the patient urine. Differences in TGF_A/TGF_E ratios could be discerned when normal controls and patients were examined. In addition to examining EGF-related TGFs, we detected EGF dependent, non-EGF related TGF activity. This has never been described in urine before. No differences were observed for this type of TGF activity between normal individuals and cancer patients. Studies on the 30,000 dalton tumor-associated TGF revealed upon rechromatography by gel filtration that it had been broken down to 6000 dalton activity, suggesting that 30 Kd TGF may be present because of incomplete metabolic processing.

II. Studies on a Human Lung Tumor-Associated Antigen

Solid phase ELISA using 503-D8 monoclonal antibody was developed and showed that the antigen detected by this antibody is elevated on all types of lung tumor cell line, except small cell carcinoma, and on fresh human lung tumor explants. The antigen was also expressed at high levels in lung, liver and kidney of patients with a variety of tumors, in the lung of a patient with non-malignant lung disease and in a colon tumor. An immunoperoxidase technique was developed to allow examination of paraffin-embedded tissues. Thus, we found that only tumor cells in adenocarcinoma and squamous cell carcinoma of the lung and adenocarcinoma of the colon scored positive. However unrelated tissues expressed the antigen in glandular epithelial cells, particularly the bile duct cells in the liver of a trauma victim with no recent history of disease. Immunoprecipitation and SDS-PAGE analysis revealed that all 503-D8 positive tumor cell lines, and fresh lung tissue explants expressed the same bimolecular complex of 15000 and 69000 dalton proteins.

SIGNIFICANCE

The TGF studies show that another means of cancer diagnosis and prognosis may be available. The rapid purification of TGF by HPLC and the EGF-competing activity they display has a number of applications. EGF competition assays are rapid overnight assays whereas soft agar growth assays take up to 14 days. The solid phase EGF competition assay we developed utilized membrane fragments, and is more consistent and more convenient than assay systems which use freshly grown cells. Recent evidence of unique receptors for TGF suggests that the above solid phase assay can be adapted to examining TGF-receptor interactions via TGF receptors instead of using a clonogenic Bioassay or indirect EGF-binding assays. This will further simplify assays for TGF activity. We have shown that EGF-competition assay on HPLC fractions of patients' urine could be used as a rapid, indirect diagnostic assay. Two applications come out of the HPLC purification. First, the purified TGFs can be used as pure immunogens for development of monoclonal antibodies that will allow rapid, direct assay for TGFs to be performed. Second, TGF-drug conjugates can be developed and possibly used in vivo as cytotoxic agents. This is predicated on the possibility that growing tumors have a high density of available receptors for TGFs. Finally,

availability of purified TGF will facilitate further biochemical studies on TGFs and comparison to EGF and other growth factors. Such studies will provide a greater understanding of the structure-activity relationships required for transforming growth ability. The finding that 30,000 molecular weight tumor-associated TGF will decompose in vitro to 6000 molecular weight TGF seen in normal controls and cancer patients suggests that its original presence in vivo is due to metabolic alterations. It also may be an embryonic or precursor form of EGF-related TGF and might provide information regarding biosynthesis of these molecules. The putative lung tumor antigen recognized by antibody 503-D8 is actually a marker for glandular epithelial cells and may be valuable as such. Its expression is elevated in a number of tumor types, particularly adenocarcinoma of the lung, colon and breast. Its expression by cells which are histologically not glandular epithelial demonstrates that This antigen may be a differentiation antigen. Since this data suggests that this antigen is of limited utility as a tumor marker, this problem will not be pursued any further.

PROPOSED COURSE

During the next year, we plan to continue the studies on TGFs as follows:

A) Biochemical Studies: we plan to further characterize TGFs in terms of amino acid composition and amino acid sequence. Purified TGF will also be used to develop a TGF solid-phase assay similar to the one developed for EGF. Cell lines with little or no EGF receptors but with high numbers of TGF receptors are available for this purpose. The conversion of high molecular weight tumor-associated TGF to low molecular TGF will be studied more thoroughly to determine the molecular relationship between the two forms of TGF. Inhibitors of enzyme activity, as well as specific substrates for enzymes will be used to determine whether the cleavage is enzyme-driven or not. Monoclonal antibody will be raised to highly purified TGF. This will eventually allow another rapid, highly sensitive and specific assay for TGFs to be performed. It should also allow a finer level of discrimination to be achieved among the various types of TGFs.

B) Clinical Relevance: the diagnostic potential of examining urinary TGF levels after HPLC fractionation has already been demonstrated. However, the potential for using TGF in a treatment methodology has not been explored. Accordingly, purified TGF will be trace-labeled and introduced into tumor-bearing animals. Autoradiography can then be used to follow the localization of labeled TGF to determine the feasibility of using TGF conjugated to cytotoxic agents.

PUBLICATIONS

Two papers on the TGF studies and one on the 503-D8 study have been submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09242-07 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Serological Studies of Tumor-Associated Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James A. Braatz, Exp. Cons., LMI, NCI

COOPERATING UNITS (if any)

Abbott Laboratories; Hoffmann-La Roche; Litton Institute of Applied
Biotechnology; Georgetown University Hospital

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

LTA, a human lung tumor-associated antigen which is found in 86% of lung tumors of all major histologic types, but not in non-lung tumors, normal adult or fetal lung, and other normal tissues, has been further evaluated in terms of its usefulness as a lung cancer marker. We have previously demonstrated increased circulating levels in lung cancer patients using an improved radioimmunoassay and have now extended these results with a solid phase enzyme-linked immunosorbent assay (ELISA). Serum LTA levels were determined in three terminal lung cancer patients and compared to CEA levels for monitoring capability. In all these patients, LTA levels rose dramatically 2-4 months prior to death while CEA levels, at least in two patients, were not predictive of disease recurrence. A major finding was the presence of LTA in bronchial washings from lung cancer patients in concentrations up to 40 times higher than in normal samples. LTA has been further compared to known proteins in the circulation and is not related to any of the 53 serum proteins examined. These studies made use of a second generation antiserum which was raised in a goat against a highly purified LTA preparation. Large quantities of this antiserum were collected and will replace the existing rabbit anti-LTA which has been used in this project since its inception.

PROJECT DESCRIPTION

PERSONNEL

David T. Hua, Ph.D.	Visiting Fellow	BS LMI NCI
Gerald L. Princler, M.S.	Chemist	BS LMI NCI

OBJECTIVES

The objectives of this study are: (1) to purify and characterize a human lung tumor-associated antigen (LTA), (2) compare antigens purified from various sources in terms of their physicochemical properties and their structural and immunochemical relationships, (3) develop radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) for quantitating these antigens, (4) assess the usefulness of these immunoassays in the diagnosis of lung cancer and for monitoring the course of disease, (5) study the biological role of these antigens in the tumor, and (6) develop monoclonal antibodies against the purified antigen which would be useful in Objectives 1-5.

METHODS EMPLOYED

Radioiodination of purified protein antigens. Radioimmunoassays using the purified, trace-labeled protein antigens. ELISA based on immobilized, highly purified antigen. Standard biochemical isolation and analytical techniques including gel filtration; ion exchange chromatography; affinity chromatography; acrylamide gel electrophoresis; isoelectric focusing, chromatofocusing; ultraviolet, visible and fluorescence spectroscopy; ultracentrifugation; lyophilization; etc. High performance liquid chromatography (HPLC) in both the size exclusion and the reverse phase modes. Autoradiography. Immunochemical techniques such as double diffusion in gel, radial immunodiffusion and immunoelectrophoresis. Techniques related to cell hybridization studies directed at the hybridization of murine lymphoid cells and long term maintenance of hybrid cells in culture. Monoclonal antibody production by B cell hybridomas and detection by ELISA and RIA. Techniques related to cell-free translation of mRNA including the isolation and characterization of mRNA from tissue culture cell lines and characterization of the products of its translation in vitro. Computerized analysis and graphic presentation of RIA, ELISA and other results using personal programs as well as public programs, such as MLAB and TELL-A-GRAF which are supported by the NIH computer systems.

MAJOR FINDINGS

During the fiscal year significant progress was made in several areas of investigation concerning the basic biology and clinical utility of a human lung tumor-associated antigen (LTA). This antigen which has previously been demonstrated in 84 of 98 primary lung tumors as well as 7 of 10 lung tumor tissue culture cell lines but not in 12 of 13 nonlung tumors or normal tissues including

adult and fetal lung, has now been shown to be immunologically distinct from each of 53 individual normal human serum proteins. A suspected cross reactivity with one of these, alpha-1-acid glycoprotein (AGP), was shown to be due to trace contamination of the commercially-prepared AGP with LTA, or an LTA-like activity which is present in normal serum. Useful in these and other studies has been a second generation antiserum which was raised in a goat against a very highly purified preparation of LTA. Plasmapheresis of the animal on a regular schedule permitted the collection of 15 liters of antiserum. It was found, in contrast to rabbit anti-LTA, which was raised against a crude lung tumor extract and consequently reacted with most normal tissues, that the goat anti-LTA was much more specific in an Ouchterlony assay. This goat antiserum has been used to develop an enzyme-linked immunosorbent assay (ELISA) which because of greater convenience and shelf life, has replaced the more cumbersome RIA for detecting LTA. Using the ELISA we have now identified LTA in bronchial washings from patients with carcinoma of the lung. These patients were easily distinguished on this basis from normal smokers and nonsmokers as well as patients with benign lung diseases in a series of blind studies. In another clinical application of the ELISA, serial serum samples from three patients with terminal lung cancer were assayed for LTA levels. In all three cases, LTA rose dramatically 2-4 months before the patients died. In contrast, CEA levels in two of these patients remained unchanged throughout and were not predictive of disease recurrence.

I. Preparation of a second generation antiserum to LTA

Antisera previously used in studies on LTA were raised in rabbits against crude tumor extracts. The availability of purified LTA provided a much cleaner protein preparation which could serve as immunogen. Administration of 30 micrograms of purified LTA lead to an anti-LTA response which remained essentially constant over a 30 week period. Plasmapheresis and collection of 1 liter of plasma at weekly intervals seemed not to drastically reduce the titer. This goat antiserum, referred to as G1270, produced precipitin lines of identity with those formed using rabbit anti-LTA. The multiplicity of reactions against normal tissues observed with the rabbit anticrude lung tumor extract, which required extensive adsorption to remove, were not observed with G1270. While the titer of the goat antiserum was about three-fold lower, the binding affinities of goat versus rabbit anti-LTA were similar, $4.6 \times 10^9 \text{ M}^{-1}$ and $3.4 \times 10^9 \text{ M}^{-1}$, respectively.

II. Development of an ELISA for LTA

Because of limited availability of purified LTA, development of a solid phase ELISA was attempted using subnanogram quantities of immobilized target antigen. By using 0.5 nanograms of purified LTA per well in a 96 well polyvinylchloride microtiter plate we have been able to establish a quantitative assay sensitive to less than 1 nanogram of LTA. The real advantages of this assay are that for an equivalent amount of antigen we can assay 10 times as many samples as with the RIA. In addition, radioiodinations are not required and immobilized target antigen was stable to storage. Thus with this assay format we can quantitate as many or as few samples as are necessary in contrast to the RIA, which once the antigen was radiolabeled, had to be used as quickly as possible. G1270 is the

antiserum used in this assay and it was found necessary to affinity purify it over an immobilized, partially purified LTA preparation to eliminate a high background. That the assay in fact measures the antigen in question was demonstrated by showing coincidence of ELISA-inhibitory activity with authentic LTA electrophoretic mobility after LTA was electrophoresed in 7% nondetergent polyacrylamide gels. Standard displacement curves generated with pure LTA preparations indicate the ELISA is 5-10 times as sensitive as the former RIA. The reproducibility of the ELISA is suggested by a series of six separate experiments in which a constant amount of pure LTA, 1.6 ng, produced $70.8 \pm 7.3\%$ inhibition. As a technical note the assay is based on the glucose oxidase-avidin system in which 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) is the chromogenic substrate.

III. Evaluation of LTA as a Lung Tumor Marker

Last fiscal year we reported on a study performed using the RIA which examined a panel of 215 serum samples from (a) patients with lung cancer, (b) patients with nonlung cancers, (c) patients with benign lung disease, and (d) normal individuals. Frequent elevations were demonstrated in the lung cancer group, especially in those with squamous cell carcinoma and adenocarcinoma. With the newly developed ELISA we have continued this approach and have made the discovery, in collaboration with Dr. Henry Yeager of Georgetown University Hospital, that LTA can be demonstrated in bronchial lavage fluids from patients with lung cancer. In the limited panel so far tested (blind) the following results were obtained: (a) lung cancer, 12 patients, 6 had levels > 125 ng/ml, mean = 235 ng/ml; (b) benign lung disease, 4 patients, 0 had levels > 125 ng/ml; mean = 73 ng/ml; (c) normals, 31 patients, 1 had levels > 125 ng/ml, mean (of 30 negatives) = 22 ng/ml. It was of interest that the one false positive had a repeatedly high value of > 800 ng/ml and, although otherwise normal, in addition was positive in a test which measures benzpyrene binding to alveolar macrophage DNA, a test thought to indicate a predisposition to lung cancer.

In another early attempt to apply the new assay to clinical specimens, LTA was determined retrospectively in a series of serum samples from three patients with advanced lung cancer. Since these samples had been in frozen storage for several years they were not considered ideal for this purpose. Nevertheless, some interesting results were obtained. In all cases, LTA levels rose progressively during the last few months of life. Two patients LTA levels exceeded 3000 ng/ml. For comparison, in a limited panel of normal sera, 20 of 23 samples contained less than 100 ng/ml. In contrast to the rise in LTA levels in these three cancer patients, CEA increased in only one, but remained essentially within normal limits in the other two. Efforts are continuing to further evaluate the usefulness of this assay for measuring circulating LTA levels in the diagnosis and monitoring of lung cancer.

IV. Immunochemical Relationship of LTA to Normal Serum Proteins

In order to determine whether LTA is related to a normal serum component, purified and radioiodinated LTA was incubated with antisera to individual serum proteins and control antisera. Positive control antisera, which included both rabbit and goat anti-LTA, as well as two rabbit antisera raised against ChaGo,

a cell line know to express LTA, all bound significantly to LTA. Of 53 antisera to serum proteins, only one produced significant and reproducible binding to LTA: antiserum to alpha-1-acid glycoprotein (AGP). This result suggested that previously observed inhibition by normal human serum in the RIA for LTA was due to AGP. Additional evidence supporting this hypothesis was our observation that purified AGP was capable of inhibiting the RIA for LTA, although the amounts required were extremely high. On the order of 1000 times as much AGP was needed to give equivalent inhibition obtained with LTA. A comparison of the properties of the two proteins revealed that they differ with respect to size (both native and in detergent), lectin binding patterns, iodination patterns, isoelectric points, sedimentation and diffusion coefficients, and their electrophoretic mobilities in the absence of detergent. Another observation which suggested AGP and LTA were distinct was the inability of anti-LTA to bind radiolabeled AGP. These seemingly conflicting results lead us to suspect that commercially obtained AGP was contaminated with trace quantities of LTA or LTA-like activity which originated in serum. This possibility was confirmed in an experiment which took advantage of the large differences in electrophoretic mobilities of the two proteins and demonstrated that AGP and LTA-activity could in fact be separated. Thus LTA is not related to AGP, but interestingly, some unknown serum substance which inhibits the ELISA for LTA contaminates commercial AGP preparations.

