Division of

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1984 - September 30, 1985

The Clinical Oncology Program is the intramural treatment research arm of the National Cancer Institute. The program is composed of 6 Branches, and a summary of their scholarly accomplishments for fiscal year 1985 is listed separately for each Branch. A laboratory under the supervision of Dr. Samuel Broder operates within the Office of the Director. The OAD also contains a Biostatistics Data Management Section supervised by Dr. Robert Makuch.

Laboratory activities of the OAD

The OAD has reprogrammed much of its scholarly activities in response to the AIDS epidemic and the assignment of AIDS as the Department's number one priority. Dr. Broder's laboratory activities can be summarized under three headings.

1. Screening and development of anti-retroviral agents for use in patients with AIDS and related diseases caused by HTLV-III/LAV.

   Dr. Broder's lab was the first to recognize the in vitro anti-viral activity of Suramin against HTLV-III and the Program has launched several studies to test Suramin in vivo. The preliminary research suggest that Suramin can inhibit the in vivo replication of HTLV-III/LAV. During the past year a number of rapid-screening systems have been devised by Dr. Broder's lab to evaluate new anti-retroviral agents. These rapid-screening systems have explored pharmaceutical agents already in the public domain as well as those provided by private sector manufacturers. These studies have yielded a number of purine and pyrimide nucleoside analogues which have a potent anti-viral effect against the replication of HTLV-III in vivo. Moreover, these studies have yielded a set of chemical rules for converting essentially any normal purine or pyrimidine nucleoside into an anti-retroviral drug by a simple "modification" of the sugar moiety. A phase I trial of a new anti-viral agent, 3'-azid-3'-deoxythymidine(manufactured by the Burroughs-Wellcome corporation) has been initiated in patients with AIDS in the COP on the basis of these studies. It is highly likely that other agents will also be developed for Phase I testing similarly within the program. These trials represent the first of their kind.

2. Development of human monoclonal antibodies to human retro-viruses

During the past year, Dr. Broder's lab has explored ways of generating human monoclonal antibodies against viruses in the HTLV family of pathogenic retroviruses. The lab has been able to generate a human B-lymphoblastoid line from the lymph node of a patient with adult T-cell leukemia, whose serum contained antibodies to HTLV-I. The line was generated by a process of EBV-immortalization followed by cloning and screening for antibody activity against HTLV-I. The resultant line was found to be a stable producer of an Ig G k antibody reactive against an epitode on th gp46 envelope glycoprotein of HTLV-I. This is the first report of a monoclonal antibody to a pathogenic retrovirus and the first report of a human monoclonal antibody (either human or animal) reactive against the major gp46 antigen of HTLV-I. These strategies will now be applied to the problem of developing a human monoclonal antibody against HTLV-III/LAV, the retrovirus etiologically linked to AIDS.

3. Configuration and expression of T-cell antigen receptor genes in HTLV-I infected cells.

In collaboration with Drs. Ruth Jarett and Marvin Reitz in Dr. Gallo's laboratory, Dr. Broder's lab has studied the configuration and expression of the gene encoding the beta-chain (and more recently, the alpha-chain) of the T-cell receptor. First, HTLV-I infection per se does not alter the configuration of either the alpha or Beta gene in antigen-specific T cells. Second, almost all HTLV-I infected cell lines and all primary tumor cells that bear the pro-virus showed rearrangement of the B gene and in each case the rearrangement is distinct (i.e, clonal). Finally, a major factor of Dr. Broder's lab has been to analyze the factors which contribute to the altered immune reactivity (loss of specificity) exhibited by certain T cell clones following HTLV-I infection. An antigen-specific T-cell clone which developed an indiscriminate pattern of immune reactivity after HTLV-I infection expressed an unusually small 1.0 kilobase b-chain transcript in addition to the four length mRNA. The relationship between aberrant message and the development of deranged immune reactivity will be explored in future research.

Program Accomplishments

Biostatistics and Data Management Section

The Section is the statistical and data management component of the Clinical Oncology Program (COP). The Section provides statistical leadership and data management consultation for major activities of the Program, and is involved in the design, conduct, monitoring, and statistical analyses of intramural and national multicenter clinical trials of experimental treatments for cancer. The Section also is involved in the scientific monitoring of national, multi-center studies which are not funded by the NCI. Other major collaborative efforts include studies to identify important prognostic and treatment selection factors, evaluate diagnostic procedures, develop improved staging

systems, and assist investigators in the design, execution, and analyses of major in vitro drug testing studies. The Section develops new statistical designs and biometric methods related to the development and evaluation of new cancer treatments. Current research is focused on the evaluation of the person-years index to summarize the incidence of second neoplasms after initial cancer treatment, and the effects of early termination on the interpretation of clinical trial results. The Section maintains computerized data collection systems for intramural and national multicenter clinical protocols, and it works closely with interested branches to improve data recording and retrieval. The Section provides liaison with the Clinical Center Medical Information System team and the Clinical Center Pharmacy, allowing COP input into decisions which directly impact patient care and protocol management. The Section assists the Deputy Clinical Director to insure adequate monitoring of protocols through the MIS Toxicity and Protocol Monitoring screens and other mechanisms.

Clinical Pharmacology Branch

This is the first year in which the Branch has had all of its planned sections in place and operating. It is also 18 months since our last site visit and the activities of the last year reflect full implementation of the site visit recommendations. In this summary, I will not detail all of the activities of each section, but rather touch upon the highlights.

1. Pharmacokinetics Section

In terms of program priorities, this group has made two major contributions. First, this group, in collaboration with Dr. Broder and Dr. Gallo, have established that suramin can be given to AIDS patients and will suppress the replication of HTLV-III in vivo. This group, in particular, have shown that because of the unique pharmacology of this drug, it need only be given every 2-4 weeks. A second group of patients is now being treated with a drug schedule designed according to initial pharmacokinetic analysis. We are very optimistic that this will prove to be truly effective therapy for AIDS; ARC and related syndromes if given early in the disease. We also feel that the remarkable pharmacology of this drug might suggest new avenues in cancer drug design. In addition to suramin, this group is collaborating with Dr. Broder in the development of other drugs active against HTLV-III. The second major contribution has been Dr. Collins development of the blood level working group concept. At present, it appears that this approach will be a far more accurate predictor of starting dose in man than in the mouse lethality test.

2. Biophysical Pharmacology Section

This is the first year this group has been adequately staffed and has had its NMR in place. The Varian machine has proved to be exceptionally reliable for such a high tech device. This is especially remarkable because we received one of the first models. As a result, this has been a very productive year in the Biophysical Pharmacology Section. The various projects are detailed below. There are two major advances. First, Dr. Cohen has developed a very

promising group of NMR imaging agents based upon the observation that hematoporphyrins selectively localize in carcinomas. The concept is to use the porphyrin to carry paramagnetic metal ions into tumor cells. The paramagnetic metal ions then cause enhanced relaxation of water protons within the tumor, thus creating difference in contrast with surrounding tissue. Of the metalloporphyrins tried to date, manganese III complexes of TPPS and TM PyP have proven most effective. Both localize to human colon tumor xenographs in nude mice. Furthermore, they appear to bind and stay in the tumor for prolonged periods, like lymphangiogram dye. I think this will prove to be a remarkably fertile area for long term development because the chemical synthesis of porphyrin analogs is remarkably well developed and concepts and test systems are also well developed.

The second area revolves around the ability of NMR to study DNA structure in solution. The evolution of the concepts of drug-DNA interaction have been held back by the fact that the only way to get detailed 3D information of drug-DNA complexes was through X-ray crystallography. Not only is this technique very laborious and expensive, the results reflect crystal geometry in the absence of solute effect--a major determinant of DNA structure in solution. Over the past year Dr. Cohen has been able to use two dimensional NMR to get equivalent three dimensional information of DNA and DNA-drug complexes under physiologic solute conditions. The result has been the resolution of several conflicts in the field. I view the long term value of this approach in that it will allow us to develop agents selective for their attack on special regions of the DNA. One can envision attack on regulatory regions such as the TATA box, zDNA sequences or retroviral LTR's. Work now in progress is aimed at achieving just such goals.

3. Biochemical Pharmacology

This group has spent the last 8 years investigating the mechanism of adriamycin action. Over the past year, two major observations have come to light. The determination of mechanism of action of a drug is difficult when the drug is known to participate in a wide range of biochemical reactions. The problem has been to develop an experimental approach which effectively parses through the epiphenomenon to spot those biochemical events responsible for cytotoxicity. We have made the assumption that the genetic changes associated with the development of drug resistance provides valuable insight into how a drug acts. We have applied this approach to MCF-7 the human breast cancer cell line and an adriamycin resistant clone of this line obtained by Dr. Ken Cowan. We find that the resistant cell line has a number of changes including increased glutathione transferase, glutathione peroxidase and sulfo transferase activity. In addition, the resistant line has a much more active HMP shunt, which is needed to supply reduced glutathione to the above enzymes. All of these changes would be expected to increase the tolerance of the cell line for oxygen free radical attack. Indeed, we find that this cell line is markedly resistance to direct addition of toxic peroxides. These changes suggest that adriamycin kills wild type MCF-7 via the generation of toxic oxygen species. Dr. Ken Cowan is now in the process of cloning the genes in this cell line which confirm adriamycin resistance.

4. Office of the Chief

There are two groups within the Office of the Chief: one under the direction of Dr. Bruce Chabner, the other under Dr. Ken Cowan.

A. Dr. Cowan - Dr. Cowan has continued to characterize the DHFR gene he has cloned from MTX resistant human breast cancer cells. This gene was under estrogen regulation in the original host cell and preserves estrogen regulation now that it is cloned. By selectively deleting 5' upstream flanking sequences. He has been able to show that the hormonally responsive sequence is 110 bases 5' to the start of transcription. In order to confirm this, the putative regulatory sequence was ligated 5' to the end of the bacterial chloramphenicol transferase gene. This hybrid construct expresses well in human and hamster cells. With this construct, CAT expression is now regulated by estrogen. These studies now have clearly defined a system in which hormonal regulation of a human gene responsible for drug resistance may be studied.

Finally, Dr. Cowan has inserted this DHFR gene into a defective retroviral vector which now allows highly efficient transfer of the DHFR gene into mammalian cells. The plan is now to use this to transfer DHFR gene into human marrow and cloned human lymphocyte cell lines. The clinical implications of this are now obvious.

B. Dr. Chabner - There are two major developments from this group. First, this group has continued its elegant work on antifolates. Over the past year they have been able to show that MTX polyglutamates (1) inhibit thymidylate synthase, (2) inhibit AICAR transformlyase a key enzyme in purine synthesis and (3) are the major determinant of sensitivity of human small-cell lung cancer cell lines to MTX. This work and that which has preceded it are revolutionizing our concepts of MTX action.

The second development is the discovery that a 20K dalton membrane protein is frequently phosphorylated in adriamycin resistant cells. Current results suggest that this protein phosphorylation is by protein-C kinase.

Medicine Branch

In 1984-1985 the Medicine Branch staff published 109 papers, articles, or book chapters and has in press or accepted 32 additional publications. This continues the productivity record of publications established over the past several years by the Branch. Thirty-five active protocols are maintained primarily by the Medicine Branch with 1070 patients on clinical trials, an increase of 21% from last year. Details of the clinical and laboratory studies are presented in detail in the Annual Report Document.

Major Accomplishments in 1984-1985:

1. Early Hodgkin's disease: Radiation therapy vs. combination chemotherapy: While radiation therapy is generally successful in the management of early stage Hodgkin's disease, as many as 25% of patients relapse from

radiation induced complete remissions and, although many can be salvaged by chemotherapy, this is accomplished at some risk of induced second malignancy. Furthermore, successful radiotherapeutic management of early stage disease demands considerable technical expertise and access to sophisticated equipment not always available to all patients. Because combination chemotherapy is curative in advanced disease and can salvage many patients who relapse after radiation therapy and because small trials with MOPP chemotherapy in early stage Hodgkin's disease appeared successful, the Medicine Branch and Radiation Oncology Branch are performing a randomized comparison between these treatments. Thus far, 41 patients have been randomized to chemotherapy and 37 to radiation therapy. The complete remission rates are 100% for combination chemotherapy, and 94% for radiation therapy. With a median follow-up in excess of 32 months, 8% of the MOPP treated patients have relapsed compared to 36% for those treated with radiation therapy. Disease-free survival in randomized patients is significantly different ($p = .007$), and overall survival in randomized patients is now significantly different ($p = 0.03$) in favor of the MOPP chemotherapy treatment. Initial results of this trial establish uniformly high complete remission rates with both modalities and generally equivalent results regarding overall survival. If further follow-up substantiates initial observations, this trial will establish MOPP chemotherapy as an excellent alternative to radiation therapy in the management of early Hodgkin's disease.

2. Inoperable Stage III and Inflammatory Breast Cancer: Inoperable Stage III breast cancer and inflammatory breast cancer have long been unsatisfactorily managed by local surgery and/or radiation. Forty-six such patients, 18 Stage IIIA, 27 Stage IIIB,(21/27 inflammatory) have been treated with combination chemotherapy (CAMF) with hormonal synchronization with tamoxifen and premarin. Forty-one out of the 46 patients with inoperable breast cancer have achieved objective response (CR + PR = 89%), 46% of patients have achieved a clinical complete remission with chemotherapy alone. Seventy-one percent of patients achieving a complete remission with chemotherapy were documented to be in a pathologic complete remission at surgery and fully 40% of patients that completed local therapy have been rendered NED with the entire breast intact. Patients free of disease after induction chemotherapy receive local radiation therapy if pathologically free of disease and surgery if residual disease remains. Median disease-free intervals are 28 months for the Stage IIIB patients and median overall survival is 30 months. For Stage IIIA patients, none have relapsed with a median follow-up of approximately 14 months. This study, when completed, will likely establish this sequence of treatment as the preferred approach to the management of inflammatory or unresectable Stage III breast cancer.

3. Antineoplastic Drug Resistance in Human Ovarian Cancer: Human ovarian cancer cell lines are an extremely useful model for investigating drug resistance in human solid tumors. Four new ovarian cell lines have been characterized and additional cell lines have been developed with drug resistant variants 6-100 times more resistant than primary cultures. A new transplantable intraperitoneal model of human ovarian cancer in nude mice which produces ascites, pulmonary metastases, and death from

intra-abdominal carcinomatosis has been established which appears to be the most accurate model of the human disease yet established. It has been established that melphalan resistance is linked to increased glutathione levels in these human ovarian cancer cells and drugs such as butathionine sulfoximine (BSO) which inhibit glutathione synthesis, restore drug sensitivity in melphalan, cisplatin, and adriamycin resistant cell lines. In addition, it has been demonstrated that the calcium channel blocker, verapamil, alters adriamycin efflux increasing the intracellular concentration of adriamycin and restoring adriamycin sensitivity in resistant cell lines. Further studies with BSO plus melphalan in the new mouse model demonstrated prolongation of survival in mice bearing resistant human ovarian cancer implants and these results have led to evaluation of BSO by the Decision Network of the NCI as a prelude to clinical trials in man.

4. Molecular Mechanisms of Growth in Human Breast Cancer: The viral onc genes (ras and myc) have been introduced into human breast cancer cells and these retroviruses have been stably integrated and viral mRNA is expressed at high levels. Ras induces a hormone independent phenotype. This occurs through increased secretion of several specific growth factors in an autonomous fashion. Differential hybridization techniques are being used to identify specific estrogen-regulated genes for cloning and subsequent analysis and several estrogen-induced growth factors which are secreted by breast cancer cells have been identified and partially purified. Some of these activities cross-react with EGF receptor but others are apparently novel transforming growth factors. Purification and cDNA studies have further documented the novel nature of these growth factors. Breast cancer cells also secrete TGF, a PDGF-like competency factor and an epithelial growth factor. Monoclonal antibodies are being prepared to these growth factors and both in vivo and in vitro studies are being performed assessing the therapeutic effect of these monoclonal antibodies on inhibiting growth factors in human breast cancer. In addition, a novel 39K secreted glycoprotein whose secretion is induced by anti-estrogens has been identified and may play a role in the action of anti-estrogens in breast cancer.

5. Stage I Diffuse Aggressive Lymphoma: Twenty-five patients with clinically staged early (Stage I) diffuse aggressive lymphoma have been treated with a modified ProMACE-MOPP regimen at 75% doses on a monthly basis for 4 months followed by involved field radiation therapy. This treatment is carried on entirely as an outpatient. There have been no treatment related deaths, few hospitalizations for leukopenia and 22/23 (96%) of patients have entered a complete remission. To date none of the complete remissions has relapsed. This regimen appears to eliminate the necessity for surgical staging in these early disease patients and has produced an outpatient regimen with modest toxicity producing a complete remission rate of 96% with no relapses to date.

6. AIDS and Kaposi's Sarcoma: The Medicine Branch has actively supplied clinical specimens to Dr. Gallo's laboratory which have been contributory to the isolation and characterization of HTLV-III as the etiologic agent of AIDS. In addition, a series of clinical trials has been performed on patients with AIDS/Kaposi's sarcoma involving therapies including interferon, combination chemotherapy, DFMO + interferon. More recently, study has focused upon inhibitors of reverse transcriptase and recently a trial of suramin in

collaboration with the Clinical Pharmacology Branch and the Associate Director, COP, has been completed which has defined the pharmacokinetics, toxicity, and activity of the drug and has allowed expedited study of this agent extramurally. Trials of a new, very promising reverse transcriptase inhibitor, azidodeoxythymidine have just been initiated.