SIGNIFICANCE

Studies on LTA are relevant to both clinical and basic science. The significance of the work directed at developing a clinically useful immunoassay for the detection or monitoring of lung cancer is readily apparent, as earlier detection of the disease or a recurrence is essential if survival rates are to be improved. Our continuing evaluation of LTA levels in lung cancer patients sera and, as reported this year, in bronchial washings, strongly suggests that LTA will find some role as a lung tumor marker; whether for diagnosis, monitoring, or both remains to be decided.

In addition to its clinical potential, the basic biological features of LTA render it an interesting protein for further study. Its restricted expression by lung tumor cells suggests a possible role in the initiation, propagation or maintenance of the transformed state. Fundamental studies concerning biosynthesis and subcellular localization of LTA are required for any understanding of the protein's function.

PROPOSED COURSE

A serious effort was made in the latter part of this fiscal year to raise a monoclonal antibody to LTA. The primary difficulty encountered in the past which still plagues these attempts is the unavailability of sufficient quantities of highly purified LTA. Additional antigen is being isolated from tissue culture lines but a complete purification scheme for this source still needs to be worked out. The development of a reliable ELISA will greatly enhance the possibility of selecting an effective anti-LTA monoclonal antibody. A battery

of monoclonal antibodies will be useful for varied purposes, including antigen purification, immunoassay development, immunohistochemical localization of LTA, and studies concerning the biosynthesis and subcellular localization of LTA. Thus a high priority is assigned to the production of such monoclonal antibodies.

Continued evaluation of the clinical usefulness of LTA will remain a high priority project in the coming fiscal year. Collaboration with Abbott Laboratories on this project has been very active this year and is expected to continue. Additionally, Hoffmann-La Roche and the Litton Institute for Applied Biotechnology have also secured nonexclusive license rights to the U.S. Government patent covering this process.

PUBLICATIONS

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Braatz, J. A., Hua, D. T. and Princler, G. L.: Serum levels of a human lung tumor-associated antigen using an improved radioimmunoassay. Cancer Res. 43: 110-113, 1983.

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Jones, C. M. and Braatz, J. A.: Plasminogen activator: a rapid sensitive screening assay for macrophage activation. J. Clin. Hematol. Oncol. (In Press)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201CM09215-03 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Macrophage Migration Inhibitory Factors

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Joost J. Oppenheim, Lab Chief, LMI, NCI

COOPERATING UNITS (if any)

BTB, BRMP, DTC, NCI; Harvard University Medical School; Fermentation Program, FCRF

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokines/Cytokines Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A variety of highly purified and partially purified lymphokines have been tested for their ability to inhibit the migration of human or guinea pig macrophages. These lymphokines have been obtained from normal human peripheral blood cells, from human T-T hybridomas and from human B-cell and T-cell lines. Three types of migration inhibitory factors (MIF) have been identified: T-cell derived, B-cell derived and interferon (IFN)-related classes. The relationships between these three types of MIF's are being investigated in this study by comparing their physiochemical characteristics, their modes of action, and their antigenic properties using monoclonal antibodies (MoAb). The relationships between MIF's and other lymphokine activities are also being investigated. MoAb against human alpha IFN, human gamma IFN and human IL-2, and antisera specific against human beta IFN, human CSF and human IL-1 are being used in the evaluations. Some cytokines, such as IL-1, IL-2, lymphotoxin and thymosin alpha-1 have been found to have no effect on macrophage migration. Alpha and gamma IFN have been shown to inhibit macrophage migration with gamma IFN having the most potent MIF-like activity per unit of antiviral activity (significant inhibition at 1-10 units/ml). Human gamma IFN produced in *E. coli* by recombinant-DNA technology also was found to have potent MIF-like activity indicating that the MIF activity of gamma IFN was not caused by a contaminant. This was confirmed by showing that the MoAb to gamma IFN neutralized all of the activity present in gamma IFN preparations. MIF activity has been obtained from the human B-cell line RPMI-1788. This activity was not neutralized by the anti-gamma IFN MoAb. However, 3 different partially purified MIF preparations having different molecular weights and PI's obtained from Harvard University were all neutralized by the anti-gamma IFN MoAb when tested on guinea pig macrophages. These observations have led us to postulate that T-cell derived MIF and gamma IFN may be structurally closely related and may actually represent 2 different lymphokine activities present within a single molecular type.

PERSONNEL

Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Eugenie S. Kleinerman	Senior Investigator	IS	LMI	NCI
James A. Braatz	Acting Head	BS	LMI	NCI
Sue F. Pickeral	Biologist	LS	LMI	NCI
Robert K. Oldham	Associate Director		BRMP	NCI
Heinet Remold	Associate Professor			Harvard Univ. Med. School

OBJECTIVES

1. To evaluate sources of specific human lymphokines (MIF, MAF and IFN) for levels of constitutive or inducible production, minimal serum requirements and feasibility for large scale production. Sources presently contemplated for analysis are (a) peripheral blood mononuclear cell lines from cytopheresis or thoracic duct drainage of renal transplant candidates; (b) lymphoblastoid cell lines such as RPMI-1788; (c) established T-cell tumor lines; and (d) T-T hybridomas.
2. To evaluate, standardize, and automate several assays for measuring various parameters of the mode of action of these lymphokines.
3. To obtain partially purified preparations of these lymphokines for use in production of monoclonal antibodies, for use in further purification and delineation of modes of action.
4. To initiate in vitro studies on the molecular and cellular mechanisms of action, and in vivo studies of antitumor efficacy of these lymphokines.
5. To utilize monoclonal antibodies to various lymphokines to extract certain lymphokine activities from crude or partially purified lymphokine preparations to determine effects on activities and potency.

METHODS EMPLOYED:Production

Peripheral blood mononuclear cells or thoracic duct lymphocytes are stimulated by a pulse exposure to a mitogenic agent, such as Concanavalin A, and then cultured for two days. Supernatants are rendered cell-free by centrifugation and filtration. This supernatant is concentrated, desalted, ultracentrifuged, aliquoted, and lyophilized for use as the crude material for further purification efforts. Such supernatants can be rendered free of specific lymphokine activities by several passages over specific monoclonal antibody conjugated to sepharose.

Another method of production is the fusion of lymphokine-producing T-cells with a continuous T-cell line and evaluating the hybridomas for MIF and MAF production. In collaboration with the Cell Fusion Lab of the BRMP, with Sloan-Kettering Institute and with Harvard University, we have initiated evaluations of crude and partial purified supernatants from T-T hybridomas.

Assays

A variety of assays for measuring various parameters of macrophage function are available within the Branch. Assays are being evaluated for measuring and quantitating human MIF. Human monocytes purified by elutriation are being used as indicator cells in a miniaturized agarose-droplet MIF assay. Techniques for automation of this assay using a computer-assisted reading system have been developed. The criteria of sensitivity, efficient use of effector cells, variance of replicate samples, assay set-up and evaluation time, accuracy and reproducibility are routinely evaluated during assay development. The MAF assay being utilized is a radioisotope-release human-tumor-cell cytotoxicity assay using Percoll-separated or elutriated monocytes. Other biochemically oriented short-term assays, measuring parameters such as the oxidative burst, are being evaluated for correlation with the induction of cytolytic capability, to possibly be used for initial screening of samples for MAF activity.

MAJOR FINDINGS

As reported at the 3rd International Lymphokine Workshop, one of the major findings of this study is that interferons have potent migration inhibitory activity against human monocytes and guinea pig macrophage. Alpha and gamma interferon both have MIF-like activity with gamma interferon being 100-1000 times more potent. This observation may indicate that much of the difficulty in purification of MIF centers around the fact that several distinct lymphokines, including interferons, can have MIF-like activity. Further work has indicated that MIF activity does not always correlate with antiviral activity of interferon suggesting that MIF activity and antiviral activity may reside on two separate regions of the interferon molecule.

All of the MIF-like activity present in clinical-grade gamma IFN preparations can be neutralized by MoAb to gamma IFN, indicating that gamma IFN is the sole source of MIF-like activities in those preparations. This same MoAb can neutralize the MIF activity of the three MIF prepared at Harvard University by classical techniques. These three MIF's differ somewhat in their PI value and molecular weights and none have antiviral activity. This suggest that the Harvard MIF's are either structurally related to gamma IFN or are different activities on different parts of the same molecule. Other MIF-like factors from cell lines (like RPMI-1788) are not neutralized by this MoAb. MIF-like activity of alpha IFN is also not neutralized by MoAb anti-gamma IFN.

This suggests that a family of factors in crude supernatants are responsible for the migration inhibition activity present and indicates that the MIF-assay, once used as a simple correlate of cell-mediated immunity, is actually a complex interaction of a variety of lymphokines with macrophages. The relationship

of these MIF-like factors one-to-another and to other lymphokine activities are the basis of this study. Similar tests using the MAF assay has indicated that human gamma interferon does not give MAF-like activity and MAF activity cannot be neutralized by anti-gamma IFN MoAb. This indicates that MAF and MIF are distinct activities and probably are not antigenically or functionally related.

PROPOSED COURSE

This project will continue to evaluate existing cell lines and produce and evaluate new cell lines (or hybridomas) as potential sources of lymphokine production. Major emphasis will continue to focus on MAF and MIF production and further refinement of the assays to quantitate these factors will be placed on automation, sensitivity, specificity and reproducibility as desirable aspects of the assays.

Initial efforts into the production of MIF and MAF by a known producing cell line, RPMI-1788, will continue in collaboration with the Fermentation Program at NCI-FCRF.

Investigation into the Possible Roles of Interferon in Macrophage Function:

To further investigate the question of the possible sequential relationship of IFN and MIF, experiments will be done by adding anti-IFN antisera or monoclonal antibody into direct MIF assays. If MIF is independent of IFN, then migration inhibition should be evident in the MIF-driven system in the presence of anti-IFN antibodies. If MIF activity is diminished by the anti-IFN, it could imply a sequential relationship for IFN with these factors. If IFN acts by inducing lymphocytes to make MIF, then ultrapurified macrophages or monocytes should not be affected by IFN as they are with MIF. Retesting with added purified lymphocytes or NK cells might indicate which cell population is responsible for the factor production following IFN addition.

To test whether IFN is an essential factor produced during macrophage migration inhibition will be tested by utilizing the adsorption of IFN by monoclonal antibody linked to Sepharose. This will give us information on the number of family members of molecules with MIF activity and will help dissect the contribution made by each specific IFN or other factor.

Investigations in Animal Tumor Models of Lymphokine Mediated Antitumor Therapy:

As various human lymphokines are prepared or received in semi-purified form, protocols will be initiated to evaluate the effects of these lymphokines on tumor growth and survival of tumor-bearing hosts. Existing tumor models presently in use within the BRMP will be used and both systemic and intratumor effects of lymphokine treatment will be evaluated. Of particular interest will be the sequential administration of partially purified lymphokines designed to attract and localize macrophages at the tumor site, followed by the administration of activating agents (such as MAF) which will activate the cytotoxic activity of macrophages against tumor cells.

PUBLICATIONS

Smalley, R. V., Talmadge, J., Oldham, R. K. and Thurman, G. B. The Biological Response Modifiers Program: Preclinical and clinical studies with thymosin preparations. In International Symposium of Thymic Factor Therapy. London, Sero Symposium. (In Press)

Stevenson, H. C., Favilla, T., Beman, J. A., Akiyama, Y., Miller, P., Herberman, R. B., Oldham, R. K., Stull, H. and Thurman, G. B. A system for obtaining large numbers of highly purified cryopreserved human monocytes and monocyte-depleted lymphocytes by leukoporesis/counter-current centrifugal elutriation (CCE). J. Immunol. Methods. (In Press)

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Thurman, G. B., Stull, H. B. and Oldham, R. K. The effects of human interferon on the migration of guinea pig peritoneal exudate cells. In Oppenheim, J. J. and Cohen, S. (Eds.): Interleukins, Lymphokines and Cytokines. New York, Academic Press. (In Press)

Yukio, A., Miller, P. J., Thurman, G. B., Neubauer, R. H., Oliver, C., Favilla, T., Beman, J. A., Oldham, R. K. and Stevenson, H. C. Characterization of human blood monocyte subset with low peroxidase activity. J. Clin. Invest. (In Press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1CM09243-02 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Production of Human MoAbs Based on In Vitro Sensitization of Human Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Joost J. Oppenheim, Chief, LMI, NCI

COOPERATING UNITS (if any)

NCI-FCRF; Monoclonal Antibody Section, BTB, BRMP, DCT; Clinical Investigations Section, BTB, BRMP, DCT

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokines/Cytokines Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to develop methods to effectively immunize human cells in vitro against tumor antigens (or model antigens in well-documented systems), and to use these cells as fusion partners to produce human-human or human-mouse hybridomas secreting antitumor antibody. During this year, we have studied patients being treated in the anti-melanoma monoclonal antibody clinical trial conducted by the Clinical Investigations Section, BTB, BRMP, DCT. Various strategies of antigen presentation, timing, and culture modifications were evaluated.

The first four patients treated with the 9.2.27 murine anti-melanoma antibody were evaluated prior to and following antibody therapy. None of the patients' peripheral blood lymphoid cells produced specific anti-melanoma antibody. However, lymphocytes obtained following therapy could be stimulated to produce anti-M14 antibody by incubating these cells in vitro for 8-14 days in the presence of a solid-phase complex of melanoma cell (M14) extract presented on 9.2.27 antibody. This was true in all four of the patients studied thus far.

Recently, human-human hybridoma fusions were performed using such in vitro immunized cells and the fusion parent line HF-2. Although some human B-B hybridomas have been found to produce anti-melanoma antibody, the hybridoma cultures are not yet cloned. The oldest hybridoma culture is 80 days old (May, 1983).

PROJECT DESCRIPTION

PERSONNEL

Susan F. Pickeral	Biologist	LS	LMI, NCI
Teresa L. Delawter	Work-Study Student	LS	LMI, NCI
A. Charles Morgan	Expert	MAS	BTB, NCI
Paul G. Abrams	Expert	CIS	BTB, NCI
Helen Rager		CFL	BTB, NCI

OBJECTIVES

- (1) Optimization of conditions for the in vitro immunization of human cell to model antigens and tumor antigens to circumvent the need for in vivo sensitization of patients with tumor cell products.
- (2) Use of in vitro immunized cells as fusion sources in the production of human-human or human-mouse hybridomas to circumvent human allergic reactions to mouse monoclonal antibodies.
- (3) Investigation of the capacity of lymphokines such as interleukins, interferons and growth factors to improve in vitro immunizations or better hybridoma fusion efficiency or growth.

METHODS EMPLOYED

Methods used in this study include collection of human peripheral blood lymphoid cells from venipuncture or from leukapheresis, isolation of various lymphoid cell populations, and culture of these populations with antigens in vitro. The antigens studied include tetanus toxoid, chosen as a well-documented model system, melanoma cells (lines M14, A2058, A375), extracts or membrane fragments from these tumor cell lines, and control tumor antigen preparations from colon tumor lines, lung tumor lines, lymphomas, and others. Antibody production by lymphoid cells in culture has been evaluated in a number of ways, including enzyme-linked immunoassays (ELISA's) and hemolytic plaque-forming cell assays in agarose. Fusions were performed by the personnel of the Monoclonal Antibody Section, but all supernatant testing and bioassays and biochemical analyses have been done in our laboratories. Hybridomas have been screened primarily through ELISA testing, with confirmations of specificity on various unrelated cell types. Antibodies have been typed and classified through ELISA testing, and using polyacrylamide and agarose gel electrophoresis and isoelectric focusing procedures.

MAJOR FINDINGS

- (1) Peripheral blood cells from four melanoma patients could not be induced in vitro to produce antibody reactive specifically with the M14 melanoma cell line. Several different extracts of M14 were used as antigens, both in soluble form, presented on lectins of various types, or immobilized in solid phase on plastic or on anti-melanoma antibody (9.2.27).
- (2) All four of the patients were treated for two weeks with increasing doses of 9.2.27 antibody, as described in the Clinical Investigations Section, BTB, BRMP, DCT report. Peripheral blood was obtained following the last 9.2.27 injection, and the in vitro immunizations were again attempted. This time, in every case, antibody production could be induced, using M14 extract presented in solid phase on the 9.2.27 as an immune complex immobilized to plastic. The antibody production was specific to melanoma antigen in that reactivity was positive by ELISA against M14 cells or extracts of the M14 cell, but no reactivity was seen against lung or colon-tumor derived cell lines.
- (3) Hybridoma fusions have been performed with three of the patients' peripheral blood cells following the successful in vitro immunization procedure. Although these studies are still in the early stages, the fusions with the human fusion partner HF-2 have resulted in successful hybridoma production. That is, specific anti-melanoma antibody was detected in the hybridoma supernatants following fusion. The hybridomas have not been cloned as yet, however, and this, of course, is a critical step. The oldest culture was 80 days old at the end of May, 1983.

SIGNIFICANCE

- (1) It would be desirable to obtain human-human hybridomas that produce antibodies which are reactive against human tumors, since these might have therapeutic potential in the treatment of cancer patients. The development of anti-mouse antibodies and anaphylactic reactions reported to be associated with the administration of repeated doses of mouse immunoglobulin in immunocompetent patients (such as melanoma patients) may be lessened by the availability of such human reagents, although anti-allotype reactivity may still be a problem.
- (2) The scope of antigens to which hybridomas could be made would be greatly increase if the immunizations could be performed in vitro, since immunizations of human subjects with tumor extracts or tumor cells would not be feasible as a general practice. There is also the opportunity to use in vitro immunization for the production of anti-idiotypic antibody for the therapy of lymphoid tumors.

PROPOSED COURSE

The proposed course of this research is to continue the studies of the melanoma patients, as new patients are admitted to the clinical protocol. Among the questions which need to be answered are, what is the best antigen dose and form

for in vitro immunization; what is the optimum time during in vitro immunization for the production of a good hybridoma; what manipulations in culture (lymphokines, cytokines, growth factors) will improve the quality and/or quantity of antibodies produced in vitro or after fusion?

As mentioned above, we would like to attempt to sensitize human lymphoid cells against other antigens, such as in the production of anti-idiotypic against lymphoid tumor idiotypes, and to produce hybridoma fusions to obtain a monoclonal anti-idiotypic, if possible.

Through the production of anti-tumor monoclonals, we can, in a limited way, study the nature of the tumor antigens themselves, such as identification of the membrane structures on the tumor cells which react with the monoclonal antibodies obtained from the fusions.

PUBLICATIONS

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09251-01 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modulation of Hematopoietic Growth Factors by Biological Response Modifiers

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Erich A. Schlick, Visiting Associate, LMI, NCI

COOPERATING UNITS (if any)

BTB, BRMP, NCI

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokines/Cytokines Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antineoplastic treatment regimens consisting of chemotherapy and/or irradiation often result in a dysfunction of hematopoietic precursor cells of the granulocyte-macrophage lineage, with an increased morbidity and mortality in cancer patients. We were therefore interested in testing the ability of selected Biological Response Modifiers (BRMs) to modulate growth and differentiation of granulocyte-macrophage precursor cells (GM-CFU-C) by stimulating the release of specific granulocyte-macrophage colony-stimulating factors (GM-CSF). Release of GM-CSF by murine and human monocytes/macrophages and bone marrow cells in vitro could be induced by Poly ICLC, human and murine interferons (hIFN/mIFN), the 2-cyanaziridin BM41.332 and lipopolysaccharide (LPS). By using IFN neutralizing antibodies it was further shown, that the induction of GM-CSF release by monocytes/macrophages is independent of IFN secretion by these cells. The above BRMs, except hIFN, simultaneously stimulated the release of prostaglandin (PgE), which contributes to a negative feedback mechanism for GM-CFU-C growth. In vivo treatment with these BRMs resulted also in an increase in serum GM-CSF titers, as well as increased release of GM-CSF and PgE by macrophages and bone marrow cells. The in vitro/in vivo release of the inhibitory factor PgE could be selectively inhibited by Indomethacin, leaving the release of GM-CSF intact. The present results support the concept that some BRMs might be of value in reconstituting or protecting granulocyte and monocyte/macrophage function.

PROJECT DESCRIPTION

PERSONNEL

Michael A. Chirigos	Section Chief	IS	BTB	NCI
Craig W. Reynolds	Staff Fellow	NIS	BTB	NCI
Henry C. Stevenson	Medical Officer	CIS	BTB	NCI
Kenneth A. Foon	Section Chief	CIS	BTB	NCI

OBJECTIVES

The objective of this project is to assess the capacity of selected Biological Response Modifiers (BRMs) to modulate growth and differentiation of hematopoietic precursor cells in rodents and humans under various host conditions: 1) normal, 2) tumor bearing, and 3) tumor bearing undergoing cytoreductive therapy and to examine the immunological and pharmacological mechanisms involved.

METHODS EMPLOYED

In vitro treatment of peritoneal macrophages and bone marrow cells of mice as well as human peripheral monocytes and bone marrow cells with selected BRMs. Determination of granulocyte-macrophage colony stimulating factor (GM-CSF) levels by bioassay and of prostaglandin E (PgE) by radioimmunoassay.

The in vivo treatment of mice with BRMs is followed by determination of GM-CSF and PgE titers in serum, GM-CSF/PgE release by macrophages and bone marrow cells, as well as number and function of hematopoietic precursor cells.

MAJOR FINDINGSI. In Vitro Modulation of GM-CSF and PgE: Mouse and Human

Resident peritoneal macrophages of mice spontaneously release about 35 U/ml GM-CSF. The following BRMs at their optimal concentration stimulated up to a 4-fold increase in GM-CSF release by macrophages: Poly ICLC (50 µg/ml); α,β-mIFN (500 U/ml), β-mIFN (500 U/ml); γ-mIFN (50 U/ml); BM41.332 (25 µg/ml) and LPS (10 µg/ml). Optimal levels of GM-CSF were obtained at day 2 of the incubation period, with a plateau or slowly decreasing GM-CSF levels thereafter. Kinetic studies showed that most of the GM-CSF is released during the first 48 hours of the culture period and that macrophages subsequently lose their ability to respond to a second signal with a further release of GM-CSF. IFN neutralizing antibodies completely block the GM-CSF inducing effect of IFN. However, anti-IFN lacks any inhibiting effect on the stimulatory capability of the other BRMs. Of all the BRMs tested, only Poly ICLC was able to induce significant titers of IFN in the culture. Both results seem to exclude the possibility of an IFN mediated two step induction of GM-CSF release. Peritoneal macrophages also

spontaneously release relevant titers of PgE. They can be stimulated by the same BRMs to increase significantly their PgE release. Kinetic studies showed that the PgE release by macrophages is generally confined to the first 24 hours of the culture period. Preincubation of the cells with Indomethacin blocks the stimulatory effect of the BRMs on PgE release, but leaves the release of GM-CSF intact. Similar patterns concerning BRM induced release of GM-CSF/PgE were obtained by using human peripheral blood monocytes, as well as human and murine bone marrow cells. In contrast to mIFNs, the hIFNs tested (α ; β ; AD-Bgl) have the advantage of stimulating only the release of the growth factor GM-CSF, but not the growth inhibitor PgE.

II. In Vivo Modulation of GM-CSF and PgE: Mouse

Sera of mice injected with an optimal dose of any of the following BRMs: Poly ICLC (4 mg/kg); α , β -mIFN (10^4 U/mouse); MVE-2 (25 mg/kg); BM41.332 (25 mg/kg) and Azimexone (50 mg/kg) contained considerable amounts of GM-CSF, but showed no increase in their PgE content. The GM-CSF levels are increased as early as one hour post injection, peak at days one to three (depending upon the BRM used), and persisted for as long as up to seven days. Peritoneal macrophages from these treated mice release significant amounts of GM-CSF, when kept in culture. The kinetic of GM-CSF release by the macrophages parallels the serum GM-CSF levels. The release of PgE by these in vivo treated macrophages is confined to the first 24 hours after injection and can be blocked by pretreating the mice with Indomethacin. A similar pattern for GM-CSF release is obtained from bone marrow cells from BRM treated mice; however, with no significant increase in their PgE levels. Results of current experiments indicate that the BRM induced increase in GM-CSF release is followed by an increased function of GM-CFU-C.

III. In Vitro Modulation of Rat Bone Marrow Precursor Cells by Large Granular Lymphocytes

Recently, we obtained indications that growth and differentiation of GM-CFU-C is not only controlled by GM-CSF and PgE, but might also be directly regulated by natural killer cells (NK cells). To test the influence of large granular lymphocytes, which mediate NK activity in rats, on GM-CFU-C, bone marrow cells were separated into five different fractions according to their density by using a five step Percoll gradient. When grown in soft agar under stimulation of GM-CSF, these fractionated cells differentiate into pure granulocyte, pure macrophage or mixed granulocyte-macrophage colonies. There exists a specific pattern amongst these fractions. Some grow mostly granulocyte and mixed colonies, whereas others give rise almost exclusively to macrophage colonies only. In collaboration with Dr. Craig Reynolds the influence of large granular lymphocytes on growth and differentiation of these different hematopoietic precursor cells is presently being investigated.

SIGNIFICANCE

A major treatment modality for various cancers is chemotherapy and/or irradiation. They often result in a dysfunction of hematopoietic precursor cells, as well as in the cellular components of the immune system, which then precludes further therapy. BRMs capable of protecting or stimulating proliferation/differentiation of hematopoietic precursors would therefore be advantageous to the tumor-bearing host undergoing cytoreductive therapy. Basic research studies, which determine extent and kinetic of the bone marrow restoring or inhibiting capacity of a given BRM (e.g., through stimulation of GM-CSF or PgE release) are thus a prerequisite for designing combined therapy modalities (e.g., chemotherapeutic agents and BRMs) in tumor-bearing hosts.

PROPOSED COURSE

Studies will be continued to assess the capacity of selected BRMs to protect/restore depressed bone marrow function in mice. BRMs will be selected based on their ability to induce high titers of GM-CSF *in vivo*. Experiments will include the determination of the pharmacokinetics of these BRMs on number and function of hematopoietic precursor cells in normal mice and normal mice treated with cytotoxic agents (e.g., Cytoxan). After determination of an optimal schedule for such a combined therapy we will use tumor-bearing mice (in collaboration with Dr. M. A. Chirigos) to test the hypothesis that BRMs might help to prolong survival time or to cure tumor bearing animals through their enhanced bone marrow activity.

The studies evaluating the potential regulatory role of large granular lymphocytes (LGL) on growth/differentiation of hematopoietic precursors (GM-CFU-C) will be continued. It is planned to incubate LGL together with different fractions of the bone marrow to test for growth inhibition and/or direct cytotoxicity of LGL on the precursor cells.

PUBLICATIONS

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PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interrelationship of Neuroendocrine Hormones and Lymphokines

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

William L. Farrar, Jr., Senior Staff Fellow, LMI, NCI

COOPERATING UNITS (if any)

NIMH

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokines/Cytokines Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1.2

PROFESSIONAL:

0.6

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the 2.5 months of this position, with the aid of Mr. Harold Stull, we have established a completely operational laboratory in the Lymphokines/Cytokines Section of the LMI. Functional assays for lymphokine activities such as interferon, IL-1, IL-2, IL-3 are operational. We have initiated studies concerning the biological activity of endorphins on human lymphocyte subpopulations. Preliminary data demonstrate that large granular lymphocytes (LGL) as well as T lymphocytes possess a large number of receptors for beta-endorphin. The binding of endorphins to the receptor apparently triggers intracellular events mediated through the metabolism of arachidonic acid. This metabolic pathway also is involved in the cellular response to many other stimulants including IL-2. Conversely, inhibitors of arachidonic acid metabolites change the extracellular levels of cyclic GMP and result in the down-regulation of immunoenhancing lymphokines.

PROJECT DESCRIPTION

PERSONNEL

Candace Pert		NIMH
Joost J. Oppenheim	Chief	LMI, NCI
Harold Stull	Biology Lab Technician	LS LMI, NCI

OBJECTIVES

The objectives of this project are: 1) to examine the effects purified human lymphokines have on intracellular metabolic events; 2) to isolate human lymphocyte subpopulations to utilize as cellular targets of lymphokine activity; 3) to investigate the physiological signals (lymphokines, etc.) involved in the activation or expression of differentiation antigens on cells of the T cell lineage and; 4) to examine the structure/function relationships between neuroendocrine hormones and lymphokines.