7. Cytogenetic Studies in Ewing's Sarcoma: Cytogenetic studies of 27 cases of Ewing's sarcoma, Askin's tumor, and peripheral neuroepithelioma have been completed. All direct tumors and tissue culture lines were shown to carry the specific chromosomal translocation t(11;22)(q24;q12). This specific chromosomal defect has not been previously described in this tumor group.

8. Poor Prognosis Advanced Testicular Cancer - PVBV vs PVB: A new four-drug combination, PVBV, composed of high dose cisplatin (40 mg/M^2 qd x 5), velban, bleomycin, and VP-16, in initial trials produced a high (89%) complete remission rate in patients with poor prognosis advanced non-seminomatous testicular cancer. In a subsequent randomized comparison between PVBV and PVB, 46 poor prognosis testicular cancer patients have been treated. Newly established hydration techniques prevented renal toxicity with high dose cisplatin. The complete remission rate for PVBV was 25/29 (86%) compared to 10/16 (62%) for PVB, $p = 0.16$. Currently, 5/16 (31%) of patients on PVB are alive and continuously disease-free after induction compared to 20/29 (69%) for PVBV, $p = 0.034$. These results indicate that PVBV treatment of poor prognosis non-seminomatous testicular patients results in more patients alive and continuously disease-free than previously published standard regimens.

9. Molecular Characterization of a Mutant Human c-myc Gene. It has been possible to describe the mutations in a human c-myc gene involved in a case of Burkitt lymphoma. In this particular case, PA682, the Burkitt cells have a 8;22 chromosomal translocation and the human c-myc gene is neither translocated nor rearranged. We have succeeded in cloning the c-myc gene from the abnormal chromosome and characterized it as having at least a 50-100 nucleotide deletion between the first and second exons of the myc gene. Currently experiments are underway to sequence the entire abnormal myc gene to characterize mutations that can occur independent of myc gene rearrangement and to try to define more clearly the regions of the myc gene that are essential for oncogenic activation.

10. Monoclonal Antibody Specific for Hodgkin's Disease: We have developed a monoclonal antibody specific for a Hodgkin's disease cell line. This monoclonal antibody identifies a cell surface structure with limited distribution on normal hemopoietic cells. A rare cell in a normal lymph node in the pericortical region bears this antigen, and occasional T cell lymphomas bear this antigen. In addition, certain EBV-infected B cell lines can be induced to express this antigen. The function of the antigen is not clear, but we have managed to isolate a variant of the Reed-Sternberg cell line which does not express the antigen recognized by the monoclonal antibody. Interestingly, the morphology of the antibody negative variant is less dendritic than the typical Hodgkin's disease cell line. The cells of the variant line grow as smooth cells instead of having the projections that are characteristic of the dendritic cell. We are now examining the phenotypic differences between the

antibody reactive and nonreactive variants and are moving to get the monoclonal antibody into clinical trials.

11. CPR for Advanced Ovarian Cancer: Cyclophosphamide, high dose cisplatin and total abdominal irradiation are now being used in advanced previously untreated patients. Twenty-two patients have been treated and 18 are evaluable. After only 3-4 months of induction chemotherapy, 67% of patients achieved a clinical complete remission and 78% of those have been pathologically free of disease at second look surgery. The majority of patients free of disease at second look had significant residual disease prior to starting chemotherapy. No patient free of disease at second look surgery has died with a median follow-up of 14+ months. This intensive 3-4 month induction regimen results in a high complete remission rate and a very high percentage of pathologically documented complete remissions. There is significant hematologic toxicity and dose-limiting peripheral neuropathy.

12. Nodular Lymphoma Trial: Eighty patients with advanced stage favorable prognosis non-Hodgkin's lymphomas have been randomized to receive either "watch and wait" therapy or intensive chemotherapy with ProMACE-MOPP and total nodal irradiation. Of those randomized to aggressive combination chemotherapy and evaluable, 90% of patients have entered a complete remission and only 2 patients have relapsed both in unirradiated, extranodal sites. All others remain in continuous complete remission with a median of 22+ months. Of those patients randomized to "watch and wait", 60% remain off therapy or with limited radiation treatment with a median of 24+ months. Forty percent of patients have been crossed over to the intensive treatment arm and, of those, 36% have entered a complete remission with intensive treatment. Of interest, 3 patients have undergone histologic progression without exposure to any chemotherapy. Several preliminary conclusions can already be derived from this study. (1) Approximately half of patients with minimal therapy will remain relatively asymptomatic for periods of time in excess of two years. (2) Initial aggressive chemotherapy and radiation treatment will produce a high complete remission rate and appears to have reduced the incidence of relapse after complete remission. (3) Histologic progression occurs in some patients without exposure to cytotoxic therapy and is therefore a part of the intrinsic natural history of disease.

13. Cytotoxic Tumor-Specific T Cell Clones: T cell clones specific for retrovirus induced syngeneic leukemias have been produced. Proliferative T cell clones specific for the FBL-3 leukemia and restricted to both class I and class II self major histocompatibility antigens have been propagated in vitro. We have been able to demonstrate significant in vivo activity of the proliferative cytotoxic T cell clones specific for the FBL-3 leukemia. In particular, we can reproducibly cure more than 50% of mice bearing established tumor by the intravenous administration of 2×10^7 cloned T lymphocytes followed by twice daily i.p. injections of recombinant interleukin 2 (IL-2) for 1 week. IL-2 administered alone was shown to have no effect on tumor growth or survival. Of major importance was the discovery that the in vivo antitumor activity of the T cell clones was markedly enhanced by pre-activation of the clones with antigen in vitro prior to in vivo administration. This was shown to be due to the fact that such antigen activation markedly increased the clone's expression of IL-2 receptors and dramatically enhanced

the proliferative response of the clone to IL-2 both in vitro and in vivo. Ongoing studies are investigating the mechanism of tumor elimination in vivo by T cell clones and the in vivo activity of T cell clones of distinct function and phenotype.

These 13 highlighted studies represent only a small portion of the clinical and laboratory investigations completed or initiated 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

The NCI-Medical Oncology Branch is conducting a series of clinical investigations into the treatment and biology of human lung cancer. Dr. Ihde and Dr. Mulshine lead the clinical trial effort, while Dr. Gazdar leads the in vitro chemotherapy sensitivity testing effort for the clinical trials. In extensive stage small cell lung cancer, we have demonstrated that tumor can be safetly obtained from patients and have in vitro chemotherapy testing performed. In nonsmall cell lung cancer this has also been possible, but the results in growing the lines have, as yet, not been as successful as in small cell lung cancer. However, new techniques for growing nonsmall cell lung cancer in serum free hormone supplemented media have been worked out, and the ability to grow nonsmall cell lung cancer successfully and perform these tests remains a major objective of the Branch. A panel of well-defined lung cancer cell lines of both small cell and nonsmall cell lung cancer has been made available to the drug therapy evaluation program to use for in vitro screening for new agents. The NCI-Navy Branch is cooperating with these efforts and is anxious to participate in the clinical trial of any newly discovered agents. In addition to in vitro chemotherapy sensitivity testing of patient samples, a prospective study of biochemical and immunohistochemical markers is being undertaken in work led by Dr. Linnoila. These potentially could be of widespread clinical diagnostic use in the typing of lung cancer.

In adult cutaneous T-cell lymphomas (mycosis fungoides), combined modality therapy studies have shown a significant fraction of long-term disease free survivors, up to five to seven years after receiving combined modality therapy. In addition, radiolabeled monoclonal antibody scanning with T101 given both intravenously and subcutaneously has showed impressive uptake in tumor bearing lymph nodes.

Dr. Johnson has correlated the presence of C and N myc amplification in small cell lung cancer with impaired survival. This could represent some of the first data in an adult tumor correlating oncogene status with the clinical course.

In the tumor cell biology laboratory, Dr. Gazdar leads the work on supporting of clinical trials of lung cancer by in vitro drug sensitivity testing. In addition to establishing techniques for growing both small cell and nonsmall cell lung cancer in serum free hormone supplemented media, he has discovered great heterogeneity of the nonsmall cell lung cancer lines. In nude mouse

xenografts, he has found that some nonsmall cell lung cancers are widely metastatic directly upon transplantation from the patient. These studies, in collaboration with the DTEP program of Dr. Mayo, provide an entirely new model of metastatic human cancer in xenografts. The molecular biology of this phenomenon specifically dealing with oncogenes and their amplification is under study.

Dr. Linnoila immunohistochemically, besides supporting the clinical lung cancer trials with the prospective studies of markers, has demonstrated the greatly depressed or absent expression of beta 2 microglobulin in a series of human small cell lung cancers and carcinoid tumors. In collaboration with a group in Finland, she has demonstrated that beta 2 microglobulin dramatically increases after Interferon therapy of the patient. This would be the first demonstration in a patient of induction of beta 2 microglobulin in a previously negative tumor. It has potential treatment implications using Interferon.

Dr. Gazdar has recently established a human IgA kappa secreting human myeloma line that is of potential tremendous significance. This tumor line grows well in culture and makes enormous amounts of immunoglobulin which it secretes into the culture fluid. He has also been able to demonstrate that it has a rearranged c-myc oncogene and expresses this oncogene. This line should be of use in the preparation of human hybridomas, the study of human B-cell development (which Dr. Michael Kuehl of our laboratory is beginning), and the cloning of a new rearranged gene adjacent to the c-myc gene (being undertaken by Drs. Hollis and Kirsch of our laboratory).

In the molecular genetics and immunology laboratory, Dr. Minna has led work on the isolation and characterization of a new proto-oncogene in human lung cancer. This gene is part of the myc family of oncogenes and has been named L-myc. In several small cell lung cancers, it has been found to be amplified and or tremendously over expressed. The structure and role of this gene in the biology of lung cancer is being explored, as well as its developmental expression. In addition, the method used to isolate the gene was based on the finding of a small homologous region between the various myc genes. Using the same strategy, Dr. Minna's group has been able to identify even further additional members of this myc family, and they are beginning the cloning and isolation of these genes.

Dr. Minna has also led the work, conducted by Dr. Cuttitta, of the role of bombesin (gastrin releasing peptide) in the physiology of small cell lung cancer. A monoclonal antibody against bombesin has been established, and this antibody will inhibit the clonal growth of these cells in vitro and the growth of small cell lung cancer xenografts in nude mice. This suggests the exciting possibility of using the antibody in a clinical therapeutic trial. Such a trial is being planned right now, and appropriate preparations of the antibody instituted to obtain approval from the FDA. In addition, an anti-idiotype antibody against the antibombesin antibody has been prepared. This anti-idiotype antibody appears to react with the bombesin receptor on the surface of tumor cells, and will actually stimulate their growth. It should provide a new reagent for isolating and characterizing the bombesin receptor, and also could be of potential therapeutic value.

Dr. Battey and Dr. Sausville have done elegant work on the structure and expression of peptide hormone genes in human lung cancer cells. They have cloned and sequenced the genes for arginine, vasopressin and oxytocin, and shown that they are tightly linked, within 50 killobase of each other, but in opposite orientations. In addition, they have cloned and sequenced, using cDNA clones, the bombesin (GRP) from human small cell lung cancer. Using these probes, they have established S1 nuclease assays that allow the detection of GRP messenger RNA in tumor samples. These studies should lead to a molecular genetic analysis of the expression of this important peptide hormone gene. In addition, plans using the antiserum against the bombesin receptor are being made to clone the bombesin receptor in an expression vector.

Drs. Hollis and Kirsch have recently isolated and cloned the gene for galactosyl transferase. This should allow a molecular genetic study of an important multigene family in cancer cells. These molecules determine the cell surface structure of cancer cells. In addition, they have identified a rearranged c-myc gene in the human myeloma cell line described above, and have cloned across the break point to identify a new piece of DNA involved in the rearrangement. They have also documented the regulation of myc and myb oncogenes in mouse erythroleukemia cells during differentiation. They have cloned and sequenced new members of the lambda family of genes, and have found a completely new set of genes that are not linked to the previously known genes on human chromosome 22. This will allow a molecular evolutionary picture of this lambda family of genes, and provide potential new targets for finding gene rearrangements in human lymphomas.

Dr. Kuehl's laboratory has studied the structure and function of the myb gene during the development of mouse lymphomas in B-cell development. This is one of the first detailed studies of the structure and expression of this gene being carried out. Dr. Segal, with Dr. Kuehl, has established transvection techniques for introducing genes into a variety of differentiated cell types, including lung cancer cells and mouse erythroleukemia cells. She is defining the controlling and enhancing regions of genes needed for the gene expression after transvection. This should provide powerful new tools for studying the expression of genes in a variety of different cell types, and the influence of genes on the behavior of their cellular biology. An excellent example of this is the effect of transvecting the c-myc oncogene into lung cancer cells that do not express this oncogene. The presence of the gene dramatically changes the phenotype and growth characteristics of human small cell lung cancer.

In summary, the NCI-Navy Medical Oncology Branch has integrated its clinical and laboratory research. The clinical protocols are built around the discoveries in the cell biology and molecular genetics labs. In addition, both cell biology and molecular genetics are being used to establish new ways to diagnose, treat and study the biology of human malignancy.

Pediatric Branch

A. Clinical Studies

1. In our study of acute lymphoblastic leukemia, we have 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, 176 patients have been randomized on this study: 58 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 approximately 75% at two years for the entire study group. With a median duration on study of 43 months, there is no significant difference in the CNS relapse rate for either treatment group. Long-term follow-up evaluation of neurotoxicity (by CT scan, neuroendocrine evaluation and psychometric testing) is underway. These data indicate 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.

   The results of the above study have helped to identify a subset of patients at particularly high risk of extramedullary relapse. Based on these observations and their overall treatment results, two new treatment studies for patients with acute lymphoblastic leukemia have been initiated. The first, a protocol specifically designed to treat high risk patients, involves the use of an aggressive, early intensification phase of therapy and an intensified systemic maintenance therapy, together with additional CNS specific chemotherapy. The second protocol is designed to treat patients in the average risk category and involves a randomization to one of two forms of CNS preventive therapy--either high dose systemic methotrexate infusions or intrathecal methotrexate alone.

2. Relapse during maintenance therapy remains a major reason for failure in children with ALL. Studies on the bioavailability of 6-mercaptopurine (6-MP) and methotrexate have demonstrated demonstrated poor and variable bioavailability of these agents following oral administration, raising the question of whether oral maintenance chemotherapy for patients with ALL is optimal. These observations have formed the basis for a newly instituted primary ALL protocol which will attempt to correlate the results of prospective periodic pharmacokinetic bioavailability studies with relapse rate and remission

duration. To date, approximately 35 patients have been entered into this planned multi-year study.

3. Our in vitro observations regarding the optimal cytotoxic concentration of 6-MP, together with our data regarding the poor bioavailability of this agent when administered orally, led us to the development of a Phase I trial of intravenous 6-MP administered by prolonged infusion. This mode of administration reduces inter-patient variability of drug levels and achieves therapeutic levels of 6-MP in the CSF. Following successful completion of this Phase I study and identification of a safe infusion dose rate, we have embarked upon three separate Phase II studies evaluating this approach to treat pediatric patients with brain tumors, with solid tumors, and with refractory acute lymphoblastic leukemia.

4. We have successfully completed a pediatric Phase I study of Tiazofurin. A total of 22 patients were entered onto this study in which the drug was given daily for five consecutive days at three-week intervals. A maximally tolerated dose of 2200 mg/m^2/day was identified. This dose has been suggested for future Phase II studies. We have instituted two additional Phase I studies. Trimetrexate, a non-classical folate antagonist, is being studied on a weekly I.V. bolus schedule in pediatric patients. To date, 8 patients have been entered on this study and no dose limiting toxicities have as yet been identified. We are also performing a Phase I study of Spirohydantoin Mustard in pediatric malignancies. This drug is being given intravenously on a weekly x 3 schedule. To date a total of 7 patients have been entered on this protocol. No dose-limiting toxicities have been observed at the doses utilized thus far.

5. Utilizing our previously described subhuman primate model for studying CSF pharmacokinetics, we demonstrated the feasibility of administering a newly developed aziridinyl benzoquinone (AZQ) by intrathecal and intraventricular injection. We developed an ongoing Phase I-II trial of intraventricular AZQ in pediatric patients. To date, 12 patients have been entered onto this study and 3 patients have attained complete remission. The intraventricular therapy with this agent appears to offer promise for patients with refractory meningeal disease.

6. We have established a late effects team to aid in the evaluation of the long-term effects of antineoplastic therapy in children. Recent studies in this area have demonstrated that 1) CT scan abnormalities may first appear as late as 8 years from the time of initiation of CNS prophylaxis 2) measurement of basal pulsatile growth hormone output is a sensitive indicator of hypothalamic-pituitary dysfunction in leukemic children who have received CNS irradiation, and 3) the use of a multidisciplinary approach to study late effects permits a comprehensive evalution which facilitates the rehabilitation of effected children.