METHODS EMPLOYED

Human blood is obtained from the NIH Blood Bank leukapheresis unit. Lymphocyte subpopulations are prepared by successive Ficoll-Hypaque gradient separations, plastic adherence, nylon wool adherence and Percoll discontinuous gradients. The resulting populations are monocyte-depleted purified NK or T lymphocytes. Cyclic GMP or AMP are determined by the methods of Steiner et al. utilizing standard RIA procedures. In order to evaluate the role of arachidonic acid metabolism in lymphokine-induced immune reactivities, specific inhibitors of arachidonic metabolism are used. Effects of arachidonic acid metabolism as well as cyclic nucleotide levels on biological activity are evaluated using in vitro bioassays for IL-2 activity and gamma interferon, routinely performed in our laboratories.

Determination of neuroendocrine activity is done by evaluation of biological effects on human lymphocyte activities in vitro as well as radioligand binding assays for specific lymphocyte subpopulations.

MAJOR FINDINGS

We have examined various subpopulations of the human T cell lineage for receptors for the neuroendocrine hormone beta-endorphin. The data demonstrate high affinity of beta-endorphin binding to large granular lymphocytes functionally identical with NK cells in the human. This supports a previous report that beta-endorphin enhances NK cytotoxicity. We are currently investigating the cellular pathways activated by endorphin binding to this cell subpopulation.

The addition of prostaglandin E2 (PGE2) apparently down-regulates or inhibits production of IL-2 and gamma interferon. This corresponds to an increase in cyclic AMP, since other cyclic AMP enhancers (e.g., isoproterenol) have a similar effect. Production of IL-2 or gamma interferon is regulated via the lipoxigenase arm of the arachadonic acid metabolic pathway. Enhancement of lymphokine production as well as associated activity correlates with elevation of cyclic GMP.

Utilizing highly purified T lymphocytes and the antigens tetanus toxoid and streptolysin O we have examined the regulatory activity of homogeneous preparations of IL-2 and gamma interferon on "activation" of cell membrane antigen expression. Preliminary data suggests that these lymphokines govern the expression of TAC, OKT-9 (transferrin receptor) and OKT-10 antigen expression.

SIGNIFICANCE

The development of cellular systems with well-characterized populations as well as human tumor cell models that are reactive with homogeneous preparations of lymphokines is requisite for studies of the cellular and molecular mechanisms of immunoregulation.

Two homogenous lymphokines are currently available for study, IL-2 and gamma interferon. In these investigations we have delineated discretely responsive populations of cells and have been able to determine specific metabolic events that control lymphokines activity. Knowledge gained from these experiments will enhance our ability to pharmacologically modify the immunoregulatory activity of lymphokines and specifically those regulatory activities which result in anti-tumor immunity.

The causal linkage between neurological activities and immunity are not clearly understood. Based on the data from this investigator demonstrating the activities of the neuroendocrine hormones vasopressin, acetylcholine and oxytocin, it seems clear that specific neuroregulatory substances may influence immune status. We are attempting to determine which of these neuroendocrine hormones exhibit binding activity to functional subsets of the immunocytes, the cellular activities modulated and the biological significance of the resulting modulations. This area may change our understanding of neurological regulation of the immunological status of an individual. The potential use of neuroendocrine activites as possible biological response modifiers will also be an obvious benefit of these investigations.

PROPOSED COURSE

We propose to continue these investigations, concentrating on: 1) cellular mechanisms of lymphokine activity, and 2) the neuroendocrine structure/function relationship to the immune system.

PUBLICATIONS

Z01CM09254-01 LMI

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09264-01 LMI

PERIOD COVERED

May 16, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Normal and Neoplastic T Cells by T Cell Growth Factor (TCGF)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Francis W. Ruscetti, Cancer Expert, LMI, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokines/Cytokines Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland, 21701

TOTAL MANYEARS:

5.12

PROFESSIONAL:

5.12

OTHER:

0

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Four different types of growth regulation of normal and neoplastic T cells have been recognized by us and others: 1) TCGF dependent normal and neoplastic T cells; 2) TCGF independent normal and neoplastic T cells; 3) normal and neoplastic T cells which initially require the factor, but on repeated incubation become factor independent; and 4) neoplastic T cells which appear to be dependent on biochemically distinct variants of TCGF. Clones of neoplastic TCGF dependent T cells have been developed that turn off TCGF production in response to glucocortical steroids. As a result, their proliferation is also drastically reduced. The addition of homogeneously purified TCGF prepared from the cell line in question overcomes the block in proliferation caused by the glucocorticoids. Thus, TCGF has autocrine activity and can promote growth of neoplastic cells. Considerable progress has also been made in characterizing the biochemically distinct variants of TCGF produced by several malignant T cell lines. The variants have a much more acidic pI and higher molecular weights than normal TCGF. The data suggest that these differences are neither post-translational modifications nor do they result from improper precursor processing, but differ in primary structure. The biological significance of these variants is being studied.

PROJECT DESCRIPTION

PERSONNEL

Hayao Abe

Guest Scientist

BS LMI, NCI

OBJECTIVES

The objectives of this project are: 1) to determine the steps involved in TCGF stimulation of T cell growth; 2) to develop methods for the quantitation of intracellular levels of TCGF messenger RNA's and TCGF protein, and 3) to determine the role of TCGF in the development of the transformed state.

METHODS EMPLOYED

In vitro cell culture. Production, identification and characterization of lymphokines involved in the growth of normal and neoplastic T cells. Tissue culture techniques designed to develop unique new cell lines are utilized.

Assay for lymphokines. TCGF, BCGF, CSF, etc. are used.

Biochemistry. Purification of radio-labeled TCGF using immunoaffinity adherence, HPLC, anion-exchange chromatographic techniques. The techniques of Western and Southern blotting are utilized.

MAJOR FINDINGS

Clones of neoplastic T cells have been developed that turn off TCGF proliferation in response to glucocorticoids. As a result, proliferation is also drastically reduced. The addition of homogeneously purified TCGF prepared from the same cell overcomes the block in proliferation caused by the glucocorticoids. This is direct evidence that in some T cell malignancies, an "autocrine model" can be invoked to explain the growth of the leukemic cells. This system provides a unique opportunity to investigate the mechanism of action and quantity of TCGF necessary to stimulate growth to overcome this inhibition.

In addition, considerable progress has been made in characterizing the biochemical variants of TCGF produced by malignant T cell lines. In general these proteins have a much more acidic pI (4.0-5.0) than natural TCGF (6.8-8.0) and slightly larger molecular weights (23000-26000) than normal TCGF. These differences are not due to sialylation, glycosylation, or phosphorylation as with post-translational modification. They are also not improperly processed precursor molecules. The biological significance of the variants needs to be resolved.

SIGNIFICANCE

Gaining an understanding in the similarity and differences in growth regulation between normal and neoplastic cells may lead to a better understanding of how the initiation and maintenance of the transformed state occurs. This information is needed to understand how to use biological response modifiers in altering the transformed state. Also, learning the steps involved in TCGF stimulation of T cell growth will enable the development of new biologic response modifiers and immunopharmacologic agents that are both agonistic and antagonistic to T cell growth.

PROPOSED COURSE

We propose to continue these investigations concentrating on: 1) delineating the steps involved in TCGF stimulation of T cell growth; 2) develop technologies to study the mechanism of growth regulation in TCGF-independent normal and malignant T cell lines, and 4) study the role of TCGF in the development of the transformed T cell.

PUBLICATIONS

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1CM09216-03 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Response of Macrophage-Monocytes to BRM: Mechanisms & Pharmacological Modulation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Luigi Varesio, Acting Section Head, LMI, NCI

COOPERATING UNITS (if any)

Walter Reed Hospital, Washington, D.C.; McGill Univ., Montreal, Canada

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Macrophages and monocytes become effector and immunoregulatory cells when activated by biological response modifiers (BRM). We studied the molecular mechanisms of the activation process. Because of the major role of RNA in any process of cellular differentiation, we analyzed the changes in RNA metabolism occurring during the transition from resting to tumoricidal macrophage. We found a direct correlation between expression of tumoricidal activity and decrease of RNA synthesis. Molecular analysis of the species of RNA affected revealed a selective inhibition of the 28S ribosomal RNA in activated macrophages. The down-regulation of RNA synthesis was observed in macrophages activated in vitro by endotoxins, lymphokines and interferon, and in vivo by endotoxins and *C. parvum*. Moreover, studies of macrophages from different strains of mice showed that the cytotoxic response to activating signals and the decrease in RNA synthesis followed the same genetic distribution. We concluded that the decrease in RNA synthesis is a marker for the acquisition of cytotoxic activity by macrophages exposed to BRM. We reasoned that if the down-regulation of RNA synthesis was causally related to the activation, drugs affecting RNA synthesis would exert a positive effect in the generation of cytotoxic macrophages. Indeed we found that inhibitors of RNA synthesis, such as actinomycin D and picolinic acid can activate macrophages to a tumoricidal state. Moreover, increasing evidence has been obtained on the positive effects of inhibitors of RNA synthesis on macrophage activation by demonstrating synergy between these drugs and suboptimal doses of BRM. These findings demonstrate for the first time that the down-regulation of RNA synthesis is involved in the generation of cytotoxic macrophages. Moreover, they suggest that combined treatments of patients with BRM and inhibitors of nucleic acid synthesis could be designed in order to exploit the macrophage-mediate antitumor defenses in addition to the direct therapeutic effects of the drugs.

PROJECT DESCRIPTION

PERSONNEL

Elisabetta Blasi	Visiting Fellow	IS	LMI, NCI
Ezio Bonvini	Visiting Fellow	IS	LMI, NCI
Mike Clayton	Chemist	IS	LMI, NCI
Monte Meltzer	Internist		Walter Reed Hospital
Emil Skamene	Prof. Med.		McGill University Canada

OBJECTIVES

The objectives of this project are to study first the molecular mechanisms by which macrophages and monocytes became activated and exert functional activities relevant to the host defenses against tumors in response to biological response modifiers (BRM). These results will lay the ground for the use of drugs, interfering with the metabolic pathways responsible for macrophage activation, to modulate the response of macrophages to BRM. This approach should provide insights in the biology of cell differentiation and rationale for designing strategies to maximize the antitumor effect of macrophages by combined treatments with BRM and drugs. The final objective will be to verify the predictions derived from the in vitro findings on mouse macrophage and human monocytes, in our in vivo tumor model.

MAJOR FINDINGSI. Changes in RNA Synthesis During Activation of Murine Macrophages

Peritoneal murine macrophages, activated in vivo or in vitro to a cytolytic stage, have a depressed rate of RNA synthesis. The correlation between expression tumoricidal activity and decrease in RNA synthesis has been verified in a variety of experimental conditions, and a close association between the two phenomena has always been found. We investigated the species of RNA affected in the activation process. By size fractionation of macrophage RNA on agarose gels, we found that a selective decrease in 28S ribosomal RNA (rRNA) but a normal synthesis of 18S rRNA or high molecular weight precursors occurs in activated macrophages. Similar results were found when RNA, was extracted from macrophages activated in vitro by lymphokines or in vivo by injection of C. parvum. Overall our data showed that the activation process involves a down-regulation of the 28S ribosomal RNA, the major component of the larger (60S) subunit of the eukariotic ribosomes.

II. Synergy Between Inhibitors of RNA Synthesis and BRM

The inhibition of RNA synthesis in activated macrophages suggested the possibility that inhibitors of RNA synthesis could activate cytotoxic macrophages and/or synergize with BRM. We found that Actinomycin-D and picolinic acid could induce cytolytic macrophages at concentrations in which they selectively inhibited the synthesis of ribosomal RNA. Moreover, very low doses of the above inhibitors could synergize with lymphokines and γ -IFN in inducing cytotoxic macrophages. These findings bear a number of implications. First they suggest that the decrease in RNA synthesis observed in BRM-treated M ϕ may be causally related to the activation since the effects of BRM can be mimicked by inhibitors of RNA synthesis. Second that low doses of inhibitors of RNA synthesis together with BRM, can be of therapeutic value in protocols aimed to the activation of macrophages.

III. Effects of Inhibitors of Protein Synthesis on the Activation of Cytotoxic Macrophages

Incubation of murine peritoneal macrophages (M ϕ) with LK (supernatants from mitogen-stimulate murine spleen cells) resulted in the activation of tumoricidal activity, assessed by an 18 hour ¹¹¹indium-release assay against L5178Y lymphoma target cells. We evaluated the effects of reversible (cycloheximide and puromycin) and nonreversible (emetine and pactamycin) inhibitors of protein synthesis on the activation of macrophages by LK and on the lytic activity of activated M ϕ . We found that M ϕ can be activated to a cytolytic stage in the absence of active protein synthesis. In contrast, protein synthesis is required by tumoricidal M ϕ to perform the killing. These results suggest that chemotherapeutic protocols with protein synthesis inhibitor may be established in such a way that the M ϕ reactivity remains intact.

SIGNIFICANCE

Macrophages and monocytes play an important role in the host defense against tumors since they can directly kill and inhibit the growth of tumor cells, and modulate the activity of other lymphoid cells in a positive or negative manner. Depending on the BRM, on the level of activation of the macrophage-monocyte and on the protocol of activation, a specific function may be preferentially expressed or more than one activity can be concomitantly present. Our findings indicated that the in vivo and in vitro activation of tumoricidal macrophages is accomplished through the down-regulation of ribosomal RNA synthesis. This observation is very relevant since it evidentiates a critical step in the activation process that could be modulated by specific drugs, and we have shown that this is indeed true. Therefore, our results suggest that combined administration of drugs and BRM may result in optimal protocols where the tumor is concomitantly the target of drugs and activated macrophages. Studies of the molecular and cellular biology of BRM-treated macrophages may contribute to the elucidation not only of the mechanisms of action of BRMs, but also of new aspects of the regulation of the gene expression during cellular differentiation. The macrophage model could be of particular relevance since these cells do not

proliferate under our experimental conditions and the molecular bases of phenotypic changes can be easily identified.

PROPOSED COURSE

The relationship of the inhibition of ribosomal RNA induced by BRM on macrophages to the expression of cytotoxic activity and the molecular mechanisms causing these changes remain unclear.

Since the 28S RNA is a major component of ribosomes, we will analyze the ribosomal functions in BRMs treated macrophages and primarily their translational activity to investigate the role of translational modifications in the BRM-mediated macrophage activation. Particular emphasis will be put on the effects of α - β - and γ -IFN on translation in an attempt to distinguish between the induction of cytotoxicity and antiviral activity by these molecules.

To address the question of the mechanisms of rRNA modulation in BRM-treated macrophages, we will first study the nucleic acid methylation because of its essential role in gene expression. Also the maturation of RNA and its transport from nucleus to cytoplasm will be analyzed.

Changes in messenger RNA will be the next question addressed. Specific messenger RNA will be studied during the activation process using cloned cDNA already available such as plasminogen activator, histocompatibility antigens and IFN.

The recent availability of purified lymphokines will make possible to address the problem of the interaction among lymphokines, cytokines and interferon, in the activation of macrophages to gain insights into the "in vivo" situation in which macrophages are concomitantly exposed to a number of different signals.

It is our intention to verify our results on human cells. Initial studies will deal with the differentiation of monocytes into macrophages and its modulation by specific drugs and/or BRM. This information will provide the ground to approach the problem of the control of human monocyte/macrophage activation for antitumor activity.

PUBLICATIONS

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Herberman, R. B., Brunda, M. J., Domzig, W., Fagnani, R., Goldfarb, R. H., Holden, H. T., Ortaldo, J. R., Reynolds, C. W., Riccardi, C., Santoni, A., Stadler, B. M., Taramelli, D., Timonen, T. and Varesio L.: Immunoregulation involving macrophages and natural killer cells. In Gershwin, M. E. and Ruben, L. N. (Eds.): Immune Regulation: Evolutionary and Biological Significance. New York, Marcel Dekker, Inc., 1982, pp. 139-166.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09225-03 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Monocyte-Mediated Cytotoxicity (In Vitro and In Vivo)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Eugenie Kleinerman, C.O., LMI, NCI

COOPERATING UNITS (if any)

Cancer Metastasis and Treatment Laboratory NCI-FCRF;
TCB, NCI; LMO, NCI;

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In an attempt to increase the in vivo effectiveness of macrophage activating factor (MAF) our laboratory has focused on the use of multilamellar liposomes as carrier vehicles for delivering MAF and other activating agents directly to the phagocytic cell. In order for liposomes to serve as vehicles for the delivery of compounds to monocytes and macrophages, they must bind to and become endocytosed by the cells. Phagocytosis of liposomes by human blood monocytes was influenced by the chemical composition and surface charge. Negatively charged vesicles consisting of phosphatidylcholine and phosphatidylserine admixed in a 7:3 mole ratio were rapidly and efficiently internalized. Incubation of human monocytes with these liposomes containing MAF, muramyl dipeptide (MDP) or MTP-PE (a lipophilic derivative of MDP) rendered them tumoricidal. However these activated monocytes did not harm normal embryonic fibroblasts even under conditions of co-cultivation. Likewise, activated monocytes lysed HSV-2 infected but not uninfected fibroblasts. Any successful approach to the eradication of metastases not only must circumvent the biological heterogeneity and cellular resistance of neoplastic cells but it must also be able to selectively destroy tumor but not normal cells. We believe that tumoricidal macrophages fulfill all of these criteria and their activation in vivo may offer a novel approach in the treatment of metastatic tumors. The *in situ* activation of tumoricidal macrophages may thus provide an attractive approach for the treatment of disseminated cancer. The mechanism by which MAF activates monocytes is thought to involve a 2-step process. The first step is binding to surface receptors followed by internalization, and the second step is interaction with an intracellular site to bring about activation. Using rodent and human MAF, we have shown that species-specific surface receptor binding can be bypassed using liposome-encapsulated MAF. Furthermore, it is our interpretation that MAF may have at least 2 separate "functional" parts of the molecule: one for cell surface binding and a second for initiating the activation process once internalized.

PROJECT DESCRIPTION

PERSONNEL

Randy Zicht	Biologist	LS	LMI	NCI
Ronald B. Herberman, M.D.	Chief		BTB	NCI
Robert Gallo, M.D.	Chief		LTCB	NCI
Prem S. Sarin	Senior Investigator		LTCB	NCI
Berge Hampar	P.O.		DCCP	NCI-FCRF
Larry Showalter	Biologist		LMO	NCI
Gregory Green	Student	IS	LMI	NCI

OBJECTIVES

The objectives of this project are: (1) To develop a monocyte-mediated tumoricidal assay. (2) To elucidate the mechanisms by which monocytes recognize and kill tumor cells. (3) To study the ability of these cells to kill other types of abnormal target cells, i.e., virally infected (Herpes Simplex Virus Type 2 [HSV-2]) fibroblasts. (4) To examine the specificity of the cytotoxic function of these activated cells by testing their ability to kill normal vs tumor targets and uninfected vs. virally (i.e., HSV-2) infected targets. (5) To characterize the lymphokine "macrophage activating factor" (MAF), and its properties, i.e., species specificity, heat stability, pH stability, etc.

METHODS EMPLOYED

We have developed an assay measuring monocyte-mediated cytotoxicity against a human melanoma tumor target, A375. Monocytes are isolated by combining the isolation techniques of continuous Percoll gradients and plastic adherence to FCS-coated plates, plus extensive washing, to obtain monocyte populations with greater than 99% purity. We find that this assay is reproducible and gives low levels of spontaneous tumor cell killing.

MAJOR FINDINGSI. Effect of Isolation Procedures on Spontaneous Monocyte Tumoricidal Activity

When comparing the isolation techniques of Percoll and adherence, we find that while Percoll-purified monocytes have low levels of spontaneous cytotoxicity (0-15%), the same individuals monocytes isolated by adherence, then removal with EDTA and scraping gave much higher cytotoxic values (20-50%). We have further shown that these two monocyte preparations respond differently to various immunomodulators. While Percolled monocytes are very sensitive to ng quantities of LPS which will activate them to kill tumor cells, plated/scraped monocytes respond only to much higher doses of LPS (1 µg). Likewise Percolled cells are not activated by gamma interferon; however, the tumoricidal function of plated/scraped monocytes can be augmented with gamma interferon. When our

Percoll-purified monocyte preparations were treated with B73.1 + complement to remove the possible residual NK activity, then treated with MAF, no decrease in tumoricidal activity was observed. Therefore, we feel confident that what we are measuring is indeed monocyte-mediated tumoricidal activity and not NK activity.

II. Production of Human Lymphokine-Containing Supernatant that Induces Monocyte Tumoricidal Activity

Human MAF is produced from MNL using Sepharose-bound Con A. This MAF activity is not due to endotoxin, soluble Con A, α , β or γ interferon. None of the interferons directly activated monocyte tumoricidal activity in this assay. We have also identified ~ 9 of 100 screened human T-T hybridomas and 2 of 30 HTLV-positive cell lines derived from patients that produce MAF as a constitutive product.

III. Activation of Human Monocyte-Mediated Tumoricidal Function by Liposomes Containing Human Lymphokines or Lipophilic Muramyl Tripeptide

MAF encapsulated within liposomes efficiently activates human monocyte cytotoxicity requiring only a 1-2 hour interaction phase and a 2-4 hour lag phase. Liposomes containing a lipophilic derivative of MDP (MTP-PE) also efficiently activate human monocytes. Human monocytes activated by liposome-encapsulated MAF or MTP-PE lyse only tumor target and not normal targets even under conditions of co-cultivation.

IV. Species Specificity of MAF

Using mouse, rat and human MAF to activate mouse, rat and human macrophages, we found that while both mouse and rat MAF was able to activate all three macrophages to kill tumor cells, human MAF only activated human monocytes. However, once encapsulated within liposomes, human MAF rendered both rat and mouse macrophages tumoricidal in vitro. This may indicate that MAF has two separate "functional" parts of the molecule: one for cell surface binding and internalization which is species specific and a second which is responsible for initial-activation and can cross species barriers.

V. Human Blood Monocytes Activated by MAF can Also Suppress Herpes Simplex Virus Type 2 (HSV-2) Infection In Vitro

These data demonstrate that human blood monocytes activated by MAF can not only discriminate between tumor cells and normal cells, but can also destroy HSV-2 infected cells and discriminate these infected cells from uninfected ones. Furthermore, in addition to lysing HSV-2 infected target cells, MAF-activated monocytes decrease the production of infectious virus particles from these cells. MAF, MTP-PE and gamma interferon encapsulated within liposomes efficiently activated human monocytes to lyse HSV-2 infected IOE2 cells. Once again, uninfected cells were left unharmed. At the present time, there is no effective chemotherapeutic treatment available for the eradication of HSV-2 induced verereal disease. The ability to activate monocytes in vivo by immunomodulators,

particularly in the liposome-encapsulated form may provide an additional treatment for HSV-2 infections and a method for selective destruction of HSV-2 infected cells.

SIGNIFICANCE

We have identified several agents (MAF, MDP, MTP-PE) that can activate normal human monocytes in vitro to kill allogeneic tumor targets. However, the administration in vivo of free MAF, lymphokines, MDP or other soluble activating factors has proven difficult due to the development of undesirable side effects such as anaphylaxis and granuloma development. The concept of devising a carrier vehicle to transport the activating compound directly into the effector cell would seem to circumvent the problem of undesirable side effects. Biologically active materials such as MDP or MAF can be encapsulated inside liposomes, and we have been shown that human monocytes incorporate these liposomes intracellularly. After cellular uptake of the liposome, the encapsulated material is released intracellularly in an active form. Subsequent to the phagocytic uptake of liposomes containing activating agents, human monocytes are rendered cytotoxic to both tumor cells and virally infected cells. Our in vitro human studies are very encouraging and have demonstrated that human monocytes respond well to activation by MAF entrapped within liposomes. These findings, coupled with the successful treatment of mice bearing metastases using systemic administration of liposomes containing MAF, suggest that further clinical trials are warranted.

PROPOSED COURSE

During the next year we would like to determine the mechanisms by which the monocytes recognize and kill tumor cells. Monocyte-tumor cell and monocyte-normal cell binding assays are now being developed in a collaborative effort with Dr. Robert Wiltrout in an effort to ascertain if there is a specific receptor or recognition site for a component on tumor cell membranes which is not on normal cells.

Although several hypotheses linking monocyte secretion of H_2O_2 and oxygen radicals to tumor cell destruction have been proposed, the experimental evidence is inconclusive. We plan to examine the ability of monocytes from patients with Chronic Granulomatous Disease (CGD) to kill tumor cells and HSV-2 infected cells. These patients, by virtue of their genetic disorder, do not produce H_2O_2 or O_2^- radicals and therefore offer a unique way to address this issue. In addition, with the identification of several human cell lines that produce MAF as a constitutive source, work will begin to further characterize and purify human MAF in collaboration with Drs. Schultz and Onzaki.

PUBLICATIONS

Bucana, C. D., Hoyer, L. C., Schroit, A. J., Kleinerman, E. S. and Fidler, I. J.: Ultrastructural studies of the interaction between liposome-activated human blood monocytes and allogeneic tumor cells in vitro. Am. J. Pathol. (In Press)

Kleinerman, E. S., Erickson, K. L., Schroit, A. J., Fogler, W. E. and Fidler, I. J.: Activation of tumoricidal properties in human blood monocytes by liposomes containing lipophilic muramyl tripeptide. Cancer Res. 43: 2010-2014, 1983.

Kleinerman, E. S. and Fidler, I. J.: Production and utilization of human lymphokines containing macrophage activation factor (MAF) activity. Lymphokine Res. 2: 7-12, 1983.

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Muchmore, A. V. and Kleinerman, E. S.: Spontaneous monocyte-mediated cytotoxicity in man: Evidence for T helper activity. In Herberman, R. B (Ed.): NK Cells and Other Natural Effector Cells. New York, Academic Press, 1982, pp. 1423-1430.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09249-04 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Interferon on Membrane Lipid Composition of Human Leucocytes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Philippe Bougnoux, Visiting Fellow, LMI, NCI

COOPERATING UNITS (if any)

LCS-DICBR, NIAAA; DBB-NCDB, FDA

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Changes in membrane lipid composition secondary to interferon-induced cell surface modifications are being examined. Fatty acid composition of cell surface lipids after interferon treatment was measured by chromatographic procedures, using a membrane impermeable chemical probe to covalently derivatize the cell surface aminophospholipids. In resting lymphocytes, an asymmetry of phosphatidylethanolamine molecular species was observed, with a more saturated pattern in the fatty acid composition of the external leaflet of the plasma membrane. Both α - and β -interferon caused the increase of arachidonic acid, as well as of other polyunsaturated fatty acids in the lymphocyte's surface phospholipids, resulting in a more unsaturated pattern in their fatty acid composition. These changes may result in an alteration of the plasma membrane motional state and may modulate, by this mechanism, certain membrane-dependent cell functions, including cytotoxicity. The effect of interferon on the lipids of the NK-susceptible target cell K562 was also examined. The development of the interferon-induced alterations in lipid composition of K562 cells was time-dependent, and essentially biphasic, with an early decrease in saturated fatty acids, and a later increase in saturated fatty acids while polyunsaturated fatty acids were decreasing. The sensitivity of K562 cells to NK lysis was also modified by interferon pretreatment, in a time-dependent and biphasic manner. A short-term interferon pretreatment resulted in an increased sensitivity of these cells to lysis, while they became more resistant to lysis after long-term interferon pretreatment, their sensitivity correlating with the changes in lipid composition. These findings, documenting the involvement of cell membrane lipids in sensitivity of tumor cells to cellular cytotoxicity opens up possibilities in cancer treatment for manipulating tumor cells to make them more susceptible to elimination by the host's defenses.

PERSONNEL

Norman Salem Jr.		LCS	DICBR, NIAAA
Ezio Bonvini	Visiting Fellow	IS	LMI, NCI
Thomas Hoffman		DBB	NCDB, FDA

OBJECTIVES

1. To investigate the molecular mechanisms by which interferon alters the surface of sensitive cells, specifically with respect to membrane lipids.
2. To obtain insights into the mechanisms of transmembrane transmission of the signal generated after interferon's binding to its receptors, to the intracellular effector machinery.
3. To examine the contribution of changes in membrane lipids to changes in functions of interferon-treated cells: e.g. boosting of the cytotoxic capabilities of peripheral lymphocytes, and modifications of the sensitivity of NK-sensitive target cells to lysis.

METHODS EMPLOYED

Peripheral blood mononuclear cells were obtained by Ficoll-Hypaque separation. Purification of lymphocytes was achieved by elutriation. In vitro cell culture. NK-cytotoxicity was assayed by ⁵¹Cr release. Interferon receptors were measured by competitive ligand binding assay of ¹³¹I labeled interferon. Membranes preparation was performed by cell fractionation and plasma membrane purification by sucrose gradient centrifugation. Membrane lipids were prepared by organic solvent extraction. Phospholipid classes were purified by chromatographic procedures (HPLC and 2 dimensional TLC). Fatty acid methyl esters were prepared from phospholipids by methanolysis in presence of boron trifluoride. Fatty acid identification was performed by capillary gas-liquid chromatography and quantification by internal standardization.

MAJOR FINDINGS

1. Asymmetry of phosphatidylethanolamine molecular species in the human lymphocyte plasma membrane.

The use of the covalent chemical reaction of the membrane-impermeable reagent trinitrophenylbenzenesulfonate, TNBS, allows a selective labeling of cell-surface phosphatidylethanolamine (TNP-PE), and its comparison with the non-derivatized PE. Analysis of the fatty acid of the TNP-PE fraction therefore reflects the composition of the PE species of the external side of the plasma membrane. The fatty acids of the TNP-PE were found to be more saturated than those of the non-derivatized PE, with a ratio of saturated/unsaturated fatty acids 1.2 to 3 times higher in TNP-PE than in PE in 5 different donor's lymphocytes. Arachidonic acid and other polyunsaturates were much lower in the cell surface PE. Similar findings, with respect to their Sat/Unsat fatty acid ratios were obtained in phosphatidylcholine and sphingomyelin, phospholipids mainly distributed in the external leaflet of the plasma membrane.