7. Our primary study (77-C-145) for patients with undifferentiated lymphomas (both Burkitt's and lymphoblastic types) employs alternating cycles of a high-dose methotrexate infusions with CHOP, administered on approximately 10-day intervals without delays for neutropenia. Our analysis of 85 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, 12 of 13 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. On the basis of this information two new pilot protocols for patients with undifferentiated lymphomas have been initiated. One is a study of a new drug combination in relapsing patients (ifosfamide, VP16 and high dose ara-C). The other is an intensified version of protocol 77-C-145. If the new drug combination proves to be active in relapsed patients, a protocol incorporating this combination for very high risk, untreated patients, will be developed. Patient numbers are too small at present for further comment.

8. We have initiated 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. This protocol combines high dose chemotherapy during induction (emphasizing intensive adriamycin) in combination with cyclophosphamide and vincristine. Following induction, patients undergo high dose total body irradiation (800 rads) in conjunction with autologous bone marrow reconstitution. To date, 59 patients have been enrolled in this protocol and the results suggest that early intensive therapy is well tolerated and highly effective (93%) in achieving a successful induction. Evaluation of the bone marrow transplant component of this protocol is currently underway.

9. 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, we randomized patients to either our conventional combination of cephalothin, gentamicin, carbenicillin (KGC) versus a new third generation cephalosporin, ceftazidime (CTZ). To date, 608 granulocytopenic episodes have been randomized to either antibiotic regimen when they become febrile. This represents the largest clinical trial done in this area. The initial response during the first 72 hours was evaluated according to whether the patient had an unexplained fever (FUO) or a documented infection: For patients with FUO, 98% of patients treated with KGC or CTZ were successfully treated; for patients with documented infection, the initial response was 98% for

KGC and 97% for CTZ patients. The overall response, at the resolution of the granulocytopenia was 98% for the FUOs randomized to KGC and CTZ and 91% for the patients with documented infections randomized to KGC vs. 89% for documented infectious patients randomized to CTZ. Thus, monotherapy may be as successful as combined therapy, particularly for initial period of empiric management. Overall, our results are superior to any other reported to date.

10. We have contributed 16 patients of the 36 eligible randomized patients on the Pediatric Oncology Group multi-institutional osteosarcoma study which randomized patients with totally resected, high-grade, extremity osteosarcoma between no chemotherapy and immediate adjuvant chemotherapy using first-line agents. This study has demonstrated statistical superiority of the immediate chemotherapy group in time to first relapse, although there is no survival difference to date between the two arms. The results of this crucial study will form the basis of future trials.

11. We have initiated a series of studies looking at pediatric pain measurement techniques, and epidemiologic and therapeutic aspects of pain in children with cancer. The epidemiologic studies are detailing firstly the incidence, duration and nature of pain in newly-diagnosed cancer patients, and secondly the predictive factors and nature of phantom limb pain and sensations in amputees. The therapeutic studies have documented dosage, toxicity and kinetic data of morphine given via the continuous intravenous or subcutaneous routes in 26 patients. Ongoing therapeutic studies are examing firstly the efficacy and kinetics of continuous intravenous or transdermal infusions of fentanyl in children with cancer experiencing pain, and secondly the efficacy and feasibility of administration of nitrous oxide in children undergoing painful diagnostic or therapeutic procedures.

B. Pre-Clinical Studies

1. In our sub-human primate model for CSF pharmacokinetics studies, we have recently evaluated 3 agents of potential utility in treating meningeal malignancy. Tiazofurin (TCAR), a C-nucleoside which produces guanine nucleotide depletion by the inhibition of inosine monophosphate dehydrogenase, was demonstrated to have excellent penetration into the CSF following IV administration. This drug is currently being studied in Phase II clinical trials. Studies of Trimetrexate, another non-classical antifol which has been demonstrated to be active in vitro against leukemic cells resistant to methotrexate, has been studied for its CSF pharmacokinetics. Its enhanced lipophilicity holds the prospect for using this agent in the treatment of meningeal leukemia or meningeal carcinomotosis. We have also studied the pharmacokinetics of intravenous thiotepa. This latter agent has been used with extremely limited success via the intra-CSF route of administration. Our studies have demonstrated for the first time that substantial amounts of its metabolite, TEPA, are present in the cerebrospinal fluid for prolonged periods of time

following intravenous administration, suggesting that this route of administration may be a more optimal one to approach CNS disease with this agent. A clinical trial of this approach will be instituted shortly.

2. To study mechanisms responsible for clinical resistance to 6-MP, leukemic cells were obtained from 10 patients with ALL at diagnosis and on the same patients at the time of their initial marrow relapse. Four of these patients had biochemical evidence of 6-MP resistance in relapse. Three of four patients had a greater than 50% decrease in intracellular HPRT activity, four of four had a greater than 50% increase in intra-cellular PRPP, and two of four had a greater than 9-fold increase in intracellular alkaline phosphatase activity at relapse. These results indicate that clinical resistance to 6-MP may be related to alterations in HPRT, PRPP or alkaline phosphatase activity. These findings may have implications for the manner in which 6-MP is given during ALL maintenance therapy.

3. We studied the pharmacokinetics of Trimetrexate in rhesus monkeys and found that Trimetrexate is characterized by a slower clearance rate than methotrexate, by poor CSF penetration and elimination primarily by metabolism. In addition, we have identified two new active metabolites of Trimetrexate both in plasma and urine. These metabolites are currently being identified. Knowledge of their existence may be of value in the design of Phase I and Phase II studies of this agent in man.

4. We have begun to explore the association of c-myc and Cμ expression in Burkitt's lymphoma cells and lymphoblastoid cell lines treated with retinoic acid and ethanol. Preliminary results suggest that expression of these two genes is not always concordant in Burkitt's lymphoma cells, and that whereas in B-cells without a chromosomal translocation, c-myc expression is diminished during differentiation, this is not the case in Burkitt cells. These findings have implications for the regulation of the c-myc gene in Burkitt's lymphoma cells.

5. We have developed an in situ hybridization technique, using a biotinylated probe, able to demonstrate the presence of EBV genomes in individual cells. We shall be exploring the utility of this technique in detecting the presence of EBV DNA in Burkitt tumors from various parts of the world.

6. We have determined that $pp60^{c\text{-}src}$ molecules bound to the polyoma virus middle T antigen in polyoma virus transformed rodent cells are phosphorylated on tyrosine residues close to the aminoterminal end of the molecule. This post-translational modification is likely to be closely associated with the enhanced $pp60^{c\text{-}src}$ tyrosyl kinase activity which we previously demonstrated to occur during polyoma virus transformation of cells. We have also found that the level of $pp60^{c\text{-}src}$ tyrosyl kinase activity is high in neuroblastoma and dramatically altered in other human tumors. Activated $pp60^{c\text{-}src}$

tyrosyl kinase molecules found in human neuroblastoma also bear a N-terminal phosphorylation indistinguishable from that found in polyoma transformed cells. However, activated $pp60^{c\text{-}src}$ in other human tumors is not modified in this manner suggesting the possibility that this enzymatic activity may be modulated by multiple molecular mechanisms.

7. We have found that the rcp(11;22)(q24;q12) translocation of Ewing's sarcoma and peripheral neuroepithelioma also characterizes Askin's tumor of the chest wall. Subsequent analysis of this tumor has revealed it to be indistinguishable from peripheral neuroepithelioma and provides a rationale for treatment of these patients, whose clinical course also parallels that observed in patients with Ewing's sarcoma, with therapy which is effective treatment for this tumor. Molecular studies of tissue from these tumors has allowed us to place the translocation breakpoint in these tumors at a site distal to the lambda light chain gene locus but proximal to the site of the c-sis proto-oncogene on the long arm of chromosome 22. We are currently characterizing other molecular clones in order to more precisely describe the breakpoint site and initiate an evaluation of the physiologic alterations resulting from this chromosomal rearrangement.

Radiation Oncology Branch

The three major goals of the ROB are: 1) major emphasis on clinical trials of a combined modality nature, predominantly collaborative with other clinical branches; 2) strong radiation-biology program with heavy emphasis on basic science, radiologic physics and questions of clinical relevance; 3) a training program in radiation therapy, equivalent in stature to the programs of training in medical, surgical and pediatric branches within the NCI.

Concerning the training program, approval has been obtained from the AMA Residency Review Committee for the Uniformed Services University of Health Sciences, working through the NCI, as well as Walter Reed Army Medical Center and the National Naval Medical Center in Bethesda. We have a three year program approved for the residency in Radiation Oncology, directed by Dr. Eli Glatstein. Half of the time is spent within the NCI and half within the military complex. This integrated program is required because of the complementary nature of the clinical material at the various hospitals, with GYN, head and neck and GU cancers in abundance at the military hospitals, whereas they are virtually completely lacking within the NCI. Each year we take four individuals for training, two of the positions being reserved for military personnel.

The clinical program within the ROB is centered on combined modality studies. Most of these are in collaboration with other branches. The most important of these are combined modality studies on small cell carcinoma of the lung, and on mycosis fungoides, both of which are in collaboration with the NCI-Navy Medical Oncology Branch. The findings in small cell carcinoma of the lung are strongly suggestive of benefit of combined modality treatment

for limited stage patients over chemotherapy alone. A number of interesting observations have been made which will be reported by the Navy Medical Oncology Branch. There are also important collaborative studies going on with the Surgery Branch in the soft tissue sarcomas, and also with the Pediatric Oncology Branch in areas of pediatric sarcomas. These latter pediatric studies appear to be extraordinarily promising, and if they continue to hold up they will represent a major step forward in the management of these patients. Again, the early findings will be reported by the POB rather than the ROB. There are also studies with the Medicine Branch in lymphomas and Hodgkin's disease.

Primary ROB studies center around intraoperative radiation therapy. A new treatment base/operating room has been opened, and the Microtron has been used for intraoperative therapy. Large single doses of electron beam treatment are applied intraoperatively to the tumor bed with critical normal viscera moved out of the way. There is also a dog program going on concurrently to determine tolerance levels. These programs represent major integration of surgery and radiation therapy in a cooperative way. Gastric cancer, retroperitoneal sarcomas and other retroperitoneal problems are under investigation on randomized studies; in addition, a study has just begun on locally advanced lung cancer utilizing intraoperative radiotherapy.

Another major ROB study centers on Stage I and II breast cancer. In this randomized study, radical surgery is compared to definitive irradiation with preservation of the breasts following a lumpectomy. This study was originally organized by Dr. Allen Lichter, but following his departure to the University of Michigan, it is now headed by Dr. Peggy Findlay. There are now over 190 patients randomized, despite the very difficult randomization. In the first six years of this study, there is no obvious superiority of either arm, suggesting that the long-term results of treatment will be comparable. This study differs from the studies of Dr. Fisher of the NSABP in that the surgical excision makes no attempt to make the surgical margins negative but just simply remove the lump. Cosmesis is a major endpoint in addition to survival and freedom from relapse. In addition, the protocol is also open to patients who have masses up to 5 cm. This makes this approach of breast preservation applicable to the vast majority of patients who present with breast cancer initially. All patients with positive nodes receive adjuvant chemotherapy, regardless of the primary treatment.

Another area of intense clinical investigation has been that of radiosensitizers, with special attention directed to halogenated pyrimidines, specifically BUdR and IUdR. BUdR Phase I studies have been completed and unequivocal radiosensitization was demonstrated in human cells and human patients. However, hematologic and especially cutaneous toxicity due to photosensitivity limited the applicability of intravenous BUdR. We were able to demonstrate here that IUdR was an equivalent to radiosensitizer but far less of a photosensitizer. Consequently, we have now completed our first Phase I study of intravenous IUdR with special attention in unresectable sarcomas and gliomas. The glioma information is difficult to interpret thus far, but we have seen some striking regressions of unresectable sarcomas and, indeed, have five unresectable masses that have gone away completely with IUdR and radiation in a Phase I study. We have additionally planned

Phase I investigations to carry out with this compound, mostly seeing if it can be potentiated by having other agents which eliminate de novo thymidine synthesis. Ultimately, it is our aim to take patients with unresectable sarcomas and gliomas and randomize them to receive the radiosensitizer program or not, in conjunction with twice a day radiation fractionation. Under the direction of Dr. Jan van de Geijn our CT scanning has been fully incorporated into our radiotherapeutic treatment planning. Virtually all patients treated with curative intent are scanned in the treatment position and computerized treatment plans are routinely generated, superimposed on CT cross-sections. The program allows for adequate dose calculations, even accounting for tissue inhomogeneities and blocks within the radiation field.

A final clinical program of interest is interstitial implantation for patients who have relatively small gliomas. In this study, selected patients with lesions that are appropriately located will undergo radioactive interstitial implantation therapy inserted stereotactically in conjunction with the Surgical Neurology Branch of the NINCDS.

In the laboratory, our major emphasis has been on mechanisms of sensitization and protection, resulting from radiation modifiers, and also investigation of the mechanism of the action of several different chemotherapeutic agents. Our interests have centered on sulfhydryl compounds, especially glutathione, its relationship to cell killing or protection, either by radiation or chemotherapy. Additional work has gone on in heat shock proteins and the characterization of human tumor cell lines (in conjunction with other branches). An embryonic program in molecular biology is also going on through Dr. Fornace, whose chief work is aimed at trying to clone heat shock protein genes, and also attempting to clone genes involved in radiosensitivity. Radioimmunoglobulin work has been going on through Dr. Gansow. His work is focused on pursuits of various chelates and being able to attach the chelate with radioactive heavy metals to immunoglobulin. We are especially interested in alpha emitters as a potential means of delivering radiotherapy. There is also a large dog program run in conjunction with the Surgery Branch, in which we are investigating the tolerance of various organs to intraoperative radiation therapy.

The ROB has a major research program exploring the use of phototherapy (coupled with hematoporphyrins) in the therapy of cancer. The goal of this research is to learn whether non-ionizing photons can be harnessed to treat cancer.

The key lab observations in the past year center on (1) the demonstration that cells which are pleiotropically resistant to drugs have, at least in most cases, no resistance to radiation, (2) the remarkable efficacy of phototherapy to cure mice with advanced ovarian cancer in the abdomen, and (3) lympholysis in animals treated with phototherapy.

Surgery Branch

Laboratory efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients. The most significant laboratory accomplishments of the Surgery Branch in the last year are as follows:

1. Therapy with lymphokine activated killer (LAK) cells and recombinant interleukin-2 has been shown to effectively treat established pulmonary and hepatic metastases in a variety of animal tumor models.

2. The mechanisms of the therapeutic effectiveness of LAK cells plus IL-2 has been shown to be due to the in vivo expression of transferred LAK cells.

3. Techniques have been established for the isolation of lymphoid cells with specific anti-tumor activity obtained from cells infiltrating growing murine and human tumors.

4. The exact phenotypes of the precursor and effector LAK cells in the mouse and human have been elucidated.

5. The toxicity of recombinant IL-2 administered to rodents has been defined.

6. Monoclonal antibodies have been produced that recognize antigens newly expressed by NIH 3T3 fibroblasts transfected with oncogenes from human tumors. These cell surface antigens result from the transformation of these lines and may be useful in defining the mechanism of oncogene-related transformation.

7. Several hamster animal models of ductal adenocarcinoma of the pancreas and several lines of human pancreatic cancer have been established as experimental models.

8. Several monoclonal antibodies with specificity for pancreatic and other gastrointestinal cancers have been developed and are currently being tested for reactivity in animal models and against panels of human tissues.

9. Monoclonal antibodies conjugated with alpha-emitting heavy metal radionuclides have been developed and successfully used to localize pancreatic cancers in tumor-bearing experimental animals and to destroy pancreatic cancer cell lines in vitro.

10. Studies in dogs have defined the tissue tolerance of various normal and surgically manipulated tissues to intraoperative radiation therapy and have enabled dose guidelines to be established for therapy in human patients.

11. Insulin treatment can reverse cancer cachexia in rats, improve host body composition, and survival following tumor resection.

12. Adriamycin impairs wound healing in rats. Chemoattroctants and growth factors, of which transforming growth factor-B is most important, reverse the healing impairment.

13. Hepatocytes from tumor-bearing rats with small tumors exhibit increased rates of gluconeogenesis. Lactic acid may be the cause of this abnormal energy-wasting metabolism.

14. The cytoplasm of human breast cancer cells has been found to contain several molecular weight species of the estrogen receptor. The distribution of molecular weight species from cell lines of differing hormone sensitivity are being studied.

15. The pineal gland hormone melatonin has been found to alter the growth of human breast cancer cells in vitro and in vivo. The plasma hormone melatonin has been found to alter the steroid binding and the nuclear binding of the estrogen receptor in human breast cancer cells.

16. A parathyroid hormone-like factor which causes bone resorption and is produced by human prostate carcinoma has been identified. This factor may provide a more basic understanding about the effect of prostate carcinoma on calcium metabolism in humans and may further our understanding of the pathophysiology of metastatic prostate carcinoma.

17. A prostate tumor derived growth factor which stimulates osteoblastic cells is being evaluated. This factor may have an autocrine effect and should further our understanding of the effect of prostate carcinoma on bone and other tissues.

18. The usefulness of a substance which inhibits 5-alpha reductase has been found to inhibit the growth of human prostate carcinoma in nude mice. This substance may provide a new direction for the treatment of metastatic prostate carcinoma in humans.

Clinical efforts in the Surgery Branch continue to emphasize combined modality treatments.

1. The successful regression of established cancer metastases in humans has been accomplished using therapy with lymphokine activated killer cells plus recombinant IL-2.

2. Clinical trials have been completed defining the immunologic effects of the administration of RIL-2 to cancer patients. Significant findings have been 1) a dramatic increase in IL-2 receptor bearing cells, 2) a 2-12 fold expansion of lymphoid cells in vivo and 3) early migration of IL-2 precursors out of the peripheral blood following IL-2 administration.

3. Clinical trials of recombinant IL-2 have been completed evaluating a range of doses administered intravenously or intraperitoneally, which define dose limiting toxicity. Unusual side effects including pronounced eosinophilia and weight gain have been reported.

4. Prospective randomized trials have demonstrated that adjuvant chemotherapy improves disease-free and overall survival in patients with high grade soft tissue sarcomas of the extremities. "Short" course chemotherapy is as effective as our previously used "long-course" treatment.

5. Prospective randomized trials have demonstrated that limb-sparing surgery is the equivalent of amputation in the treatment of patients with high grade soft tissue sarcomas of the extremities. Disease and overall survival rates in both groups are the same.

6. Analysis of eight years experience with the resection of pulmonary metastases in patients with osteogenic and soft tissue sarcomas has been completed. Improved survival resulting from resection of these metastases has been demonstrated and factors that determine prognosis in these patients has been defined.

7. A prospective randomized trial evaluating neoadjuvant chemotherapy for epidermoid carcinoma of the esophagus is in its third year. To date, 34 patients have been randomized.

8. A prospective randomized trial has demonstrated that local excision, axillary dissection and primary radiation therapy is as effective as mastectomy in the treatment of stage I, II breast cancer in women. Further follow-up is necessary.

9. A prospective randomized trial has demonstrated that the psychosocial aspects of primary radiation therapy for breast cancer are well accepted and relieve many of the emotional problems associated with mastectomy.

10. A prospective trial has demonstrated the importance of a thorough dissection of the axillary lymph nodes for staging and prognostic purposes in patients with breast cancer.

11. Eleven patients have been evaluated in a protocol designed to test the use of radionuclide coupled antibodies to melanoma injected subcutaneously for the evaluation of nodal disease. 3/3 true negatives and 1/8 true positives were found using this technique. Further work using whole antibodies are planned.

12. A prospective randomized trial comparing regional vs systemic FUdR continuous chemotherapy via the implantable pump for the treatment of colorectal hepatic metastases is continuing. To date, 36 patients have been accrued; no significant differences in overall survival has been seen.

13. Phase I-II studies of intraoperative radiotherapy in various advanced-stage malignancies have been proceeding for five years, providing information on efficacy of local tumor control, treatment toxicity, and tissue radiation tolerance. A dedicated intraoperative radiotherapu suite combining a fully-equipped operating room with a high-energy linear accelerator has been opened and is being used for patient treatment.

14. Various technical innovations for intraoperative radiotherapy have been developed, including treatment table, beam applicator system, monitoring devices, and procedure protocols.

15. The only prospective randomized protocols evaluating intraoperative radiation therapy that are being performed at any institution are entering their fifth year. Approximately 90 patients have been given intraoperative radiation therapy since the program was initiated. Randomized trials are proceeding in patients with pancreatic cancer, gastric cancer, and retroperitoneal sarcomas.

16. A prospective randomized trial has been completed demonstrating the efficacy of low molecular weight povidone-iodine solution in preventing intra-abdominal abscesses following surgery in the face of bacterial contamination causing peritonitis.

17. Patients with estrogen receptor or progesterone receptor positive breast cancer have been found to have lower plasma levels of melatonin than normal subjects or ER- or PR- patients. A significant inverse correlation is present between plasma melatonin and the receptor content of breast cancer.

18. An evaluation of tetracycline introduced into the axillary space at the time of axillary dissection has been carried out and demonstrated to be efficacious in decreasing amount and days of drainage through drainage catheters. Although efficacious, significant problems with decreased range of motion in the shoulder precludes its wide spread use. A prospective evaluation of its use in sarcoma patients is being considered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07200-03 CO

PERIOD COVERED

October 1, 1984 - September 30, 1985

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

Pathogenic Human Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Samuel Broder, M.D., Associate Director, COP, DCT, NCI

Associate Investigators - listed on next page

COOPERATING UNITS *(if any)*

Clinical Pharmacology Branch, COP, DCT, NCI
National Institute of Allergy and Infectious Diseases
Litton Bionetics, Kensington, Maryland

LAB/BRANCH

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

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Introduction - Project A

The Clinical Oncology Program is the intramural treatment-research arm of the National Cancer Institute. In recognition of the assignment of acquired immune deficiency disease (AIDS) as the number one priority of the DHHS, a number of research projects within the laboratory of the Office of the Associate Director have been initiated to study human T-lymphotropic virus type III (HTLV-III) and the distantly related pathogenic human retroviruses broadly grouped into what is called the HTLV family of viruses.

HTLV-III (also called LAV) is the etiologic agent of AIDS and the studies in the OAD are exploring new strategies for pharmacologic interventions against the etiologic pathogen of this disease. These studies are also exploring how viruses in the HTLV-family can perturb immune reactions even in settings where they do actually destroy cells with immune potential.

During the past year the OAD has focused on the development of new anti-viral agents and the implementation of such agents in clinical trials. We have conducted a feasibility study using one such agent - suramin - to see if it is possible to suppress the replication of HTLV-III in vivo. Suramin was selected because it had been shown to inhibit the reverse transcriptase of animal retroviruses in 1979, and because it had been used in human beings (for a

Associate Investigators:

Hiroaki Mitsuya, M.D., Expert, OAD, COP, DCT, NCI
Shuzo Matsushita, M.D., Visiting Associate, OAD, COP, DCT, NCI
Robert Yarchoan, M.D., Investigator, OAD, COP, DCT, NCI
Mary Megson, Biologist, OAD, COP, DCT, NCI
Anthony Fauci, M.D., Director, NIAID
Clifford Lane, M.D., Senior Investigator, NIAID
Phillip Markham, M.D., Investigator, Litton Bionetics, Kensington, MD
Charles E. Myers, M.D., Chief, CPB, COP, DCT, NCI
Jerry Collins, Senior Investigator, CPB, COP, DCT, NCI
Raymond Klecker, Chemist, CPB, COP, DCT, NCI

totally different reason) for many decades, thus obviating the need for pre-clinical testing. This approach may provide a model for studying other agents.

We have previously shown that suramin inhibits the in vitro infectivity and cytopathic effect of HTLV-III, the human T-cell tropic retrovirus linked to the etiology of the acquired immunodeficiency syndrome. There is considerable clinical experience with suramin as a treatment for Rhodesian trypanosomiasis and onchocerciasis, and this observation prompted us to recommend that suramin be considered as an experimental agent in the therapy of HTLV-III infection and to initiate a short-term trial of suramin in patients with AIDS or AIDS-related complex (ARC). The primary goal of this trial was to determine the feasibility of giving a six-week course of suramin to patients with HTLV-III infection. We can discuss our findings related to the clinical pharmacology, toxicity, and anti-viral effects of suramin in this pilot trial. These studies represent a collaborative effort involving the Clinical Pharmacology Branch, the Laboratory of Tumor Cell Biology, and the Laboratory of Immunoregulation, the Walter Reed Army Medical Center also cooperated in these studies.

Patients and Methods

Ten patients were entered into the current pilot study. The patients were homosexual or bisexual white males whose ages ranged from 23-53 years. Six patients had AIDS with Kaposi's sarcoma and four patients had ARC (either oral thrush or generalized lymphadenopathy with persistently decreased OK T4 cells). All patients were seropositive for HTLV-III and gave their informed consent to participate in the trial. We detected HTLV-III replication in cultures of phytohemagglutinin (PHA) activated peripheral lymphocytes derived from five patients prior to therapy as previously described, and in these individuals we followed viral replication in cultures of patients' PHA-activated lymphocytes as an index of drug effect.

Suramin was administered intravenously, and the regimen was comparable to one used in the therapy of onchocerciasis. In most cases, a total dose of 6.2 gm was administered: a test dose of 200 mg was administered over 20 minutes on day 0, followed by one-gram doses on days 3, 7, 14, 21, 28, and 35.

Heparinized plasma samples were obtained for determination of suramin levels before each dose and one the day following each dose. After the last dose, samples were obtained at intervals of one week or longer. Urine samples (24-hour collections) were obtained in two patients following the cessation of therapy.

Plasma suramin levels were determined using a high pressure liquid chromatographic (HPLC) procedure. Plasma half-life was determined for the mono-exponential disappearance following the end of therapy.

RESULTS

Side-Effects

All patients completed the full dose regimen of drug; however, in a few patients individual doses were reduced and/or delayed. Fever and photosensitizing erythematous drug eruption were the most common physical side-effects observed during the trial (7/10 each). These side-effects most commonly occurred between the second and third week of therapy. In all cases, these findings were self-limited and disappeared with continued administration of the drug, although one patient was given intravenous fluid support during his febrile reaction. Also, 3/10 patients developed a burning sensation of the skin, particularly involving the extremities, immediately after the administration of the third and fourth doses.

The most common laboratory abnormalities were low-level proteinuria (7/10), microscopic pyruia (6/10), trace hemoglobinuria (5/10), and occasional granular casts (2/10); these urinary abnormalities generally persisted during the treatment but then resolved. Creatinine clearance was not affected in any of the patients. Elevations of transaminases occurred in 6/10 patients, usually during the second and third weeks; these elevations did not correlate with plasma suramin levels and resolved with continued administration of the drug. Finally, mild eosinophilia (maximum 14%) was observed in three patients during their drug eruption. No evidence of bone marrow suppression was observed in any of the patients.

Clinical Pharmacology

We found that suramin accumulated during the five weeks of administration (loading phase), and then diminishes with a very long half-life (>40 days following the last dose. We observed that 99.7-99.8% of the drug is bound to proteins in the circulation. The half-life appears to be shorter during the initial loading phase. It was not possible to accurately describe two-compartment behavior since multiple doses were given in this study. The mean half-life, which incorporates both the loading and elimination phases, was 43 days. The initial volume of distribution is more than twice the initial volume. Total body clearance, which reflects all elimination processes, was less than 0.5 ml/min for all patients. Renal clearance of 0.38 ml/min was observed in

one patient, and 0.3 ml/min was observed in a second patient. Thus, the renal clearance accounts for essentially all drug removal from the body. (This conclusion is consistent with the lack of metabolites observed in chromatograms of patients' plasma in our study.) Although the overall removal of suramin from the body is very slow, the renal clearance of unbound suramin is actually quite efficient. If the glomerular filtration rate is 120 ml/min and only 0.3% of suramin were available for filtration, then the maximum filtration rate would be 0.36 ml/min.

Effects on HTLV-III Replication In Vivo

We detected HTLV-III replication in primary cultures of extensively washed peripheral blood lymphocytes obtained from four patients prior to therapy. These patients in effect represent a special subset of persistently viremic patients. HTLV-III replication was detected by measuring the reverse transcriptase activity released in to the supernatants by activated lymphocytes in culture. In each case, the amount of virus found in the patient's lymphocytes decreased, and at the time of maximal drug concentrations, viral replication became undetectable in three of the four cases. However, in the weeks following the cessation of therapy, viral replication was again detected in three patients. Patients 2 and 3, who had the most severe fevers and rashes early in the course of their suramin administration, also transient elevations of detectable virus during that period; the significance of that observation is not clear at this time. We then re-administered suramin to a patient whose viremia had cleared while on therapy but re-appeared after the drug was stopped. The re-administration of suramin once again cleared the patient's viremia once levels >100 mg/ml were reached in the circulation.

Effects on clinical and immunologic parameters

During this study, we did not observe a significant improvement in the clinical condition of the patients, and there was no consistent change in any of the immunologic parameters followed. In the patients with Kaposi's sarcoma, there was no change in any of the lesions in 4 individuals, slight increase in the lesions in the other two. One patient (No. 3) who had a marked decrease in Leu 3+ (OKT4+) T cells and oral candidiasis prior to therapy, developed Pneumocystis pneumonia after the completion of the therapy. All patients were alive and stable at the time of this writing (one to nine months following the completion of therapy). Total Leu 3+ cells fell by 50% in one patient (No. 2), increased by 50% in one patient (No. 4), and were essentially unchanged in the other patients.

Discussion

All retroviruses require an enzyme called reverse transcriptase in their natural cycle of replication, and therefore, agents which inhibit this enzyme are logical candidates as experimental agents for the therapy of HTLV-III

infections. In 1979, de Clercq reported that suramin--a drug used to treat African trypanosomasis and onchocerciasis--was a competitive inhibitor of the reverse transcriptase of a number of animal retroviruses, and we have recently confirmed that his agent can inhibit reverse transcriptases of diverse retroviruses, including that of HTLV-III (L. Arthur, P. Fischinger, R. Gilden el al, unpublished observation). Suramin can also protect T cells in vitro against the infectivity and cytopathic effect of HTLV-III; these protective effects, however, may well occur by a mechanism other than the inhibition of viral reverse transcriptase. The precise mechanism of action is not crucial to the analysis of drug effects at this time.

The side-effects of suramin observed in our study are similar to those reported in a similar regimen used to treat patients with onchocerciasis. Thus, we did not encounter unexpected toxicities or adverse reactions in administering the drug to patients with AIDS, as has been the case with trimethoprim/ sulfamethoxazole. Moreover, the drug inhibited detectable viral replication in these patients during therapy, and in this sense appears to be virustatic for HTLV-III in vivo. In each of the four evaluable patients, the capacity to detect virus was abolished or reduced at the time of peak circulating suramin concentrations. Similar virologic effects have been observed by Saimot et al. (AG Saimot, personal communication). These effects are not likely to result from a lack of suitable cells of viral infectivity and replication as there was little or no change in the levels of Leu3+ (OKT4+) T cells in (four) of these patients. (Also, patient lymphocytes could be infected by and support the replication of exogenously added HTLV-III virions in vitro.) In collaboration with Drs. Ruth Ruprecht and William Haseltine, we observed that suramin can suppress replication of the murine T-cell tropic virus SL3-3 and other murine retroviruses in vitro and in vivo. These observations imply that suramin's activity is not limited to one viral system.

Although we observed an effection viral replication in patients receiving suramin, we did not observe significant clinical or immunologic improvement using the current regimen. It is possible that higher doses or more prolonged administration of the drug will be necessary in order to bring about a clinical response. (In this regard, there are preliminary data from Rouvroy et al. in Rwanda that a more intensive regimen of suramin brings about clinical and immunologic responses in patients with clinical features of ARC.) It is also possible that HTLV-III infection in certain settings causes stem cell damage or autoimmume phenomena which prevent the return of normal immune function without the requirement for further viral replication per se. It may, therefore, be necessary to administer anti-viral agents early in the course of HTLV-III infection in order to observe the best clinical results.

We believe the results of this trial provide a rationale for a more prolonged period of experimental suramin therapy to learn whether the drug induces sustained viral inhibition and clinical improvement. (The pharmacokinetic data indicate that after an initial loading course of therapy, it should be possible to maintain a steady state level by administering maintenance dose 0.5 gm every week.) Also, once a virustatic concentration of suramin is achieved in a patient, it might be worthwhile to explore the addition of a T-cell

stimulating agent such as interleukin-2 to the experimental regimen. It should be stressed, however, that while there is considerable experience with the regimen of suramin used in the current report, higher doses may be associated with additional degrees of toxicity. We, therefore, recommend that the evaluation of alternate experimental regimens of suramin to patients with HTLV-III infection be undertaken only in centers with medical, pharmacologic, and immunologic and virologic support to adequately study and monitor these patients.

Project B

At the same time that our in vivo studies with suramin were underway, we initiated a crash program to screen new anti-viral agents using a rapid-assay system. This system involves the use of a highly susceptible T cell clones which die within a few days after exposure to HTLV-III in vitro. Over 100 compounds have been screened for anti-retroviral activity in this system. One such agent will be discussed below.

We report the capacity of a new thymidine analogue, BW A509U to inhibit HTLV-III/LAV replication, and to block the cytopathic effect of HTLV-III in vitro under a variety of conditions. This is an agent provided by the Burroughs-Wellcome Company for screening against HTLV-III/LAV. The project described is illustrative of the kinds of drug-development efforts that are possible in the intramural program.

Materials and Methods

HTLV-III$_B$ was obtained from the culture supernatant of HTLV-III$_B$- producing H9 (H9/HTLV-III$_B$) cells. The virus was prepared to contain approximately 10^{10} virus particles per ml. In some experiments, irradiated (10,000 rad) H9 cells, producing a Haitian variant of HTLV-III/LAV (HTLV-III/RF-II), were used as a source of infectious virions. The Haitian variant differs from the several closely related virus genotypes in H9/HTLV-III$_B$ cells about 20% in the amino sequence of its envelope gene.