2. Alterations by interferons of the lymphocyte's surface fatty acid composition.

After binding with high affinity to a specific cell surface receptor, interferon does not appear to be internalized. This raises the possibility that some of the various effects of interferon on the cell surface might be mediated by a change in the cell membrane fatty acid composition, which is a major determinant of the physical state of the membrane. After 16 hours treatment with either α - or β -human interferon, arachidonic acid, as well as other polyunsaturated fatty acids, increased in the lymphocyte's surface PE and, to a lesser extent, in phosphatidylcholine and sphingomyelin. Opposite changes were observed in the non-derivatized PE, suggesting a redistribution mechanism of cellular lipids. Thus, interferon alters the lipid composition of cell membranes in an asymmetric way. These changes are likely to result in "fluidization" of discrete membrane environments and may, by this mechanism, regulate certain membrane-dependent cell functions, including cytotoxicity.

3. Effect of interferon on the membrane lipids of the NK-susceptible target cell, K562.

In contrast to the effects observed in lymphocytes, the development of the interferon-induced alterations in lipid composition of K562 cells was dependent upon time, following two schematic phases. 1- An early effect, maximal at 3-4 hours of incubation, where the changes were similar to that observed in lymphocytes, with a decreased sat./unsat. fatty acids ratio. 2- A late effect, observed over a 8-16 hour time period, where an increase in the proportion of saturated fatty acids was apparent. These changes were not limited to the cell surface fatty acids, but rather present in all the lipid classes, resulting in the reversal of the global sat/unsat ratio.

4. Relevance of the interferon-induced membrane lipid changes to the sensitivity of K562 cells to NK cytotoxicity.

K562 target cells pretreated with interferon for a short time (2 to 4 hours), or overnight, exhibited different degrees of sensitivity to NK killing. This change in sensitivity correlated with changes observed in lipid composition: K562 treated for less than 6 hours were found to be more sensitive to killing by lymphocytes or LGL effectors than the control K562 cells, while K562 pretreated for a long time period (overnight) were more resistant to killing. This correlation makes likely that membrane lipid composition is involved in the sensitivity of tumor cells to NK-killing. Binding studies (with J. Ortaldo) showed that the percentage of LGL binding to short term treated K562 was markedly increased as compared to control K562, while no difference was apparent between overnight treated and control K562, suggesting a different mechanism behind these two opposite effect of interferon.

SIGNIFICANCE TO BIOMEDICAL AND CANCER RESEARCH

Currently, interferon is in use in numerous clinical trials in a wide range of malignancies. Although a large body of data in vitro and in animal models

exists, providing rationales for the use of interferon in cancer treatment and elucidating certain of its mechanisms of action, no experimental evidence relating to its effects on membrane composition exists. These findings documenting the involvement of tumor cell membrane lipids in sensitivity to cellular cytotoxicity open up possibilities for manipulating tumor cells to make them more susceptible to elimination by the host's defenses. These could include other membrane-modifying agents in addition to interferon, as well as dietary manipulations of membrane lipid composition. These observations provide new insights into the mechanisms of the antineoplastic activity of interferon, with potential therapeutic applications. Modifications of patient's tumor cells sensitivity by short term interferon pretreatment in a defined sequence prior to adoptive immunotherapy becomes conceivable, and suggests a requirement for testing in animal systems.

PROPOSED COURSE

1. Define thoroughly this phenomenon (time-course of interferon effects, dose effect, effect of other interferons) and test for its existence other cell lines bearing interferon receptors, but exhibiting different degrees of sensitivity to the antiviral or antiproliferative actions of interferon.
2. Extend the applications of these findings to other membrane-dependent cell functions, and examine in vitro whether interferon-induced cell membranes changes can modulate the down regulation of tumor cell surface antigens occurring during monoclonal antibody treatment (by FACS analysis of T101 MoAb coated normal and leukemic lymphocytes after appropriate interferon treatments (with Bob Schroff)).

PUBLICATIONS

- P. Bougnoux, E. Bonvini, Z.L. Chang and T. Hoffman. Effect of interferon on phospholipid methylation by peripheral blood mononuclear cells. J. Cell. Biochem. 20:215-223, 1982
- P. Bougnoux, E. Bonvini, H.C. Stevenson, S. Markey, M. Zatz and T. Hoffman. Identification of Ubiquinone 50 as the major methylated non-polar lipid in human monocytes. Regulation of its biosynthesis via methionine-dependent pathways and relationship to superoxide production. J. Biol. Chem. 258:4339-4344, 1983
- T. Hattori, M. Pack, P. Bougnoux, Z.L. Chang and T. Hoffman. Interferon-induced differentiation of U937 cells. Comparison with other agents which promote differentiation of myeloid or monocytic-like cell lines. J. Clin. Invest. 1983. In press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09250-08 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Macrophage Immunobiology in Experimental Tumor Systems

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Howard T. Holden, Acting Section Head, LMI, NCI

COOPERATING UNITS (if any)

LVC, NCI; NCI-FCRF

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies were performed to investigate the development of specific macrophage functions after differential in vitro activation and to identify cell surface markers of macrophages that could be used to identify macrophages at different stages of activation and/or differentiation. Macrophages can be differentially activated in vitro to express suppressor or antitumor functions. Cytolytic and cytostatic activities can be induced by lymphokines (LK) plus either low dose endotoxin (LPS) or by high dose LPS. However, low dose LPS will induce cytostatic, but not cytolytic function. Natural suppressor activity, as demonstrated by inhibition of lymphoproliferation, is expressed in resident and peptone-elicited macrophages. In vitro activation with LK + low dose LPS or high dose LPS reverses the suppressive effect. Two interrelated projects were pursued which examined cell surface markers associated with macrophages at different stages of activation. We found that the neutral glycolipid, asialo GM1, is found on all macrophage populations but in varying amounts. There is an increase in asialo GM1 (asGM1) in macrophages that have been activated to become tumoricidal as indicated by 1) enhanced fluorescence on activated cell populations, 2) decreased fluorescence intensity and cytolytic activity after in vivo treatment with antisera to asGM1, and 3) positive correlation between high expression of asGM1 and tumoricidal activity after the cells were sorted for this marker. Other stimuli such as thioglycollate and agar also enhanced the expression of asGM1. Biochemical analysis of selected populations of macrophages indicated that there is also an increase in asGM1 in activated and thioglycollate-elicited macrophages. In addition, there are other major shifts in neutral glycolipids and gangliosides as the cells become activated. Monoclonal antibodies against poly I:C activated and lymphokine-treated macrophages were prepared. We are screening for antibodies that react with specific macrophage markers.

PROJECT DESCRIPTION

PERSONNEL

Zong L. Chang	Visiting Scientist	IS	LMI	NCI
Denise C. Knott	Biologist	LS	LMI	NCI
Robert H. Wiltrout	Staff Fellow	LS	LMI	NCI
Craig W. Reynolds	Staff Fellow	NIS	BTB	NCI
L. Srinivas	Visiting Fellow		LVC	NCI
N. H. Colburn	Senior Investigator		LVC	NCI
W. R. Overton	Research Associate		Program Resources, Inc.	
Ezio Bonvini	Visiting Fellow	IS	LMI	NCI
Ronald B. Herberman	Chief		BTB	NCI
Luigi Varesio	Visiting Associate	IS	LMI	NCI

OBJECTIVES

The general objectives of this project are: (1) to gain insight into the functional changes that take place in macrophages during the process of activation; (2) to identify and characterize macrophage subpopulations and macrophages at different stages of differentiation and/or activation; and (3) to identify biochemical or antigenic changes that take place on the surface of macrophages during activation.

MAJOR FINDINGSI. Expression of Asialo GM₁ on Macrophages (M_φ) During Activation

Preliminary studies from this laboratory (Wiltrout *et al.*) have shown that M_φ which had been activated in vivo by the pyran co-polymer MVE-2, were more sensitive than nonactivated M_φ to treatment in vitro with a rabbit antiserum to the neutral glycolipid asialo GM₁ plus complement. These results suggested that asialo GM₁ (asGM₁) might be an antigen which could be used to follow the course of M_φ activation. Therefore, we quantitated the expression of asGM₁ on different populations of M_φ by flow microfluorometry on a Cytofluorograph. It is clear from these results (and from the biochemical analysis described below) that all the M_φ have at least some asGM₁ on their membrane. However, quantitative analysis of the different M_φ populations indicated that there were significant, reproducible differences in the level of expression of asGM₁ which appeared to correlate with changes in M_φ function. M_φ from the peritoneal cavity of normal, unstimulated animals (resident M_φ) had the lowest amount of asGM₁. About 20% of the cells showed greater fluorescence with anti-asGM₁ than with the normal rabbit serum treated control but the intensity of fluorescence was weak. Two phlogistic agents, proteose-peptone and NIH thioglycollate, when injected into the peritoneal cavity, elicited M_φ which had a higher percentage of positive cells (35-40%) with a fluorescence intensity which was slightly higher than the resident M_φ. Agents which when injected into mice activated the M_φ to express tumoricidal activity

also caused a higher expression of asGM₁ as indicated by a higher percentage of positive cells and an increase in the fluorescence intensity. The pyran co-polymer, MVE-2, and poly I:C/LC when injected i.p. in conjunction with proteose-peptone or alone, induced 45% of the M ϕ to express asGM₁, 30-50% of which had high levels of fluorescence. Corynebacterium parvum (C. parvum)-activated M ϕ had a similar percentage of cells positive, but the fluorescence intensity was slightly less than that from cells activated with MVE-2 or poly I:C/LC. Kinetic analysis of the development of cytolytic activity and asGM₁ expression indicated that asGM₁ levels increased before the development of cytolytic activity suggesting that the shift in antigen expression may precede expression of tumoricidal activity. Several strains of mice that have impaired M ϕ function were examined for the expression of asGM₁. Agents that would selectively activate the M ϕ of these animals caused enhanced expression of asGM₁. The MVE-2 activated peritoneal M ϕ which stained brightly with anti-asGM₁ were separated on the cell sorter and analyzed for their cytolytic activity. Those cells which had high intensity staining had the highest levels of tumoricidal activity. This demonstrated a correlation between the level of expression of asGM₁ and the tumoricidal activity. Mice treated with MVE-2 to activate the M ϕ at the same time that they received antibody to asGM₁, showed a decreased in tumoricidal activity. Correspondingly, fewer cells expressed asGM₁ and the intensity of fluorescence was lower. Two interesting exceptions to the above pattern of results were noted. M ϕ that were elicited with Brewer's thioglycollate had a large percentage of positive cells with high levels of fluorescent intensity. These M ϕ are biochemically activated with enhanced production of many enzymes and mediators, which may be associated with the change in asGM₁ levels. In addition alveolar M ϕ have much higher levels of asGM₁ expression. In contrast to all the other populations examined, these cells were very homogeneous in their expression of asGM₁. Since these cells are continuously stimulated by particles in the air, this may explain the enhanced expression of asGM₁. Ingestion of particulate material such as agar by peritoneal M ϕ also was associated with enhanced expression of asGM₁. In collaboration with Drs. L. Srinivas and N. H. Colburn, we have measured the levels of neutral glycolipids and gangliosides in different populations of M ϕ . The results correlate with the data obtained in the indirect immunofluorescence assays. M ϕ activated with MVE-2 or elicited with thioglycollate have a greater amount of asGM₁ as measured by isolation of neutral glycolipids that incorporated ¹⁴C-galactose. There also appears to be a corresponding decrease in the glycolipid GM₁. Shifts in the amounts of other glycolipids were noticed when the M ϕ were activated, but the identity of these glycolipids is not known.

II. Production of Monoclonal Antibodies Against M ϕ

It would be useful to identify M ϕ at various stages of activation/differentiation with monoclonal antibodies. We initiated experiments to develop monoclonal antibodies against M ϕ that were activated by poly I:C or stimulated by lymphokines. The former display tumoricidal activity and no suppressor activity in the production of lymphokines while the latter will suppress the production of lymphokines by lymphocytes but are not tumoricidal. Supernatants were tested for activity in two assays, binding and lysis of M ϕ in the presence of complement. We have isolated 12 clones that have activity in one of the assays against

one or the other activated/stimulated M ϕ . We are in the process of determining the specificity of the antibody-producing colonies and of cloning them.

III. Differential In Vitro Activation of Antitumor and Suppressor M ϕ

The in vitro modulation of mouse peritoneal M ϕ functions was compared after activation with lymphokines and/or endotoxin. M ϕ were tested for their ability to kill tumor cells, inhibit tumor cell proliferation and to suppress lymphocyte proliferation. Both cytolytic and cytostatic activities were induced in proteose peptone-elicited M ϕ by high dose endotoxin (LPS; 10 μ g/ml) or by lymphokines plus low dose LPS (10 ng/ml). Lymphokine in the absence of LPS was incapable of inducing either functional activity. However, low dose LPS, which did not induce a tumoricidal state, would impart cytostatic activity to M ϕ . M ϕ which had been incubated in control medium exhibited no cytolytic function and cytostatic activity only at high effector:target cell ratios. However, these same M ϕ suppressed lymphocyte proliferation in response to Con A or PHA, even at low effector:target cell ratios. Furthermore, treatment with several activation stimuli, low dose LPS, high dose LPS, lymphokines plus low dose LPS but not lymphokine alone, decreased the natural suppressor activity of these cells. Thus the two antitumor activities and the suppressor function of the M ϕ can be regulated independently of each other. Measurement of superoxide production suggested that the killing of tumor cells by lymphokines plus low dose LPS activated M ϕ was no dependent on this functional activity.

SIGNIFICANCE

The immune response to neoplasia is very complex and involves not only the interaction between host effector cells and tumor cells, but also interactions among the various subpopulations of leukocytes. Some cells and/or factors may serve to amplify the immune response, while others may suppress it. Macrophages are capable of modulating the host's response to the tumor, both by their ability to act as accessory cells in the development of an immune response and by their capacity to suppress development of immune functions. Understanding all these interactions, what influences them and how they can be modulated to the benefit of the host, will give us information that we can use to assist the host in dealing with its tumor. Macrophages can also have direct antitumor activity. Studies on the process by which macrophages become activated have indicated that there are multiple stimuli which are required for macrophages to become cytolytic. In addition, there appears to be several different stages of differentiation which may be represented by distinct subpopulations of macrophages. Identifying the mechanism of macrophage activation and the subpopulations involved in this process should provide information that can be used to establish immunotherapy regimens.