Nucleoside analogue

A nucleoside analogue, (BW A509U) (M.W. 267.24), was synthesized as described by Lin and Prusoff. This compound is referred to as A509U in this report. In collaboration with scientists at Burroughs-Wellcome Research Laboratories, we found that A509U is a relatively specific inhibitor of reverse transcriptase of HTLV-III/LAV as a triphosphate; but the unphosphorylated compound does not inhibit reverse transcriptase per se (unpublished data).

Cells

Clone H9 is an OKT-4+ T-cell line which is permissive to HTLV-III/LAV replication but partially resistant to its cytopathic effect as previously

described.

Tetanus toxoid-specific T-cell clones were generated as previously decribed briefly, 10^6 peripheral blood mononuclear cells (PBM) isolated from heparinized blood of two normal volunteers (who had been immunized with tetanus toxoid) were cultured with 2 limit flocculation units (LF) per ml of tetanus toxoid (Commonwealth of Massachusetts Departments of Public Health, Jamiaca, Plain, MA) in 24-well microculture plates (Costar, Cambridge, MA) at 37°C in 5% CO_2-containing humidified air in 1 ml of complete medium (RPMI 1640 supplemented with 4 mM L-glutamine, $5x10^{-5}$ M 2-mercaptoethanol, 50 U/ml penicillin, and 50 ug/ml streptomycin) containing 10% autologous plasma. After 7 days in culture, the cells were restimulated with the same concentration of tetanus toxoid plus irradiated (4,000 rad) fresh autologous PBM. On day 14 and beyond, the cells were exposed to 15% interleukin-2 (Il-2) (lectin-depleted; Cellular Products Inc., Buffalo, NY) and 15% fetal calf serum (FCS) was substituted for the autologous plasma. The cells were restimulated every 7 days as described above. On day 40 in culture, the cells were cloned by limiting dilution in the presence or absence of lethally irradiated (12,000 rad) human T-lymphotropic virus type I (HTLV-I)-producing MJ-tumor cells. A rapidly growing normal T-cell clone TM3 (derived in the absence of HTLV-I from cultures plated at 0.5 cells per well) was expanded with 15% IL-2-containing complete medium. Also, fifteen rapidly growing clones were obtained from cultures plated at 0.5 cells per well in the presence of HTLV-I. One of these, clone ATH8, was selected for this study on the basis of its rapid growth (in the absence of antigen) and its exquisite sensitivity (cells die quickly in the presence of 5 virions/cell) to the cytopathic effect of HTLV-III_B. It is worth stressing that the development of ATH8 per se has opened up new possibilities in drug-screening programs in AIDS treatment research, as this clone is very suitable for mass screening systems in vitro.

Determination of HTLV-III/LAV gag Protein Expression

$2x10^5$ H9 cells were exposed to various concentrations of A509U for 4 hours, then to 2 µg/ml polybrene (Sigma Chemical Co., St. Louis, MO) for 30 minutes, pelleted, and exposed to HTLV-III_B virus (1,000 virions/cell) for 1.5 hours. Cells were resuspended in fresh complete medium and cultured in tubes at 37°C in 5% CO_2-containing humidified air. The cells were continuously exposed to A509U. On days 8, 9, and 10 in culture, the percentage of the target H9 cells expressing P24 gag protein of HTLV-III/LAV was determined by indirect immunofluorescence microscopy as previously described by using anti-HTLV-III/LAV p24 murine monoclonal antibody (M26).

Inhibition Assay for the Cytopathic Effect of HTLV-III/LAV

HTLV-III/LAV cytopathic effect inhibition assays were performed. Briefly, clone TM3 cells were stimulated by antigen plus irradiated fresh autologous PBM and cultured in 15% IL-2- and 15% FCS-containing complete medium 6 days before assay. The FCS used in these studies was not dialysed. ATH8 cells were used without the antigen stimulation. Following preexposure to 2 µg/ml polybrene for 30 minutes, the target T-cells ($2x10^5$) were pelleted, exposed

to HTLV-III$_B$ virus for 45 minutes, resuspended in 2 ml fresh medium, and incubated in culture tubes (3033, Falcon, Oxnard, Ca.) at 37°C in 5% CO_2-containing humidifed air. Control cells were treated similarly but were not exposed to the virus. The cells were continuously exposed to IL-2 and A509U. When ATH8 cells were employed in this assay system, 5-50 virus particles/cell were minimum cytopathic dose of virus. In the cell co-culture experiments, $5x10^4$ lethally irradiated (10,000 rad) HTLV-III/RF-II-producing H9 cells or uninfected H9 cells were added to $2x10^5$ target T-cells. At various time points, the total viable cells were counted in a hemocytometer under the microscope by the trypan blue exclusion method.

Assays for Reverse Transcriptase

10^6 PBM from a healthy individual which had been cultured for 7 days after phytohemagglutinin (PHA)-stimulation were exposed to HTLV-III$_B$ and cultured in the presence and absence of various concentrations of A509U. On the following day, cells were washed, resuspended in fresh medium, and cultured further with the same concentrations of the agent. On day 5 in culture, supernatants were harvested and subjected to reverse transcriptase assay as previously described. Briefly, precipitable reverse transcriptase activity was measured by using $dT_{15}.rA_n$ as the primer-template and Mg++ as the divalent cation. Results are expressed as counts per minute of methyl-[^{3}H]-deoxythymidine triphosphate (40-60 Ci/mmol) incorporated per 10 ul of concentrated (25-fold culture supernatants).

Antigen- or Mitogen-induced Activation Assays of T-cell Function

$4x10^4$ washed responder TM3 cells were cultured for 3 days with tetanus toxoid and $8x10^4$ irradiated (4,000 rad) autologus PBM in 160 ul of 15% FCS-containing complete medium in round-bottom microtiter culture plates. In some experiments, 10^5 fresh PBM were cultured with or without polyclonal mitogen (PHA or concanavalin A (ConA)) for 3 days in flat-bottom microtiter culture plates. Since the addition of thymidine analogue A509U competively inhibited the incorporation of methyl1-[^{3}H]-thymidine by cells, the [^{3}H]-uridine incorporated into the cellular RNA was assessed as an index of antigen or mitogen-induced activation of the responder cells. All cultured cells were exposed to 0.5 uCi [5-^{3}H]-uridine (25.8 Ci/mmol) for the final 5 hours, harvested onto glass fibers, and the incorporated radioactivity was counted.

Immunoglobulin Production and Helper Assays

Indicator B-cells (consisting of B-cells and monocytes) were separated from PBM from the normal individual from whom clone TM3 was derived by using neuraminidase-treated sheep erythrocytes as previously described. 10^5 indicator B-cells were exposed to tetanus toxoid (2 Lf/ml) for 4 hours, extensively washed, and cocultured with $5x10^4$ TM3 cells in 0.3 ml of 15% of FCS-containing complete medium. On day 8 the culture supernatants were harvested and assessed for IgG production using an enzyme-linked immunosorbent assay.

Inhibition of HTLV-III/LAV p^{24} gag protein expression in H9 cells by A509U

Expression of HTLV-III/LAV p^{24} gag-protein correlates with HTLV-III replication in infected target T-cells. We first addressed the inhibitory effect of A509U on the expression of HTLV-III/LAV p24 in clone H9, a lymphoblastoid line, which is permissive for HTLV-III replication. When the target H9 cells were exposed to HTLV-III_B isolate (1,000 virus particles/cell) and cultured in the absence of A509U, by day 10, 74% of the target H9 cells became infected and expressed p24 gag protein as determined by an indirect immunofluorescence assay. A striking protective effect was observed when the H9 cells were cultured in the presence of small amounts A509U. A complete protective effect was observed at concentrations of 5 and 10 micromolar, and none of H9 cells became positive for p24 throughout the 10-day interval of culture.

Protection of Helper/Inducer T-cells by A509U against HTLV-III/LAV cytopathic effect

We then asked whether A509U could block the cytopathic effect of HTLV-III_B against a normal IL-2 dependent helper/inducer T-cell clone, TM3. This clone displays the following surface membrane phenotype: OKT3+, OKT4+, tac-antigen+, HLA-DR+, and OKT8-. Clone TM3 produces substantial quantities of IL-2 and undergoes a proliferative reaction in response to the specific antigen, tetanus toxoid, in the presence of appropriate accessory cells. In addition, TM3 shows helper activity for immunoglobulin production by normal B-cells, as discussed later.

Five thousand virus particles/cell were used to produce a cytopathic effect of HTLV-III_B in the target T-cells in a short period of time in culture. Since TM3 cells had been stimulated by soluble tetanus toxoid 6 days previously, these cells continued to grow in the presence of exogenous IL-2. In the absence of the drug, the HTLV-III_B virions exerted substantial cytopathic effect on the TM3 population by day 10 in culture, resulting in a profound decrease in the number of total viable cells, as compared to the control HTLV-III_B-unexposed TM3 population. However, the addition of 5 or 10 μM A509U protected TM3 cells and enabled them to survive and grow.

This ability of A509U to protect T-cells against the cytopathic effect of HTLV-III_B was confirmed in clone ATH8. Clone ATH8 bears several distinct copies of HTLV-I in its genome when assessed by Southern blot hybridization using a radiolabelled HTLV-I cDNA. The surface phenotype of this clone is OKT3+, OKT4+, Tac+, HLA-DR+, and OKT8-. We found this HTLV-I-infected T-cell clone, ATH8, highly susceptible to the HTLV-III_B-cytopathic effect in vitro. When cultured in the absence of A509U, by day 7 after HTLV-III_B exposure, almost all ATH8 cells were killed. The susceptibility of this clone to the HTLV-III_B cytopathic effect is much greater than that of clone TM3. However, the addition of 5 and 10 μM of A509U could again completely protect ATH8 cells against the cytopathic effect of HTLV-III_B.

Reversal of A509U Inhibition of HTLV-III/LAV cytopathic effect by Thymidine

We attempted to test the possibility that A509U inhibits the HTLV-III/LAV cytopathic effect by acting as a competitive analog of thymidine. When exposed to HTLV-III_B (5,000 virus particles/cell) together with 1 uM A509U, ATH8 cells were moderately protected against the virus. The addition of thymidine reversed the protective effect of A509U and the target ATH8 cells were lysed in a dose-response fashion. When added alone, thymidine did not exert any cytotoxic effect.

A509U Protects the Target T-cells Against the Cytopathic Effect of a Haitian Isolate of HTLV-III/LAV (HTLV-III/RF-II)

To determine whether A509U is effective against a genetically different isolate of HTLV-III/LAV, we tested the effect of the drug against HTLV-III/RF-II, a variant of HTLV-III/LAV isolated from a Haitian patient with AIDS showing significant divergence in its genome from other isolates of HTLV-III/LAV. In this experiment HTLV-III/RF-II-producing H9 cells were used as a source of infectious virions. The results illustrate the protective effect of A509U on the survival and growth of the clone ATH8 following exposure to HTLV-III/RF-II. In the absence of A509U, lethally-irradiated (10,000 rad), HTLV-III/RF-II-producing H9 cells exerted a substantial cytopathic effect on the ATH8 population. Most of the target cells were killed by day 5 in culture. We found that 0.5 μM A509U showed a partial protective effect of the cells against the cytopathic effect. However, at concentrations of 1 to 10 μM, A509U clearly blocked the cytopathic effect of HTLV-III/RF-II, indicating that A509U inhibits the cytopathic effect of HTLV-III/RF-II. These data might also suggest that A509U is able to block HTLV-III/LAV infection caused by both extracellular and cell-associated virions.

Inhibition of Reverse Transcriptase Production by HTLV-III_B-exposed PBM

To extend our observations, we investigated whether the addition of A509U inhibits reverse transcriptase production in normal PBM exposed to HTLV-III_B. A substantial level of reverse transcriptase activity could be detected in the supernatants of normal PBM exposed to HTLV-III_B in the absence of A509U. However, addition of A509U resulted in a dose-related decrease in reverse transcriptase activity in the supernatants. Inhibition was observed at doses as low as 0.005 μM and was marked at 0.05 μM. Complete inhibition was achieved at doses of 0.5 μM and more.

A509U does not Inhibit Functions of T- and B-cells at Concentrations that Block Replication and Cytopathic Effect of HTLV-III/LAV

We tested the effects of A509U on the in vitro immunologic functions of the antigen-specific helper/inducer T-cell clone TM3 and PBM from normal individuals. When exposed to tetanus toxoid plus irradiated autologous accessory cells, clone TM3 was substantially activated and incorporated [^{3}H]-uridine to cellular RNA. A509U exerted a slight inhibitory effect on the antigen-induced activation of TM3 at a concentration of 1 μM. At a concentration of 50 μM, T-cell activation

was only partially inhibited. The mitogen-induced activation of PBM using PHA or ConA was only marginally inhibited at concentrations up to 10 μM. When cocultured with tetanus toxoid-preexposed indicator B-cells (consisting of autologous B-cells and monocytes), TM3 cells showed a significant level of helper activity in inducing IgG production by the indicator B-cells. This immunoglobulin production was partially inhibited at a concentration of 50 μM of A509U; however, it was not inhibited in a dose range of 1 to 10 μM, indicating that at these concentrations A509U exerts only moderate inhibitory effects on immunoglobulin production by B-cells in this system of helper activity. When assessed by trypan blue dye exclusion, the viability of TM3 cells which had been cultured for 14 days in the presence of various concentrations of A509U (0 to 10 μM) was approximately the same and not adversely affected. These data suggest that at concentrations which block the in vitro infectivity and cytopathic effect of HTLV-III/LAV, A509U only slightly inhibits some immunologic reactivities of normal T-cells, and that even at relatively large doses (up to 50 μM), a substantial level of in vitro immune reactivity is preserved.

Discussion

Although a number of specific and non-specific inhibitors of retroviral reverse transcriptase have been described in the literature, the recent discovery of pathogenic human retroviruses has intensified the search for new chemotherapeutic agents which may halt the replication of these viruses in vivo. The results reported here characterize the activities of one such agent (BW A509U).

There are no universally accepted, standardized techniques for testing the in vitro sensitivity of anti-viral agents. The quantitative determination of the inhibitory activity of A509U against HTLV-III/LAV poses special problems because some of the usual plaque reduction techniques which may correlate with in vivo activity in other viral systems have not been perfected for human retroviruses. We, therefore, examined several aspects of viral inhibition to address the spectrum of potential predictors of in vivo activity. Moreover, because patients infected with HTLV-III/LAV may be severely immunocompromised, we measured not only the often accepted 50% inhibition levels (ID50) but also levels that achieved complete or nearly complete inhibition because the patients' humoral and cell-mediated immunity could not be relied upon to eliminate virus not inhibited by the drug. The current approach may provide a general model for drug screening and development of anti-viral agents.

In the current in vitro studies, we observed that p24 viral gag protein synthesis was completely inhibited in H9 cells by 5 and 10 μM concentrations of A509U. Indeed, even a concentration of 1 μM permitted only 4% of the cells to express this protein, by contrast to 74% in untreated populations of cells. Although there was a sharp break between 0.1 and 1 μM, the approximate ID50 was slightly more than 0.1 μM. Furthermore, in experiments using a high multiplicity of infection (5,000 virus particles/cell) between 80% and 100% of the cells exposed to HTLV-III_B survived in the presence of 5-10 μM of A509U. In another model of infection (using the reverse transcriptase activity released in culture supernatants of normal lymphocytes following exposure to HTLV-III_B as an index

of replication), viral replication was suppressed by 50% using concentrations of A509U slightly less than 0.05 μM and was almost completely ablated by concentrations of 0.5 μM and higher.

Variation in the sensitivity of different viral isolates to individual chemotherapeutic agents is a well-known phenomenon, and it was, therefore, of interest to learn whether more than one isolates of HTLV-III/LAV are sensitive to A509U. We tested the activity of the drug against the most genetically disparate isolate to date (HTLV-III/RF-II), and the results obtained were similar to those obtained with the prototype HTLV-III$_B$, with 50% inhibition being between 0.5 and 1 μM and 99-100% inhibition occurring between 1-5 μM.

Because A509U is an analogue of thymidine, it is perhaps not surprising that reversal of its anti-viral effects was observed following the addition of exogenous thymidine. However, between 10 and 100 μM of thymidine were required to fully reverse the inhibition of the virus induced by 1 μM A509U. At present, one may conclude only that A509U exerts its anti-viral effects at some step in the various metabolic pathways utilizing thymidine. Although it is known that the triphosphate derivative of A509U is a relatively selective inhibitor of HTLV-III/LAV reverse transcriptase as opposed to mammalian cell alpha DNA polymerase (unpublished observations), it is not known whether this is the exclusive mechanism of the anti-viral effect. Irrespective of the exact mechanism(s) of the anti-viral effect, it is likely that the activity of A509U would be influenced by concentrations of enzymes (deoxycytidine monophosphate deaminase, thymidylate synthase, thymidine kinase, etc.) that govern the intracellular pools of thymidine in host cells. In this context, cellular levels of thymidine kinase might play a crucial role in generating the fully phosphorylated form of the drug, which would be active as an anti-viral agent. Retroviruses do not encode their own thymidine kinase and, therefore, unlike certain herpes viruses, they cannot adopt a strategy of diminishing viral thymidine kinase activity as a mechanism for developing drug resistance.

Of course, it is worth stressing that the activity of an agent against viruses in vitro does not ensure that the agent will be clinically useful in treating viral diseases. Toxicity, metabolic features, bioavailability, and other factors could negate the clinical utility of a given agent. Moreover, it is possible that patients with the most advanced forms of AIDS might have an immunodeficiency state that no longer depends on HTLV-III/LAV replication, and would therefore require therapeutic interventions beyond an anti-viral agent per se. Nevertheless, there are certain characteristics which would argue for cautious exploration of A509U as an experimental drug in patients with HTLV-III/LAV infection. First, the drug produces little or no toxicity even when given in high doses (85-150 mg/kg) in rats and mice for up to 4 weeks. Second, the drug can bring about essentially complete inhibition of viral replication at doses that do not substantially diminish various in vitro parameters of T-cell immune reactivity. Finally, from experience with other nucleoside analogues, it is likely that A509U will be absorbed by oral administration, making it potentially suitable for regimens that involve prolonged therapy.

Based on these considerations, the program has initiated a phase I clinical trial of A509U in patients with AIDS.

Project C

HTLV-I (Human T-lymphotropic Virus Type I) is the prototypical pathogenic human retrovirus discovered by Gallo and his co-workers in 1978. This virus is endemic in several parts of the world, including the southwestern portion of Japan, the West Indies, and the southeastern United States. The virus is the cause of adult T-cell leukemia in these known regions. The availability of monoclonal antibodies to this virus--especially antibodies of human origin--would have clinical and theoretical importance not only in understanding HTLV-I, but in providing models suitable for studying other human retroviruses including HTLV-III. We, therefore, undertook to make a human, monoclonal antibody against HTLV-I.

Specific antibodies to both gag and env antigens of HTLV-I have been detected in people who are exposed to the virus, including ATL patients and asymptomatic carriers. Moreover, it has been possible to generate a number of murine monoclonal antibodies against the p19 and p24 gag proteins of HTLV-I. By contrast, the generation of monoclonal antibodies against env proteins has proven to be extremely difficult. A murine monoclonal antibody against the HTLV-I transmembrane protein (gp21 and p21E) has been reported. However, to our knowledge, no monoclonal antibodies with reactivity against the major env glycoprotein (gp46) are currently available, and the absence of such monoclonal antibodies has limited the immunologic and biologic characterization of the env region and its products. In this report, we describe the generation and characterization of a human monoclonal antibody reactive against the major env component of HTLV-I.

We separated the B-cell fraction from lymph-node cells obtained from a seropositive patient with ATL by rosetting with sheep erythrocytes, and exposed them to Epstein-Barr virus (EBV) in vitro. We then cloned these cells by limiting dilution and obtained a B-cell clone (designated 0.5α) which produced an antibody (0.5α antibody) reactive against HTLV-I-producing cells. 0.5α grew with a doubling time of approximately 6 days. 0.5α has been continuously cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 4 mM L-glutamine, 50 U/ml penicillin and 50 ug/ml streptomycin and maintained a stable level of antibody production for over 10 months. Recloning of 0.5α cells produced 8 subclones, all secreting human IgG(k) monoclonal antibody that is specific for HTLV-I-producing cells. We observed that the 0.5α antibody had complement-fixing and Staphylococcal protein A-binding properties.

We tested the reactivity of this 0.5α antibody with preparations of disrupted HTLV-I, II and III viruses by an enzyme-linked-immunosorbent assay (ELISA). The antibody reacted only with disrupted HTLV-I virus but not with HTLV-II and III viruses. We then tested the binding activity of this antibody to the surface of living cells by fluorescence activated cell sorter (FACS). The

0.5α monoclonal antibody bound to the surface membrane of HTLV-I-producing cells but not to that of HTLV-I-negative cells. This reactivity occurred with 100% of the HTLV-I-producing cell populations. We then analyzed the complement-mediated cytolytic activity of 0.5α antibody by using a trypan blue dye exclusion test. 0.5α antibody lysed almost all HTLV-I-producing HUT 102 cells but did not lyse HTLV-I-negative Molt-4 cells even at high concentrations. Protein-A-purified polyclonal IgG from the serum of an ATL patient also lysed HUT 102 cells but it did so less effectively. The antibody failed to bind to and lyse a variety of HTLV-I-negative cell lines, HTLV-II-producing C3-44 cells and HTLV-III-producing H9 cells. The antibody also failed to stain and lyse normal peripheral T-cells purified by sheep erythrocyte rosetting as well as phytohemagglutinin (PHA)-stimulated normal peripheral T-cell blasts (PHA-blast). A normal tetanus toxoid-specific T-cell clone YTA1, which is negative for 0.5α reactivity, became positive after HTLV-I infection. The 0.5α antibody did not react with cryopreserved fresh peripheral leukemic T-cells from four ATL patients. However, following short-term culture of leukemic T-cells from two of these cases, reactivity with 0.5α appeared and those cultured leukemic T-cells were lysed. Lack of HTLV-I gene expression in fresh leukemic T-cells has been previously observed; however, viral expression may be induced under short-term culture conditions. These data indicate that the 0.5α human monoclonal antibody can bind to an antigen specifically expressed on the surface of HTLV-I-producing cells and can lyse such cells in the presence of complement.

To further characterize the antigen detected by 0.5α antibody we tested a preparation of disrupted, double-banded HTLV-I using an electroblot (Western) technique. The 0.5α antibody detected a 46-kilodalton (kD) molecule as a major band and a 42-kD molecule as a minor band. The former has a pattern of migration similar to that reported for the HTLV-I major envelope protein (gp46). The latter is presumably a variant cleavage product of the *env* gene, or a partially glycosylated product. Serum from a patient with ATL *also* detected the 46-kD antigen as a broader band. Antibodies to HTLV-I *gag* proteins, did not detect this molecule. We also analysed disrupted HTLV-II and III viruses with 0.5α antibody in the same electroblot assay and observed no reactivity.

We then treated the HTLV-I viral preparation with endoglycosidase F and analyzed the antigen detected by 0.5α antibody in an electroblot. The antibody reacted with 34-kD and 38-kD proteins after endoglycosidase F treatment, while the control buffer treatment without enzyme did not change the molecular weight of the antigen as compared with the untreated preparation. The protein moiety of major envelope glycoprotein gp-46 has been reported to be a 34-kD molecule detected by the antibody most likely represents a portion of the envelope protein inaccessible to complete digestion by endoglycosidase. These data suggest that 0.5α antibody binds to a viral glycoprotein with molecular weight of 46-kD which has been considered as the major component of HTLV-I envelope protein.

We then observed that the 0.5α antibody bound a high molecular weight determinant (>60 kilodaltons) in living cells that were producing HTLV-I. We focused on HUT 102, a long-term line producing the virus.

HUT 102 cells were then endogenously radiolabelled in the presence of tunicamycin and immunoprecipitated. Following tunicamycin treatment, the bands of the high molecular weight 61-kD molecule disappeared and two major bands of molecular size 46- and 41-kD appeared when precipitated with 0.5α antibody. The precipitation of gag-related protein by patient serum and goat anti-serum to p24 was not affected by tunicamycin treatment.

Such high molecular weight glycoproteins have been reported to represent the HTLV-I env-gene product in these cell lines by others. In our experiments with tunicamycin treatment, two proteins (p46 and p41) were immunoprecipitated by 0.5α antibody. The p46 has been reported to be the env-gene encoded precursor protein which is glycosylated to 61- to 68-kD molecules (recognized in HTLV-I-infected cells) and subsequently cleaved to the mature envelope glycoprotein gp46 and the transmembrane protein p21E. The nature of 41-kD protein in the tunicamycin-treated HUT 102 cells has not been determined; however, it clearly shares an epitope with p46, and has been previously observed using antisera to synthetic peptides deduced from the env gene sequence. These results, taken together, indicate that the antigen precipitated by the 0.5α antibody is a glycoprotein not related to the gag proteins but related to the env-gene product of HTLV-I.

Several features of the 0.5α human monoclonal B-cell line and its secreted antibody might be of special interest from a research perspective. First, the 0.5α B-cell line exhibits a stable pattern of growth and specific antibody secretion. Second, the secreted human IgG product fixes complement and binds to Staphylococcal protein A. Finally, the 0.5α antibody-producing line was derived from a patient with adult-T-cell leukemia (ATL), and the determinant detected by the antibody would, therefore, represent a viral antigen which is recognized by humans in response to HTLV-I infection in vivo. Taken together, these features make the 0.5α antibody a unique reagent for studying the natural history and biology of HTLV-I infections.

The 0.5α human monoclonal antibody could also conceivably have value in clinical interventions against diseases linked to HTLV-I infection. If viral envelope antigen were expressed on the surface of tumor stem-cells at some point in the clinical course of ATL or its smoldering variants, it might be possible to use the antibody in experimental regimens for the therapy of these diseases. By the same token, the antibody might have an experimental therapeutic role if it were possible to first induce the expression of viral envelope in tumor cells by a pharmacologic or biologic agent in vivo. It is also possible that this human monoclonal antibody could facilitate an anti-HTLV-I vaccination process; anti-idiotypic antibodies to 0.5α antibody might be used as antigen substitutes to generate immunity without the use of virus or its products as antigens per se. We have produced a murine monoclonal antibody against the 0.5α human antibody, and tests are now underway to see if this murine anti-idiotypic antibody can be used as an immunizing substance to raise antibodies to HTLV-I in animals.

We believe this approach to raising monoclonal antibodies might have general applicability in other systems including HTLV-III interactions.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07202-02 BDMS

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Biostatistics and Data Management Section

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Robert W. Makuch	Head	BDMS, COP, DCT, NCI
Others:	Robert W. Wesley	Senior Investigator	BDMS, COP, DCT, NCI
	Margaret N. Wesley	Senior Investigator	BDMS, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

Biostatistics and Data Management Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	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 Section is the statistical and data management component of the Clinical Oncology Program (COP). The Section provides statistical leadership and data management consultation for major activities of the Program, and is involved in the design, conduct, monitoring, and statistical analyses of intramural and national multicenter clinical trials of experimental treatments for cancer. The Section also is involved in the scientific monitoring of national, multi-center studies which are not funded by the NCI. Other major collaborative efforts include studies to identify important prognostic and treatment selection factors, evaluate diagnostic procedures, develop improved staging systems, and assist investigators in the design, execution, and analyses of major in vitro drug testing studies. The Section develops new statistical designs and biometric methods related to the development and evaluation of new cancer treatments. Current research is focused on the evaluation of the person-years index to summarize the incidence of second neoplasms after initial cancer treatment, and the effects of early termination on the interpretation of clinical trial results. The Section maintains computerized data collection systems for intramural and national multicenter clinical protocols, and it works closely with interested branches to improve data recording and retrieval. The Section provides liaison with the Clinical Center Medical Information System team and the Clinical Center Pharmacy, allowing COP input into decisions which directly impact patient care and protocol management. The Section assists the Deputy Clinical Director to insure adequate monitoring of protocols through the MIS Toxicity and Protocol Monitoring screens and other mechanisms.

1. Collaborative Projects Within Clinical Oncology Program

Members of the Biostatistics and Data Management Section provide to the intramural clinical research program both biostatistical and data management expertise. Our efforts in these areas are described in sections A) and B) below.

A. The Biostatistics and Data Management Section (BDMS) is organized with a designated coordinating statistician for each Clinical Oncology Program (COP) branch. A member of the BDMS participates in the development of new protocols and the interim monitoring and data collection for ongoing studies. A member of the Section also serves on the Clinical Research Sub-Panel to review all intramural clinical trials. BDMS staff collaborate in clinical and laboratory studies to evaluate prognostic and treatment selection factors and elucidate tumor biology. The Section provides statistical support for the COP as well as consultation on the best ways to use available NIH computer systems or microprocessor based professional workstations for clinical and laboratory research.

A detailed list of COP projects to which members of the Section have provided statistical input follows:
(1) Analyzed the randomized study for early breast cancer (radiation + lumpectomy vs. mastectomy) in regard to the number of patients required to complete the study, and also compared certain subgroups (e.g., ER+ vs. ER-, T1 vs. T2, age <50 vs. age >50).
(2) Determined the frequency of second primary bronchogenic carcinoma of non-small cell lung cancer in long-term survivors (40 patients with two-year disease-free survival) of small cell lung cancer.
(3) Evaluated the effects of chemotherapy and total skin electron beam irradiation in 39 patients with mycosis fungoides and sezary syndrome to determine if CMT could produce prolonged disease progression.
(4) Compared survival in patients with limited small cell lung cancer to determine whether combination chemotherapy with or without radiation therapy was more effective. Also, toxicities were compared between the two treatment regimens.
(5) Evaluated 102 previously untreated patients with stage III and IV malignant lymphoma in a randomized trial comparing combination chemotherapy to several irradiation regimens between 1968 and 1974. Also evaluated 57 patients with various histologies including nodula, poorly differentiated lymphocytic, and histiocytic lymphoma.
(6) Evaluated patients with cutaneous T-cell lymphoma to develop a method of describing lymph node histopathology in these patients. We found a significant correlation between LN class and the extent of skin, blood, visceral organ involvement, and survival.
(7) Examined the hormonal alterations of testicular function (serum FSH, LH, and testesterone concentration) in males with soft tissue sarcoma who were treated with surgery and high dose radiation to the tumor bed.
(8) Five to eleven year outcome was determined in 252 small cell lung cancer (SCLC) patients treated with combination chemotherapy with or without chest and cranial irradiation in 1973-1978 NCI therapeutic trials. Thirty month SCLC-free survival is insufficient to demonstrate cure.
(9) Twenty patients with SCLC who were alive and free of cancer at least 2.4 years were studied to evaluate the relationship between neurologic function and

cranial irradiation.
(10) Evaluated patients with cutaneous T-cell lymphoma (CTCL) to assess the clinical significance of peripheral blood infiltration by CTCL cells. Convoluted cells were divided into a small (<11u) or a mixed (>11u) cell variant, from which it was concluded that the mixed cell variant was associated with a significantly worse prognosis in terms of survival.
(11) For SCLC patients, we studied whether pulmonary toxicity (PT) limited disease SCLC was higher in patients treated in a randomized study with either CT+RT or CT alone. It was found to be higher in the combined modality group, and we found that pretreatment vital capacity, forced expiratory volume/1.0 record, and total lung capacity were significantly lower in patients eventually developing PT.
(12) Analyzed the effects of dose-time fractionation, volume to be treated, spinal cord shielding, concurrent versus sequential, radiation, and other considerations in the radiotherapy of patients with small cell lung cancer.
(13) Serve as member on Institutional Review Board for the COP.
(14) Gave several lectures to clinical associates and senior staff on biostatistical considerations in cancer clinical trials.
(15) Analyzed a variety of factors in patients with hepatocellular carcinoma to determine which factors were important and independent prognostication of survival.
(16) Analyzed small-cell lung cancer patients treated with combination chemotherapy with or without irradiation in regard to potential cures, chronic toxicities, and late relapses.
(17) Analyzed the effects of fractionated irradiation on testicular function in patients treated with radiation therapy.
(18) Analyzed patients with small cell lung cancer to evaluate the incidence of non-small cell lung cancer as a major cause of late mortality.
(19) Analyzed the prognostic importance of lymph node histology in mycosis fungoides/Sezary syndrome patients, and developed a new classification system.
(20) Analyzed patients with osteogenic and soft tissue sarcomas to determine whether CT scans or conventional linear tomograms were superior in detecting pulmonary metastases.
(21) Evaluated the incidence of pulmonary toxicity in limited stage small cell lung cancer patients treated with combined modality therapy.
(22) Two major updates of results on all soft tissue sarcoma protocols, comparing adjuvant chemotherapy to no chemotherapy in patients with extremity tumors and with head, neck, and trunk tumors; comparing limb-sparing surgery to amputation in patients receiving adjuvant chemotherapy; and comparing a short course adjuvant chemotherapy regimen (350 mg/m2 doxorubicin) with standard course (550 mg/m2).
(23) Update of all colorectal and hepatic metastases protocols for publication. These analyses compared adjuvant IP 5-FU vs. no chemotherapy in patients with hepatic metastases from colorectal cancer; IP 5-FU vs. IV 5-FU in patients with colon and rectal cancer; and adjuvant radiation therapy vs. none in these patients to assess toxicity (number of patients getting through full course of chemotherapy, number of courses until patient had thrombocytopenia or granulocytopenia); and systemic FUDR vs. intrahepatic FUDR (delivered by implantable pump) in patients with hepatic metastases from colorectal cancer.
(24) Interim analyses of intraop radiation protocols (retroperitoneal, gastric, resectable pancreas, unresectable pancreas).