PROPOSED COURSE

As of July 1, 1983, the Principal Investigator of this project will be moving to another position. Hence, much of this work will be discontinued. For segments that will be continued see the Annual Reports of Drs. R. H. Wiltrout and C. W. Reynolds.

PUBLICATIONS

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- Brunda, M. J., Taramelli, D., Holden, H. T. and Varesio, L.: Suppression of murine natural killer cell activity by normal peritoneal macrophages. In Herberman, R. B. (Ed.): NK Cells and Other Natural Effector Cells. New York, Academic Press, 1982, pp. 535-540.
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CM09260-01 LMI

PERIOD COVERED

February 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Lymphokine Cascade and Macrophages in Lymphocyte Activation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Joost J. Oppenheim, Chief, LMI, NCI

COOPERATING UNITS (if any)

Laboratory of Microbiology & Immunology, NIDR; Biological Therapeutics Branch, BRMP, NCI; Medicine Branch, NCI; NCI-FCRF; NIADDA, NIH; Hematology Dept., GWU, Wash. D.C.; LDBA, NIDR

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

2

PROFESSIONAL:

1.5

OTHER:

0.5

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying four major means by which accessory cells affect lymphocyte activation. (1) It has been observed that only IA/DR positive cells can successfully present activating agents to lymphocytes. We have established that human monocytes from cord blood or adult peripheral circulation can be induced to express DR antigens by "resting" lymphocytes or by immune interferon (IFN γ). Conversely, monocytes lose their DR expression with in vitro culture, or by incubation with prostaglandins or antibody to IFN γ . Thus this major histocompatibility product can be modulated in various ways which in turn provides a means of influencing lymphocyte reactivity. (2) Accessory cells are said to "process" stimulants. We established that paraformaldehyde-fixed human monocytes can activate T lymphocytes, provided they are preincubated with the stimulant for several hours at 37°C and subsequently supplemented with IL-1. Furthermore, if the monocytes were exposed to lysosomotropic agents during the preincubation period and then fixed, they could no longer activate lymphocytes. This suggests that stimulant processing at the lysosomal level is necessary to enable the stimulant to interact with DR antigens and the T-lymphocyte receptor. (3) We have ascertained that several cell types in addition to monocytes that can act as accessory cells, can also produce IL-1: (a) a subset of DR-positive peripheral human large granular lymphocytes with natural killer activity, and (b) 7 of 10 EBV-transformed human B-cell lines tested. Although all the B-cell lines were DR positive, only those which produced an IL-1-like activity could function as accessory cells. This data leads to the hypothesis that any cell type that is capable of activating T cells, and hence initiating immune responses, must be DR positive and an IL-1 secretor. (4) Finally, a cascade of cell-cytokine interactions serves to promote lymphocyte activation. IL-1 is known to induce the production of IL-2. We have shown that IL-2 in turn induces human IFN γ which in turn induces monocyte DR expression and IL-1 production.

PROJECT DESCRIPTION

PERSONNEL

Dr. Giuseppe Scala	Visiting Fellow	LS	LMI	NCI
Dr. Kouji Matsushima	Visiting Fellow	LS	LMI	NCI
Dr. Yan De Kuang	Guest Researcher	IS	LMI	NCI
Dr. Tadashi Kasahara	Visiting Fellow			NIDR
Dr. Hiroko Nagai	Guest Researcher			NIDR
Dr. Richard Fisher	Medicine Branch			NCI
Dr. Alfred Steinberg	Sr. Invest. Arthritis Branch			NIADDK
Dr. Marcelo Sztejn	Sr. Invest. Dept. of Hematology		GWU, Wash., D.C.	
Dr. Patricia Steeg	Postdoctoral Fellow		LDBA	NIDR
Dr. John F. Ortaldo	Sr. Invest.	NIS	BTB	NCI
Dr. Paolo Allavena	Visiting Fellow	NIS	BTB	NCI

OBJECTIVES

Understanding the mechanism of lymphocyte activation is fundamental to obtaining therapeutic means of manipulating immunological reactions with biological response modifiers (BRM). Lymphocyte activation is dependent on accessory cells for stimulant processing, presentation of the stimulants, and secretion of immunoregulatory mediators such as interleukin 1 (IL-1). We are investigating these processes using a variety of human cell types including peripheral blood monocytes, natural killer (NK) cells, B-cell lines, and keratinocytes.

METHODS EMPLOYED

We are studying the role of accessory cells in lymphocyte activation predominantly in tissue culture and occasionally in animal models. Human cells are obtained either from buffy coats or leukapheresis samples from the NIH Blood Bank or the BRMP Leukapheresis Unit. The leukocytes are fractionated using Ficoll-Hypaque or Percoll gradients to obtain the appropriate subpopulations. Leukocytes are also treated with monoclonal antibodies and complement or depleted by adherence to further purify the desired cell types. The cells are cultured for short periods and culture supernatants concentrated and chromatographed if necessary to detect cytokines. Interleukin 1 bioassays were performed using C3H/HeJ thymocytes and/or primary cultures of human foreskin fibroblasts. Interleukin 2 bioassays were performed using IL-2-dependent lymphocyte lines, and the antiviral assay using WISH cells was used to titer immune interferon activity.

MAJOR FINDINGSI. Identification of Cell Types That Produce IL-1

Interleukin 1-like activity, that enhances the proliferative response of murine C3H/HeJ thymocytes, was unexpectedly detected by Dr. G. Scala in the culture supernatants of a human "ROHA9" cell line that is EBV positive and has surface immunoglobulin receptors. This data suggesting that human B-cell lines, in addition to macrophages and keratinocytes, could also produce IL-1, was supported by the observations of Dr. Y. Kuang that 7 of 10 EBV-positive B-cell lines which can function as accessory cells also produce a thymocyte and fibroblast activating factor. Dr. K. Matsushima has demonstrated that this B-cell product has the same biochemical characteristics as monocyte-derived IL-1. In contrast, those three DR-positive B-cell lines, that could not function as accessory cells that activate T lymphocytes, either failed to produce IL-1 or, in one case, produced an inhibitor of IL-1 as well as IL-1. This inhibitor is a 100,000 MW entity. Dr. Scala also has observed that human peripheral blood Large Granular Lymphocytes (LGL) that exhibit natural killer (NK) activity also produce an IL-1-like activity. By using a battery of monoclonal antisera against leukocyte membrane antigens, IL-1 production could largely be attributed to a monomyelocytic-like subset of LGL which was OKT11⁺, OKM1⁺ and DR⁺. In contrast, Dr. T. Kasahara has observed that another subset of OKT11⁺, OKM1⁻, DR⁻ "atypical T lymphocyte-like" LGL was capable of producing IL-2. A third subset of OKT8⁺ LGL exhibited a suppressive effect on such IL-1 and IL-2 production by the other LGL subsets. In addition, Dr. Scala has observed that the DR⁺ subset of IL-1-producing LGL is even more effective than monocytes in its capacity to function as accessory cells, and can activate T lymphocytes to respond to alloantigens, antigens, and polyclonal stimulants. Thus those B lymphocytes, monocytes, and NK cells that can function as accessory cells, both express DR/Ia antigens and produce IL-1.

II. Metabolic Processing of Stimulants by Monocytes

The phenomenon of macrophage-lymphocyte interaction, in addition to requiring the presence of a surface DR antigen, and a mediator, interleukin 1, also is said to require biochemical processing of the antigen by the monocyte. Dr. Scala investigated this problem by confirming that paraformaldehyde-fixed human monocytes even when supplemented by IL-1 could not activate T lymphocytes to react to an antigen or polyclonal stimulant. However, if monocytes were preincubated with stimulants prior to gentle fixation and if supplemented by IL-1, about 50% of the maximal lymphocyte activation could be obtained. This suggests that some metabolic processing of stimulant at 37°C by monocytes is necessary to produce a stimulatory signal. This hypothesis was supported further by experiments showing that lysosomotropic agents transiently inhibited the capacity of monocytes to mediate lymphocyte activation. The lysosomotropic agents had no inhibitory effect on macrophages which were preincubated for 2 hours with stimulants. Presumably metabolic events occurring involving the phagolysosome are necessary to enable a stimulant to activate lymphocytes.

III. Effects of the Lymphokine Cascade on Monocyte DR Expression

It has previously been shown that lymphocytes activated with the help of IL-1, produce IL-2. Dr. T. Kasahara has shown that human IL-2 in turn can induce other human T lymphocytes to produce immune interferon. Further studies of this cascade effect of immunoregulatory lymphokines have revealed that gamma interferon in turn promotes monocytes to express DR antigens, thus enhancing their accessory cell capabilities. Studies by Drs. Steeg and Szein have revealed that prostaglandins reduce monocyte DR expression. Their studies are also compatible with the view that low levels (2-8 U IFN γ /ml) suffice to induce monocyte DR in vitro, that in the presence of "resting" lymphocytes monocytes maintain their DR antigen expression in vitro and that antibody to IFN γ inhibits this effect. These data identify the signals that regulate the ability of monocytes to express DR antigens which mediate lymphocyte activation.

Since neonatal human monocytes express much lower levels of DR (15-25%) than those from adults (75-85%), one can propose that perhaps environmental stimulation is the ultimate inducer of interferon-mediated monocyte DR expression and consequent accessory cell function. Dr. Nagai has assessed monocyte DR levels in patients with immunological disorders. She has observed that DR expression was diminished in a proportion of patients with Systemic Lupus Erythematosus and in patients in the more advanced stages of Hodgkin's disease. Finally, Dr. K. Matsushima is generating data showing that IFN γ can activate human monocytes to produce IL-1 in vitro. Thus we have actually established that there is an amplifying circuit of cytokine-cell interactions involving IL-1, IL-2, and IFN γ that promotes stimulant-induced lymphocyte reactions in a variety of ways.

SIGNIFICANCE

Identifying cell types that can act as accessory cells and delineating their role in processing and presentation of stimulants and elaboration of amplifying cytokines such as IL-1 will lead to a greater understanding of means of immunotherapeutic manipulation of lymphocyte-dependent as well as NK-dependent immunological reactions. Presumably tumor immunity requires the participation of accessory cells, T lymphocytes, and NK cells. Therefore agents which promote accessory cell functions, e.g., DR expression and production of immunoregulatory cytokines such as IL-1, IL-2, and IFN γ presumably can promote the rejection of neoplastic cells.

PROPOSED COURSE

Greater characterization of immunoregulatory human cytokines such as IL-1 is necessary. This will require production of IL-1 in considerable quantities, purification and the development of monoclonal antibodies to IL-1 to facilitate immunoaffinity purification and to study effects of inhibition of IL-1. Similarly, the biological effects of antibodies to IL-2 and immune IFN require further study as does the inhibitor of IL-1 which is produced by some B-cell

lines. The in vivo antitumor effects of more purified IL-1 and IL-2 will also be investigated.

PUBLICATIONS

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Stiehm, E. R., Mann, D., Newland, C., Szein, M. B., Steeg, P. S., Blaese, R. M. and Oppenheim, J. J.: Deficient DR antigen expression on human neonatal monocytes: Reversal with lymphokines. In Wedgwood, R. and Rosen F. (Eds.): Primary Immunodeficiency Diseases. Birth Defects: Original Article Series. (In Press)

PERIOD COVERED
 October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Antitumor Effects of Macrophages and Natural Killer Cells in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)
 (Name, title, laboratory, and institute affiliation)
 Robert H. Wiltrout, Staff Fellow, LMI, NCI

COOPERATING UNITS (if any)
 Hoffmann-LaRoche, Inc., Nutley, New Jersey; NCI-FCRF; NCI; Queen's University, Kingston, Ontario, Canada

LAB/BRANCH
 Laboratory of Molecular Immunoregulation

SECTION
 Immunobiology Section

INSTITUTE AND LOCATION
 NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.5	0.8

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
 Natural killer (NK) cells and macrophages (M ϕ) may inhibit formation of metastases. NK cells can perform this function in both normal or BRM-treated mice, while M ϕ probably only exert these effects in BRM-treated mice. NK cells exert antimetastatic effects during the bloodborne phase of metastasis whereas activated M ϕ exhibit antitumor activity at local tissue sites after extravasation of tumor cells from the bloodstream. We have used three approaches to study the antitumor activities of M ϕ and NK cells. (1) Anti-serum raised against the neutral glycosphingolipid asialo GM1 (asGM1) has proven effective in deleting NK cell activity in vitro and in vivo. In contrast, most M ϕ populations are resistant to anti-asGM1 serum, although some M ϕ activated with the pyran co-polymer MVE-2 are sensitive. M ϕ activated by C. parvum are refractory to effects of anti-asGM1. Therefore, we have established an in vivo model for selectively studying M ϕ -mediated antitumor effects in normal or BRM-treated mice, using anti-as GM1 to selectively suppress NK activity. (2) Molecular and cellular events required for in vivo expression of M ϕ -mediated antitumor effects are being investigated. We have demonstrated that murine recombinant γ IFN supplies both priming and triggering signals for M ϕ activation, at physiological concentrations. Therefore γ IFN may play an important role in the generation of M ϕ -mediated antitumor effects in vivo. (3) Following activation, M ϕ interact with tumor cells as a prerequisite to tumor cell lysis and preferentially lyse some tumor cells more efficiently than others. This pattern of tumor cell lysis is qualitatively different from that seen with NK effector cells. The molecular basis for these results may relate to the mechanism(s) by which M ϕ recognize tumor cells. We have found that carbohydrates are capable of inhibiting, to varying degrees, the cytolysis of tumor cells by activated M ϕ . The effects of various monosaccharides differ based on the specific tumor target cells employed. These observations imply that not all tumor cells are equally well recognized by activated M ϕ , and that these differences may relate to expression of carbohydrate moieties on tumor cells.

PROJECT DESCRIPTION

PERSONNEL

Craig W. Reynolds	Staff Fellow	NIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Howard T. Holden	Microbiologist	IS	LMI	NCI
Michael A. Chirigos	Head, Immunopharmacology	IS	BTB	NCI
John R. Ortaldo	Biologist	NIS	BTB	NCI
Joost J. Oppenheim	Chief		LMI	NCI
R.S. Kerbel	Research Scholar of Canada			
	Queen's University Kingston, Ontario, Canada			
M.J. Brunda	Research Scientist			Hoffman LaRoche, Inc., Nutley, New Jersey

OBJECTIVES

The objectives of this project are: (1) To discriminate *in vivo* antitumor effects mediated by $M\phi$ from those mediated by NK cells in normal and biological response modifier (BRM)-treated mice. (2) To examine the molecular and cellular events that occur during the activation of $M\phi$ for tumoricidal activity. (3) To evaluate the ability of cytokines to reconstitute specific and nonspecific immune responses, and antimetastatic activity in immunosuppressed mice.

MAJOR FINDINGSI. Role of $M\phi$ and NK Cells as Nonspecific Antimetastatic Effector CellsA. Effects of anti-asialo GM_1 serum on NK cells and $M\phi$

Treatment of mice with antiserum against the neutral glycosphingolipid, asialo GM_1 (as GM_1), abolishes NK cell activity *in vitro* and *in vivo*, and enhances the formation of lung and liver metastases following *i.v.* administration of B16 melanoma cells. These results imply that NK cells can have an immune surveillance role in normal mice. However, this implication is dependent on the ability of anti-as GM_1 serum to discriminate between NK cells, $M\phi$, and T cells. We found that anti-as GM_1 serum totally ablated NK activity, but had no effect on the generation of cytotoxic T lymphocyte responses. Moreover, cytolytic activity mediated by MVE-2 or *C. parvum* activated $M\phi$ was partially or totally resistant to anti-as GM_1 treatment. These findings support the use of anti-as GM_1 to delineate antitumor responses mediated by NK cells from those mediated by $M\phi$. (Cooperating units: Z01CM09246-15, Z01CM09250-08).

B. Mechanisms of BRM-mediated antimetastatic effects

The immunological mechanism(s) responsible for antitumor effects induced by BRM therapy remains unclear. We have approached this problem by studying the antitumor effects of BRMs in selectively (anti-as GM_1) or nonspecifically (cyclophosphamide: Cy) immunosuppressed mice. These immunosuppressive regimens

decrease NK activity, and increase metastasis formation by the B16 melanoma. MVE-2 administration prevents this increased metastasis formation in these immunosuppressed mice. This effect occurs in the absence of detectable NK activity, suggesting a potential role for both NK and non-NK effector cells in BRM-mediated antitumor responses. Further, the model suggests that various effector mechanism exert antitumor effects at different stages of the metastatic process, with NK cells contributing antimetastatic activity during the bloodborne phase, and non-NK cells exerting effects subsequent to extravasation of tumor cells from the bloodstream. (Cooperating units: Z01CM06146-06, Z01CM09228-03, Z01CM09247-03, Z01CM09246-15).

II. Molecular Events in M ϕ -Tumor Cell Interactions and M ϕ Activation

A. Basis for specificity of NK and M ϕ -mediated tumor cell lysis

Several murine tumor cell lines were tested by isotope release assay for susceptibility to M ϕ or NK mediated lysis. Activated M ϕ preferentially lysed L5178Y and P815, while the NK sensitive YAC-1 was relatively resistant. NK cells efficiently lysed YAC-1 but were ineffective against L5178Y and P815. These results were consistent regardless of the activating stimulus employed or H-2 haplotype of the effector cells. This differential lysis is probably due to variations in expression of target-cell structures recognized by each type of effector cell, and/or in susceptibility to the lytic mechanism(s) of the various effector populations. Two molecular approaches have been initiated to address these issues. First, specific carbohydrates inhibit cytotoxicity mediated by NK cells and M ϕ . D-mannose inhibits NK activity, while several monosaccharides inhibit M ϕ -mediated lysis, depending on the tumor target employed. None of the monosaccharides tested blocked M ϕ activation, indicating that the concentrations used were nontoxic. These results indicate that carbohydrate moieties may play a role in the recognition and/or mechanism of lysis of tumor cells by activated M ϕ and NK cells.

Second, using membrane vesicles, we are transferring cell surface components from lysis-sensitive cells to lysis-resistant cells, and vice versa, and then studying the susceptibility of these modified cells to M ϕ -mediated recognition and lysis. The feasibility of this approach has been confirmed using the Fc receptor (FcR) of L5178Y (sensitive to M ϕ -mediated lysis) as a marker for membrane transfer to the FcR⁻ B10:D2B10 (insensitive to M ϕ -mediated lysis). By this technique, up to 50% of the normally FcR⁻ B10:D2B10 cells were converted to FcR⁺. We are now using this model to study events associated with recognition and lysis of tumor cells by activated M ϕ .

B. Mechanisms of M ϕ Activation

Murine recombinant γ IFN was demonstrated to be a potent inducer of tumoricidal M ϕ . Activation by γ IFN occurred at physiological concentrations (<1 U/ml), and in the absence of exogenous lipopolysaccharide (LPS). This observation supports the hypothesis that γ IFN and M ϕ -activating factor (MAF) may be distinct entities. Further studies will investigate the ability of γ IFN and MAF to synergize with other cytokines or LPS during the M ϕ activation sequence.

SIGNIFICANCE

Our studies are designed to investigate several aspects of host antitumor defense mechanisms. Understanding the contributions of NK cells and M ϕ to antimetastatic defenses in normal mice may provide insight into their potential for immune surveillance against neoplasia. Defining the mechanisms through which BRMs mediate antimetastatic effects in experimental models, may provide a clearer perspective for the formulation and interpretation of BRM treatment regimens in clinical trials. It is also important to understand the molecular events that occur at the cellular level during the interaction of M ϕ and NK cells with tumor cells. These studies provide insight into the processes by which activated M ϕ recognize and kill tumor cells, and discriminate between neoplastic and normal cells. Furthermore, since immune response capabilities are complex, it is equally important to understand the molecular regulatory events which result ultimately in the generation of antitumor effector cells. Of particular interest are the roles of immune γ IFN, IL-1, and IL-2 as signals in the generation of highly tumoricidal M ϕ , NK cells, and T lymphocytes. By understanding the molecular and cellular interactions that occur during the generation of immune responses in experimental models, the modulation of host defense mechanisms in human cancer may be enhanced.

PROPOSED COURSE

Studies are ongoing to characterize the cells which respond to BRMs, to dissociate antitumor responses mediated by NK cells from those mediated by M ϕ , and to understand the respective potential of NK cells and M ϕ as antitumor effectors in immunoprophylaxis and immunotherapy. These studies will continue by examining antimetastatic effects of BRMs at local tumor sites in selectively or nonspecifically immunosuppressed mice. Studies into the nature of the interaction of activated M ϕ with tumor cells will also be performed. The recognition and lytic phases of M ϕ -mediated cytolysis will be investigated through the transfer of membrane components from lysis sensitive cells to lysis resistant cells, and vice versa. These modified cells will then be studied for changes in recognition by activated M ϕ , as well as for altered susceptibility to lysis. These types of experiments may provide some insight into the molecular processes by which M ϕ discriminate between neoplastic and normal cells. Molecular regulatory events related to generation and function of antitumor effector cells will be studied through the use of murine recombinant γ -IFN, crude and partially purified IL-1, and IL-2. The roles of these molecules as signals in the priming and triggering sequence of M ϕ activation, or in stimulation of T- and NK-cell responses will be investigated.

PUBLICATIONS

Brunda, M. J., Wiltrout, R. H., Holden, H. T. and Varesio, L.: Selective inhibition by monosaccharides of tumor cytotoxicity mediated by mouse macrophages, macrophage-like cell lines and natural killer cells. Int. J. Cancer. 31: 373-379, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM09263-01 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Aspects of Monocyte Differentiation and Functional Activities

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ezio Bonvini, Visiting Fellow, LMI, NCI

COOPERATING UNITS (if any)

NCI-FCRF; Division of Biochemistry and Biophysic, Bureau of Biologics, FDA

LAB/BRANCH

Laboratory Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

0

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monocytes when activated may have a role in host defense against tumors. Some biochemical mechanisms associated with activation of monocytes have been investigated. The effects of agents, such as interferon (IFN), the chemotactic peptide N-formyl-L-methionyl-L-phenylalanine (FMLP) and the tumor promoter 12-tetradecanoyl phorbol 13-acetate (TPA), have been examined. Methylation reactions are required for the expression of several cellular activities. Of these different methylation reactions, phospholipid (PL) methylation is of considerable interest for its role in modifying the physical state of cellular membranes. We have shown that IFN induced an inhibition on PL methylation by mononuclear cells, an effect that might contribute to alterations in the properties of the membrane of IF treated cells. We have demonstrated and characterized the presence of a methylated nonpolar lipid fraction in monocytes, lymphocytes and a variety of tumor cell lines. Ubiquinone-50 was identified as the major methylated nonpolar lipids and the regulation of its biosynthesis via methionine-dependent reactions by monocytes was defined. An inverse relationship between activation of the oxidative burst and the rate of phospholipid and nonpolar lipid methylation was observed. Both methylation reactions were similarly affected, suggesting a common mechanism of regulation. Oxidation of methionine to methionine sulfoxide by stimulated monocytes was observed, which led to the possibility, currently under investigation, that this phenomenon might limit the availability of intracellular methyl donors. The inhibitory effects on lipid methylations observed in stimulated monocytes contributes to our understanding of the mechanisms of regulation of methylation reactions in these cells and suggest a relevant role of these pathways in the response of monocytes to stimulants.

PROJECT DESCRIPTION

PERSONNEL

Luigi Varesio	Visiting Associate	IS	LMI, NCI
Philippe P. Bougnoux	Visiting Fellow	IS	LMI, NCI
Elisabetta Blasi	Visiting Fellow	IS	LMI, NCI
Thomas Hoffman	Senior Investigator		DBB, FDA

OBJECTIVES

General objectives are to gain insights into the biochemical events occurring during monocyte differentiation and expression of monocyte functions. Specific objectives of this project are: (1) To investigate the role of methylation reactions in driving the expression of functional activities and the differentiation process of human monocytes. (2) To investigate nonpolar lipid (NL) and phospholipid (PL) methylation of fresh and cultured monocytes stimulated by BRMs or drugs. (3) To study the changes in the intercellular levels of the methyl donor, S-adenosyl-methionine (SAM) and of its competitive inhibitor, S-adenosyl-homocysteine (SAH), following stimulation. (4) We will study superoxide anion (O_2^-) release an inducible functional activity in monocytes, to ascertain its regulatory effects on methylation. We intend to extend our study to other activities, such as DR antigen expression by monocytes.

METHODS EMPLOYED

A method has been developed during the last year for the simultaneous quantification of the rate of methyl group incorporation into nonpolar lipids and phospholipids. The technique is based on the measurement of the amounts of [3H]-methyl groups from [methyl- 3H]methionine incorporated by intact cells into cellular lipids extracted with organic solvents. The method allowed evaluation of the rate of methyl group incorporation in the femtomolar range, thus requiring limited number of cells. Identification of the methylated lipid compounds was performed employing different techniques, including thin layer chromatography HPLC and gas chromatography-mass spectrometry.

Methylation of RNA was evaluated from the incorporation of methyl groups from methionine into cellular RNA by intact cells. RNA is extracted from cells solubilized with guanidine thiocyanate and is purified by ultracentrifugation over cesium chloride gradient.

A cationic exchange HPLC system was used for the evaluation in the picomolar range of the intracellular amounts of SAM and SAH.

O_2^- release was used to measure the oxidative burst (OB) of human monocytes. A microtechnique based on the automatic spectrophotometric reading of microtiter plates employing a Microelisa[®] autoread was used for the evaluation of O_2^- release by monocytes in culture. The assay employed the superoxide dismutase-inhibitable reduction of ferricytochrome c. Its reactivity is in the nanomolar range.

MAJOR FINDINGS

I. Effects of Interferons (IFN) on PL Methylation by Mononuclear Cells

Inhibition of PL methylation by α -IFN (recombinant or natural) and β -IFN treatment of mononuclear cells was observed. Time course studies showed that the inhibitory effects were early events following IFN-cell interaction, occurring within the first 5 minutes of treatment. No effects of IFN on the PL methyltransferase activity was observed. Decreased transmethylation, with a consequent accumulation of phosphatidylethanolamine and diminished rate of synthesis of phosphatidylcholine, may be in part responsible for altered membrane physical properties of IFN-treated cells.

II. Nonpolar Lipid Methylation

In human monocytes the presence of a methylated nonpolar lipid fraction was observed. Further investigation in this area led us to identify ubiquinone-50 (coenzyme Q10) as the major methylated nonpolar lipid in human monocytes. Similar finding was observed in lymphocytes as well as in a variety of cell lines, including U937, HL60, K562 and the mouse RL-male 1 cell line. The biosynthesis of ubiquinone in monocytes was shown to be methionine-dependent.

III. Effects of the Activation of the Oxidative Burst (OB) on the Lipid Methylations

Different agents activate the OB in human monocytes, including the chemotactic peptide, N-formyl-L-methionyl-L-phenylalanine (FLMP), and the tumor promoter, 12-O-tetradecanoyl-phorbol 13-acetate (TPA). An inverse relationship between activation of the OB and the rate of methyl group incorporation into lipids was observed. This effect was not due to defective uptake of the methyl donor, methionine, either to enhanced decay of the methylated compounds, or to a reduced enzymatic activity. The decreased methylation was similar in phospholipids and neutral lipids, suggesting that monocytes have the capability to simultaneously modulate different methylation reactions. A possible explanation, currently under investigation, is that the reduced availability of methyl donor may be due to an intracellular oxidation of methionine to methionine sulfoxide, which can not act as a methyl donor.

SIGNIFICANCE

Monocytes and their differentiated counterpart, the tissue macrophages, have multiple functions in host defense, including a role in the control of tumor growth. In recent years the role of methylation reactions in the expression of cellular functions, e.g., the chemotactic response of phagocytes, the oxidative burst and the binding of activated macrophages to tumor cells, has been recognized. An understanding of the mechanisms of regulation of methylation reactions is a basic requirement for the planning of rational strategies to modulate the functional activity of monocytes. An important level of regulation of methylation reactions is represented by the concentration of intracellular methyl donor, SAM, and its competitive inhibitor, SAH. If a BRM is known to act on the levels of SAM/SAH pool or on specific methylation reactions, this provides an important tool to modulate the biological functions of monocytes.

PROPOSED COURSE

During the next year we will extend the characterization of the relationships between activation of the oxidative burst and inhibition of lipid methylation. Studies on PL methylation will be particularly pursued because of the role of this pathway on the control of the physical state of the cellular membrane.

We will start to investigate the biochemical events associated with the differentiation process of monocytes. Phenotypic changes occurring during the process of cell differentiation may be related to the modulation of the expression of genetic informations. In this respect, DNA methylation, which is involved in the regulation of gene expressions, and RNA methylation, which is relevant for the stability and processing of the precursor molecules, are of great relevance for their possible regulatory role. We plan to investigate these metabolic pathways during the next year. Studies on RNA methylation are currently in progress. Two cell lines, the monocyte-like cell line U937 and the promyelocytic cell line, HL60, that can be induced to differentiate into more mature cellular types with appropriate inducer, including IF and TPA, will also provide useful models for this study. In addition, the role of methylation reactions on cellular expression of differentiation markers, such as DR-antigens expression, will be studied. Specific emphasis will be put on the assessment of the effects of inhibitors of methylation on the expression of the above functional activities.

PUBLICATIONS

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