(25) Analysis of ability to detect localized parathyroid tissue using technetium scans in patients receiving definitive surgery.
(26) Analysis of over 200 extremity soft tissue sarcoma patients for manuscript on prognostic factors, including size of tumor, site, histology, and margins.
(27) Two updates of Surgery Branch osteosarcoma protocols comparing adjuvant chemotherapy with no chemotherapy (randomized protocols and retrospective comparisons), and comparing immunotherapy with BC6 versus none.
(28) Analyses of three separate experiments looking at effect of various antibody treatments in a hamster tumor model.
(29) Analysis for publication of retrospective study of granulocytopenic patients with anorectal infections to compare the efficacy of medical versus surgical management, and to determine prognostic factors - such as underlying malignancy, stage of infection, type of infecting microorganism, host compromise - which affected ultimate resolution of the infection.
(30) Analysis for publications of prognostic factors determining outcome in retrospective study of granulocytopenic patients needing emergency abdominal surgery.
(31) Interim analysis of esophageal protocol comparing surgery alone versus surgery + chemotherapy.
(32) Analysis of NIH experience with patients with advanced Zollinger-Ellison disease, primarily in regard to the role that surgery plays.
(33) Analysis of longitudinal data on ejection fractions of patients receiving high versus low doses of doxorubicin.
(34) Additional analyses of confidence intervals for difference in number of emetic episodes on protocol comparing IV metoclopramide and compazine.
(35) Updated analyses comparing three methods (CT, LT, CXR) for detecting lung nodules.
(36) Consult on how to do analyses on skin graft data in rhesus monkeys, looking at time to graft rejection in groups given various immunotherapy preparations.
(37) Consult on how to use Bonferroni's inequality and rank tests on analysis of IL2 and LAK cells data in rodent model.
(38) Consult on using nonparametric and paired or unpaired tests for comparing cachetic vs. normal sera levels of amino acids overall, especially alanine and leucine.
(39) Consult of appropriate statistical methods and interpretation for comparing Mayo Clinic and MSK results on islet cell tumors.
(40) Two interim analyses of ALL (leukemia) protocol 77-02, a cooperative study with 180 patients at five institutions.
(41) Initial interim analysis of two new ALL protocols for average and high risk patients; the high risk protocol is a single-arm extension of the successful chemotherapy only (no cranial irradiation) arm on the multi-institutional 77-02 protocol, with modifications in Ara-C administration to prevent CNS relapse and to more aggressively treat systemic disease. The average risk protocol is a randomized extension of 77-02, comparing two chemotherapy only arms - one with high dose methotrexate and one without.
(42) Two interim analyses of the Pediatric Branch high risk sarcoma protocol (focusing on Ewing's and rhabdomyosarcoma) evaluating an intensive chemotherapy regimen bone marrow transplant and total body irradiation.
(43) Analysis using logistic model of Phase I and Phase II results for complete CR rate and response rate to vindicine.

(44) Analysis for publication of protocol comparing open lung biopsies versus empiric therapy in patients with FUOs.
(45) Analysis for publication to look at baseline prognostic factors and factors during therapy to determine cause(s) of pneumocystis seen on ProMACE/CytaBOM arm in Medicine Branch non-Hodgkins lymphoma protocol.
(46) Updated analysis after eight years of follow-up of rhabdomyosarcoma protocol to evaluate the impact of stage, radiation actually delivered, age, and type of disease (embryonal or alveolar).
(47) Analysis of beta-endorphin levels in patients in pain who are on morphine.
(48) Analyses of effect on delivery of education to children and adolescents who have had cancer on Pediatric Branch protocols.
(49) Several sets of analyses updating Mopp/CMopp results and ProMACE/MOPP results by treatment and LDH as prognostic factor.
(50) Evaluation of and interim sample size considerations for a randomized trial of PVeBV and PVeB in poor prognosis nonseminomatous testicular cancer.
(51) Analysis of the effects of withdrawal methods on arterial cannula dysfunction.
(52) Analysis of the management of nonmetastatic locally advanced breast cancer using primary induction chemotherapy with hormonal synchronization followed by radiation therapy.
(53) Update of study of MOPP in Hodgkin's disease and re-analysis excluding patients who actually had diffuse large cell non-Hodgkin's lymphoma.
(54) Chapter on statistical considerations in the design and interpretation of clinical trials in breast cancer.
(55) Update survival and disease-free survival of mastectomy versus lumpectomy in patients with primary breast cancer.
(56) Analysis of chemotherapy in metastatic breast cancer.
(57) Analysis of intermediate dose methotrexate followed by 5-fluorouracil in the management of metastatic breast cancer.
(58) Analysis of a trial of tamoxifen plus fluoxymesterone versus tamoxifen plus danazol in metastatic breast cancer, evaluating survival, response rates, complete response duration, and baseline prognostic factors.
(59) Analysis of chemo-hormonal therapy in breast cancer patients with no evidence of disease following an excised or curatively irradiated recurrence.
(60) Analysis of adjuvant therapy of male breast cancer.
(61) Sample size considerations for carbenicillin and methotrexate pharmacokinetics.
(62) Sample size considerations for a pilot study of intensive therapy and anti-B1 and complement-treated autologous bone marrow rescue in patients with refractory diffuse aggressive lymphoma.
(63) Analysis of survival in advanced ovarian cancer, treated with aggressive multimodality regimen of cisplatin/cytoxan, cytoreductive surgery, and total abdominal irradiation.
(64) Analysis of chest wall resection for primary, recurrent, and metastatic chest wall tumors.
(65) Analysis of inhibitive effect of tumor angiogenesis with hexuronyl hexosaminoglycan sulfate and hydrocortisone in pancreatic adenocarcinoma in hamsters.
(66) Analysis of treatment of nodular lymphoma, in regard to survival, response rates, and complete response duration.
(67) Analysis of psychosocial data in a trial of mastectomy versus lumpectomy in

patients with primary breast cancer.
(68) Analysis of study to increase the response rate to cytotoxic chemotherapy by endocrine means in breast cancer patients.
(69) Comparison of lateral thoracotomy and median sternotomy in soft tissue sarcoma patients with pulmonary metastases.
(70) Analysis of patients with relapsed rhabdomyosarcoma, who had been previously treated on VAC and VACA regimens, for survival and CR duration.
(71) Analysis of prognostic factors for survival at the time of second pulmonary relapse in patients with soft tissue sarcomas.
(72) Interim analysis and sample size considerations for a randomized trial of MOPP alone versus alternating MOPP/CABS.
(73) Analysis of prognostic factors in a historical study of patients with metastatic breast cancer.
(74) Analysis of prognostic factors in granulocyte count increase following autologous bone marrow infusion.
(75) Evaluation of gluthathione peroxidase activity in RBC and cardiac tissue in patients on TPN.
(76) Statistical sections and consults on protocol design for approximately 20 COP studies and arranging for randomizations.

B. Data Management Activities

The Section has continued the development and maintenance of several systems to monitor protocols. (1) Through the MIS Toxicity and Protocol Monitoring screens, the physician can automatically record toxicity information in a computerized data base. This information is printed out daily and sent to the protocol principal investigator. (2) A file is maintained with the date of last clinic visit so that branches can be notified of patients who have not had at least yearly follow-up visits. (3) Active patient lists are sent to the Clinical Associates in the Medicine Branch and Pediatric Branch each month so that survival, response and relapse status can be verified and updated if necessary. (4) Toxicity data on Phase II studies are requested and sent to the Deputy Clinical Director every three months.

A detailed list of data management projects undertaken by members of the BDMS for the COP follows:
(1) Collection, maintenance, and reporting of basic survival and protocol entry data on Pediatric Branch and Medicine Branch active patients.
(2) Data management, retrievals and analyses as required by Surgery Branch staff.
(3) Data collection and coordination for several current protocols of the NCI-Navy Medical Oncology Branch.
(4) Maintenance of a computer file of the latest actual clinic visit for all active patients.
(5) Daily abstraction of toxicity data from the MIS Progress Notes and entry of data into the CAPRI system.
(6) Distribution of monthly summary reports of toxicity data to principal investigators.
(7) MIS toxicity and protocol monitoring project, including design of screens, instruction of physicians, monitoring of use.
(8) Support to insure that all patients receiving chemotherapy, especially

investigational drugs, have a valid Clinical Center protocol number for pharmacy records.
(9) Phase II studies quarterly toxicity report.
(10) Orientation to MIS toxicity and protocol monitoring screens for Medicine Branch, Pediatric Branch, and Surgery Branch.
(11) Maintenance of various computer packages used by the Section.
(12) Collaboration with Clinical Center computer staff on abstracting MIS data for protocol uses, and on computerized hospital data collection.
(13) Automatic printing of all data from the MIS Toxicity and Protocol Monitoring System as Progress Notes and distribution of these documents to appropriate principal investigators.
(14) Continued development of MIS protocol order sets, including: 76-C-377, 84-C-71, 84-C-85, 84-C-88, 84-C-131, 84-C-136, 84-C-153, 84-C-159, 84-C-174, 84-C-175, 84-C-177, 84-C-179, 84-C-193, 84-C-216, 84-C-217, 84-C-250, 85-C-8, 85-C-48, 85-C-62, and 85-C-66.
(15) Work with data nurses and principal investigators on using personal computers for protocol data management.
(16) Provide branches with access to computer programs to do standard statistical analyses.
(17) Evaluation of data base packages for the IBM-PC and Apple Macintosh.
(18) Specification of data collection needs for study of polyvalent pneumococcal vaccination in patients with Hodgkin's and non-Hodgkin's lymphoma.
(19) Wrote major statistical and plotting programs for PC to allow BDMS statisticians to do most routine computing: Kaplan-Meier curves, logrank tests (including stratified version), logistic regression, and Cox regression.
(20) Advice and purchasing information to several COP branches for acquiring personal computers; hands-on help in using PCs. Same help also given to Medicine Branch and Laboratory of Pathology.
(21) Made operational an archiving system whereby all toxicity information entered into MIS is stored on Mass Store system at DCRT for later computer processing.
(22) Prepared and distributed report on data resources available on Clinical Research Information Service (CRIS) and MIS system to COP Branch Chiefs.

2. Projects Outside COP

A. The BDMS also participates in biometric activities outside of the COP, including:
(1) Provided a critical appraisal of semen analysis in regard to total count, volume, motility, and morphology. Indicate which characteristics serve as the best predictive parameters of fertility potential.
(2) Examined various physical therapy parameters (e.g., range of motion, edence, date of first Jobst stocking, pain, weakness after surgery) in early breast cancer patients randomized to receive either radiation + lumpectomy or mastectomy.
(3) Patients with squamous cell carcinoma of the head and neck who were entered on a multi-center national clinical trial were analyzed to determine what pretreatment factors were associated with high tumor response rates to induction chemotherapy.
(4) Evaluated the short and long term effects of four weeks of total parenteral nutrition (TPN) on nutritional assessment parameters in a randomized, multicenter study of SCLC patients to receive or not receive TPN. Some short-term,

but no long-term, effects were noted.
(5) Served as review member for several grant and contract recompetition in various Programs of the NCI, including the COP, CTEP, and DTP.
(6) Invited to serve on committee which monitors a national, multi-center trial in head and neck cancer being conducted by the Veterans Administration Cooperative Studies Program. This committee has the responsibility for deciding when, and if, the study should be stopped prematurely and whether the goals of the study can be met.
(7) Served as grant reviewer for National Sciences and Engineering Research Council of Canada.
(8) Serve as Group Leader for the American Statistical Association in regard to developing guidelines and recommendations for positive control trials.
(9) Analyzed patients with Stage 1b squamous cell carcinoma of the uterine cervix to determine if any parameters involving histological grading were predictive for survival.
(10) Involved in an analysis of the applicability of a human tumor colony-forming assay for drug screening in terms of its feasibility, validity, and potential for discovering new antitumor drugs.
(11) Analyzed a multi-center, international clinical trial to evaluate the effect of adjuvant central IV hyperalimentation on the survival and response to treatment of patients with small cell lung cancer.
(12) Analyses for publication on pathology classification schemes in childhood rhabdomyosarcoma.
(13) Extensive consultations on design, analysis, interpretation, and presentation of study on post-therapy work/life/educational experience of pediatric cancer patients.
(14) Consult on appropriate use of parametric and nonparametric tests in the analysis of patient rehabilitation data.
(15) Organized FAES poster session on various uses of computers in clinical and laboratory work.
(16) Updated analysis of CVP versus CAVP in patients with advanced non-Hodgkin's lymphoma.

B. In addition to data management support for intramural trials, the BDMS provides data management services outside the COP. Project staff have provided Operations and/or Statistical Center support to a number of multi-institutional extramural trials. This support includes performing randomizations, design of data collection instruments, software design and development, production of regular status reports, and production of ad hoc reports and tabulations as directed by the study statistician. The extramural trials supported include:
(1) 7601/7602, Treatment of Early Stage Ovarian Cancer
(2) HNCP-178, Head and Neck Contracts Program
(3) Washington Myeloma Group Protocol
(4) CCSG-191P, CCSG Protocol for Acute Lymphoblastic Leukemia
(5) CCSG-134P, CCSG Protocol for Poor Prognosis Acute Lymphoblastic Leukemia
(6) CCSG-144P, CCSG Protocol for Average Prognosis Acute Lymphoblastic Leukemia

3. Biometric Research

Current biostatistical research being conducted includes:
(1) Published a critique on approaches to sample size estimation in the design

of clinical trials.
(2) Discussed issues as they relate to practical pitfalls in interpreting clinical trials.
(3) Evaluated the bias and skewness in the person-years index, a commonly used measure for evaluating second cancers after treatment for initial disease.
(4) Evaluated and developed role of personal workstations in patient care and clinical research.
(5) Examined methods to obtain maximum use of available sources of data for clinical studies.
(6) Evaluated and developed tools to enable researchers to become more directly involved in data exploration and analysis.

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

October 1, 1984 - September 30, 1985

This is the first year in which the Branch has had all of its planned sections in place and operating. It is also 18 months since our last site visit and the activities of the last year reflect full implementation of the site visit recommendations. In this summary, I will not detail all of the activities of each section, but rather touch upon the highlights.

1. Pharmacokinetics Section

In terms of program priorities, this group has made two major contributions. First, this group, in collaboration with Dr. Broder and Dr. Gallo, have established that suramin can be given to AIDS patients and will suppress the shedding of HYLV-III into the blood. This group, in particular, have shown that because of the unique pharmacology of this drug, it need only be given every 2-4 weeks. A second group of patients are now being treated with a drug schedule designed according to the pharmacology of the drug. We are very optimistic that this will prove to be truly effective therapy for AIDS; ARC and related syndromes if given early in the disease. We also feel that the remarkable pharmacology of this drug might suggest new avenues in cancer drug design. In addition to suramin, this group is collaborating with Dr. Broder in the development of other drugs active against HTLV-III. The second major contribution has been Dr. Collins development of the blood level working group concept. At present, it appears that this approach will be a far more accurate predictor of starting dose in man than in the mouse lethality test.

2. Biophysical Pharmacology Section

This is the first year this group has been adequately staffed and has had its NMR in place. The Varian machine has proved to be exceptionally reliable for such a high tech device. This is especially remarkable because we received one of the first models. As a result, this has been a very productive year in the Biophysical Pharmacology Section. The various projects are detailed below. There are two major advances. First, Dr. Cohen has developed a very promising group of NMR imaging agents based upon the observation that hematoporphyrins selectively localize in carcinomas. The concept is to use the porphyrin to carry paramagnetic metal ions into tumor cells. The paramagnetic metal ions then cause enhanced relaxation of water protons within the tumor, thus creating difference in contrast with surrounding tissue. Of the metalloporphyrins tried to date, manganese III complexes of TPPS and TM PyP have proven most effective. Both localize to human colon tumor xenographs in nude mice. Furthermore, they appear to bind and stay in the tumor for prolonged periods, like lymphangiogram dye. I think this will prove to be a remarkably fertile area for long term development because the chemical synthesis of porphyrin analogs is remarkably well developed and concepts and test systems are also well developed.

The second area revolves around the ability of NMR to study DNA structure in solution. The evolution of the concepts of drug-DNA interaction have been held back by the fact that the only way to get detailed 3D information of drug-DNA complexes was through X-ray crystallography. Not only is this technique very laborious and expensive, the results reflect crystal geometry in the absence of solute effect--a major determinant of DNA structure in solution. Over the past year Dr. Cohen has been able to use two dimensional NMR to get equivalent three dimensional information of DNA and DNA-drug complexes under physiologic solute conditions. The result has been the resolution of several conflicts in the field. I view the long term value of this approach in that it will allow us to develop agents selective for their attack on special regions of the DNA. One can envision attack on regulatory regions such as the TATA box, zDNA sequences or retroviral LTR's. Work now in progress is aimed at achieving just such goals.

3. Biochemical Pharmacology

This group has spent the last 8 years investigating the mechanism of adriamycin action. Over the past year, two major observations have come to light. The determination of mechanism of action of a drug is difficult when the drug is known to participate in a wide range of biochemical reactions. The problem has been to develop an experimental approach which effectively parses through the epiphenomenon to spot those biochemical events responsible for cytotoxicity. We have made the assumption that the genetic changes associated with the development of drug resistance provides valuable insight into how a drug acts. We have applied this approach to MCF-7 the human breast cancer cell line and an adriamycin resistant clone of this line obtained by Dr. Ken Cowan. We find that the resistant cell line has a number of changes including increased glutathione transferase, glutathione peroxidase and sulfo transferase activity. In addition, the resistant line has a much more active HMP shunt, which is needed to supply reduced glutathione to the above enzymes. All of these changes would be expected to increase the tolerance of the cell line for oxygen free radical attack. Indeed, we find that this cell line is markedly resistance to direct addition of toxic peroxides. These changes suggest that adriamycin kills wild type MCF-7 via the generation of toxic oxygen species. Dr. Ken Cowan is now in the process of cloning the genes in this cell line which confirm adriamyci resistance.

4. Office of the Chief

There are two groups within the Office of the Chief: one under the direction of Dr. Bruce Chabner, the other under Dr. Ken Cowan.

A. Dr. Cowan - Dr. Cowan has continued to characterize the DHFR gene he has cloned from MTX resistant human breast cancer cells. This gene was under estrogen regulation in the original host cell and preserves estrogen regulation now that it is cloned. By selectively deleting 5' upstream flanking sequences. He has been able to show that the hormonally responsive sequence is 110 bases 5' to the start of transcription. In order to confirm this, the putative regulatory sequence was ligated 5' to the end of the bacterial chloramphenicol transferase gene. This hybrid construct expresses well in human and hamster cells. With this construct, CAT expression is now regulated by estrogen. These studies now have clearly defined a system in which hormonal regulation of a human gene responsible for drug resistance may be studied.

Finally, Dr. Cowan has inserted this DHFR gene into a defective retroviral vector which now allows highly efficient transfer of the DHFR gene into mammalian cells. The plan is now to use this to transfer DHFR gene into human marrow and cloned human lymphocyte cell lines. The clinical implications of this are now obvious.

B. Dr. Chabner - There are two major developments from this group. First, this group has continued its elegant work on antifolates. Over the past year they have been able to show that MTX polyglutamates (1) inhibit thymidylate synthethase, (2) inhibit AICAR transformlyase a key enzyme in purine synthesis and (3) are the major determinant of sensitivity of human small-cell lung cancer cell lines to MTX. This work and that which has preceded it are revolutionizing our concepts of MTX action.

The second development is the discovery that a 20K dalton membrane protein is frequently phosphorylated in adriamycin resistant cells. Current results suggest that this protein phosphorylation is by protein-C kinase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-06513-09-CP

PERIOD COVERED

October 1, 1984, to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce A. Chabner, M.D., Director, DCT	DCT, NCI
Gregory A. Curt, M.D., Deputy Director, DCT	DCT, NCI
Carmen J. Allegra, M.D., Clinical Associate	CPB, DCT, NCI
Jacob Baram, M.D., Visiting Fellow	CPB, DCT, NCI
Brenda D. Bailey, M.D., Staff Fellow	CPB, DCT, NCI
Neil J. Clendeninn, M.D., Ph.D., Clinical Associate	CPB, DCT, NCI

[continued on next page]

COOPERATING UNITS *(if any)*

Uniformed Services University for the Health Sciences (USUHS)

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	5.5	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.)*

During Fiscal Year 1985 we have continued our basic projects on antifolate pharmacology and pleiotropic drug resistance. In the antifolate area, studies have been completed on the direct inhibition of thymidylate synthase and AICAR transformylase by methotrexate polyglutamates and by dihydrofolate polyglutamates. These studies have shown that several hundred- to a thousandfold increases in inhibitory activity occur when methotrexate is metabolized to its higher polyglutamate forms. The consequences of this direct inhibition of purine and thymidylate synthesis by methotrexate polyglutamates are being examined in new studies that relate effects on these pathways to intracellular folate levels. Dr. Allegra has worked out an HPLC method for separating intracellular folate cofactors and has shown in the presence of methotrexate there is a rapid rise in dihydrofolate, a fall in 5-methyltetrahydrofolate, but only minor changes in the intracellular levels of N-10-formyltetrahydrofolate (the cofactor required in purine biosynthesis). These findings indicate that folate depletion does not account for the inhibition of purine synthesis and point to the possibility of direct inhibition of this pathway by either methotrexate polyglutamates or dihydrofolate polyglutamates. Currently we favor the latter as being responsible, because the rise in dihydrofolate correlates most closely with the very rapid inhibition of this pathway in cultured cells. We are now undertaking careful studies of the methotrexate analog trimetrexate, which cannot be converted to a polyglutamate, and have shown a similar rapid increase in purine inhibition that correlates with dihydrofolate increases. In addition, Dr. Allegra has undertaken studies of leucovorin rescue. His preliminary evidence shows that cells recover the ability to synthesize purines and thymidylate only after restoration of competitive amounts of intracellular reduced folates. A simple return of the intracellular pool to pretreatment cofactor levels does not provide rescue, indicating

Professional Personnel (cont'd)

Kenneth H. Cowan, M.D., Ph.D., Senior Investigator	CPB, DCT, NCI
Robert L. Fine, M.D., Clinical Associate	CPB, DCT, NCI
Grace C. Yeh, Ph.D., Guest Researcher	USUHS
James C. Drake, Biologist	CPB, DCT, NCI
Sandra J. Occhipinti, Biologist	CPB, DCT, NCI

that folate depletion is probably not the mechanism of inhibition of purine synthesis, but that direct inhibition by polyglutamates is a more likely explanation.

We have also undertaken studies of the effects of methotrexate and the lipid-soluble antifolate trimetrexate on parasites (toxoplasmosis and pneumocystis). The current treatment for these diseases, using trimethoprim and sulfa, is often unsuccessful, particularly in patients with compromised immune function. We have found that trimetrexate and methotrexate are 10,000-fold more potent as inhibitors of dihydrofolate reductase from these parasites and, while methotrexate is not actively transported into the parasite, trimetrexate readily gains entry and is cytotoxic to the toxoplasmosis parasite in vivo and in vitro. Studies of patients with refractory pneumocystis carinii pneumonia have been initiated. In studies of pleiotropic drug resistance, Dr. Fine has identified an enhanced protein phosphorylation in drug-resistant breast cancer cells from MCF-7, ZR-75, and cell lines derived from patients with small-cell carcinoma and neuroblastoma. The enzyme appears to be a membrane-bound, calcium-stimulated kinase that is inhibitable by calmodulin antagonists. Studies of additional patient material and further characterization of the 20,000 molecular weight protein are planned for the coming year. Studies have progressed on 8-mercaptoguanosine, a stimulator of antibody synthesis. Dr. Yeh has shown that this compound is metabolized to nucleotide forms and is incorporated into nucleic acids. This metabolism appears preferentially in B cells, although it was detectable in both the normal and malignant T cells as well. Studies are planned to determine whether the level of incorporation into RNA or DNA correlates with stimulation of immune function.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06515 05 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

The Biochemistry of the Adriamycin-Iron Complexes

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Charles E. Myers, M.D.	Chief	CPB, COP, DCT, NCI
Josephia Muindi, M.D., Ph.D.	Visiting Fellow	CPB, COP, DCT, NCI
Birandra Sinha, Ph.D.	Cancer Expert	CPB, COP, DCT, NCI
Helen Eliot	Biologist	CPB, COP, DCT, NCI
Jack Cohen, Ph.D.	Chemist	CPB, COP, DCT, NCI
Babul Borah, Ph.D.	Visiting Associate	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Jay Zweier, M.D., Laboratory of Technical Development, NHLBI
Abraham Levey, M.D., Dept. of Physics, Johns Hopkins, Baltimore, MD

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	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 spece provided.)*

The adriamycin-iron chelate binds to DNA to form a ternary complex which cleaves H_2O_2 to yield $\cdot OH$. Of the anthracyclines tested, those most active in this chemistry are also cardiotoxic.

Adriamycin is able to abstract iron from such biologically important sites as ferritin and transferrin. Adriamycin reduces $Fe3^+$ to $Fe\ 2^+$ after it chelates the metal.

In previous years, we had demonstrated that adriamycin chelated Fe(III) and that the resulting complex could bind tightly to RBG ghost membranes and DNA. The resulting ternary complex can then catalyze redox chemistry leading to DNA cleavage. This past year, we have extended these observations.

1. Adriamycin reduces Fe^{+3} to Fe^{+2} - J. Biol. Chem. 260: 6820, 1985

We have been able to show that the Fe^{3+} adriamycin complex undergoes an internal electronic rearrangement to yield Fe^{2+} and the adriamycin one electron oxidized free radical. The Fe^{2+} can then react with oxygen to yield H_2O_2.

2. Hydroxyl radical formation by the drug iron complex - FEBs Letters 172: 226, 1984

We have been able to show that the drug-iron and drug-iron-DNA complex will react with H_2O_2 to yield the hydroxyl radical. Perhaps the most important aspect of this work is that the ternary complex with DNA is 4X more active in this than is the adriamycin-iron complex. The implications of this is that the very reactive and destructive hydroxyl radical is generated in the immediate vicinity of its macromolecular target.

3. Structure activity relationships - Mol. Pharm. 27: 356, 1985

We have been able to show that only those analogs which possess cardiac toxicity are able to do all of the following:

a) chelate Fe(III)

b) catalyze reduction of oxygen by GSH

c) form a ternary complex with DNA

d) the resulting ternary complex catalyzes hydroxyl radical formation from H_2O_2.

Because those agents which are cardiotoxic are also those with antitumor activity we were not able to determine the role of the iron complex in tumor cell kill.

4. Structure of the anthracycline-iron complex - We are now in the process of using NMR to define (a) the structure of the adriamycin-iron complex; (b) the structure of the adriamycin-iron-DNA complex.

5. Availability of iron to adriamycin in vivo - Iron in vivo is not free but rather bound to transferrin, ferritin and in the phorphyrin ring. We have been able to show that adriamycin abstracts iron from transferrin and can use that iron to destroy DNA.

Significance to biomedical research and the program of the Institute:

We fell that the above studies go a long way toard defining the properties of the adriamycin iron complex and that the way is now open for definitive tissue culture work on the role of these iron complexes in cell kill.

PUBLICATIONS:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06516 04 CP

PERIOD COVERED

October 1, 1984 - September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenneth H. Cowan, M.D., Ph.D.	Sr. Staff Fellow	CPB, COP, DCT, NCI
Merrill E. Goldsmith, Ph.D.	Staff Fellow	CPB, COP, DCT, NCI
Anil Tulpule, Ph.D.	Visiting Fellow	CPB, COP, DCT, NCI
Elizabeth Rubacalba, B.A.	Chemist	CPB, COP, DCT, NCI
Marie Ricciardone, M.S.	Chemist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Medicine Branch, COP, DCT, NCI
Pediatric Oncology Branch, COP, DCT, NCI

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2	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.)*

The major work in our laboratory has been in studies on the regulation of dihydrofolate reductase gene expression in human breast cancer cells. Human DHFR genomic sequences have been cloned from a gene amplified drug resistant cell line and have been used to form a DHFR minigene. The regulation of this minigene has been studied in a cellular expression systerm following transfection into mutant Chinese hamster cells. We have also developed retroviral vectors which contain an altered DHFR gene which can efficiently transfer methotrexate resistance to a wide variety of cells. Finally, we are also studying the mechanisms responsible for the development of pleiotropic drug resistance in an MCF-7 cell line isolated in our lab. These studies have identified increased levels of proteins and amplified DNA sequences which may play a role in the development of resistance.

PHS 6040 (Rev. 1/84)

GPO 914-918

A. Regulation of Dihydrofolate Reductase Gene Expression

In the past year our laboratory has been investigating the regulation of dihydrofolate reductase (DHFR) gene expression in cells. Dr. Merrill Goldsmith constructed a functional DHFR minigene using both human genomic and cDNA sequences for DHFR. Following transfection of Chinese hamster cells which lack functional DHFR activity we have shown that the minigene is able to reconstitute enzyme activity and convert these cells with each $DHFR^+$ phenotype. The enzyme is regulated in these transfected cells similar to the regulation of the endogenous gene. We have used two conditions to regulate DHFR reductase levels in these cells, i.e. addition of serum to serum deprived cells and repletion of amino acids to amino acid depleted cells. Both of these conditions regulate the level of DHFR reductase in wild type Chinese hamster and human cells as well as in minigene transfected cells. We have constructed a series of deletion mutants of the minigene and transfected these into CHO cells. Subsequent studies on the regulation of minigene transfected cells have enabled us to identify 5' and 3' regions which are involved in the cellular regulation of the DHFR gene.

We have also been studying the regulation of DHFR reducatase gene expression in human breast cancer cells in response in both estrogen and anti-estrogens. We have demonstrated that estrogen increases and tamoxfin decreases DHFR reductase in MCF-7 cells and that the regulation involves alterations in DHFR reductase enzymes synthesis. These changes also correlate with changes in the concentration of DHFR reductase messenger RNA in human breast cancer cells.

Since we do not have a mutant MCF-7 cell line which lacks DHFR activity we can not study the regulation of the minigene in these cells. In order to study the hormonal control of the regulation of this gene we have used a transient expression system in which the 5' end of the DHFR gene (promotor sequences) to the bacterial chloramphicol transferase (CAT) gene. This construct is expressed well in hamster, monkey and human cells. We are currently using this hybrid gene construct to evaluate the hormonal regulation of the DHFR promotor sequences in human breast cancer cells.

B. Retroviral Constructs

Other studies which have been done this year in our laboratory involves the development of retroviral vectors for the efficient transfer of drug resistance genes into cells. We have used a defective retroviral sequence from an amphotrophic virus and inserted an altered dihydrofolate reductase gene. This virus is capable of infecting mouse as well as human cells and has a high efficiency of transfer of methotrexate resistance. Different viral constructs are being compared in our lab with respect to their ability to transfer the altered DHFR into cells of different species. In particular, we will be trying to insert this gene into the bone marrow cells as well as into cloned lymphocyte cells to test the relative drug sensitivity of these cells *in vivo*.

C. Pleiotropic Drug Resistance

We are also in collaboration with Drs. Myers, Batist, Chabner, Fine and Yeh in the Clinical Pharmacology Branch studying the mechanisms of pleiotropic drug resistance in human breast cancer cells. An adriamycin resistant breast cancer cell line which was isolated in our lab. This cell line is markedly cross resistant to a wide spectrum of drugs and have been useful for a variety of studies ongoing in our Branch: 1) Dr. Batist has shown that the resistant cells contain over 100-fold increase in an anionic form of glutathione transferase. 2) Dr.Yeh has been studying the HMP shunt induction in this cell line and has shown that the shunt in the drug resistant line is markedly inducible by a variety of different inducers. This may have important implications regarding the role of the redox cycle in the development of drug resistance. 3) Dr. Fine has found a marked increase in a phosphorylated membrane protein in the resistant cell line. Dr. Fine is currently studying the activities of various protein kinases which may be involved in the phosphorylation of this membrane protein. 4) In collaboration with Drs. Neil Rosen and Mark Israel we have identified DNA sequences which are apparently amplified this cell line. We have made genomic and cDNA libraries of this cell line and are currently trying to isolate the amplified in DNA sequences from the resistant cells. We also intend to isolate the glutathione transferase gene from these libraries in order to study its structure and regulation.

PUBLICATIONS:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-CM-06518-04-CP

PERIOD COVERED
Oct. 1, 1984 to Sept. 30, 1985

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jerry M. Collins, Ph.D.	Pharmacologist	CPB, COP, DCT, NCI
Raymond Klecker, B.S.	Chemist	CPB, COP, DCT, NCI
Charles Myers, M.D.	Branch Chief	CPB, COP, DCT, NCI
Karl Belanger, M.D.	Visiting Fellow	CPB, COP, DCT, NCI
Jean Jenkins, R.N.	Research Nurse	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
NCI/DCT/COP: ROB, PB, MB; NCI/DCT/DTP; NCI/DCT/CTEP
Non-NCI: BEIB/DRS/NIH; University of Maryland Cancer Center

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Pharmacokinetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	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.)*

There is considerable diversity in the classes of drugs under investigation, and also the diseases which are targeted by this group. Nonetheless, the primary focus is always on the application of pharmacokinetic principles to questions of relevance to the treatment of cancer.

Over the last year, specific areas of interest included:

1. Relationships between in vitro pharmacology and clinical trials targeted against HTLV-III/AIDS. Matching of in vitro and in vivo exposure conditions, determination of clinical pharmacokinetics of suramin, and application of kinetic analysis to suggest improved schedules of administration.
2. Although intramural clinical resources are limited, Phase I trials of new agents remain as an important element in a balanced approach to the clinical pharmacology of anticancer drugs. Trials were completed for spiromustine and tiazofurin, and started for trimetrexate.
3. In collaboration with the Radiation Oncology Branch, work has shifted from the use of BUdR to IUdR, with specific focus on the manipulation of intracellular pharmacology.
4. Pharmacokinetic evaluation of established agents. Studies of cisplatin and 5-fluorouracil were completed, while work was begun on thio-TEPA.

PHS 6040 (Rev. 1/84)

GPO 914-918

(1) AIDS

This group has redirected its resources to support the DCT initiative in the treatment of AIDS with the antiparasitic drug, suramin. An HPLC method was developed by Ray Klecker and used to determine suramin levels in plasma, urine, and cells from patients. In addition, this method has been circulated to 6 extramural groups through the Cancer Therapy Evaluation Program (CTEP). The pharmacokinetic analysis of suramin was presented at ASCO in Houston and is being used to guide the design of further intramural and extramural trials. The clinical pharmacology of other drugs which might be used for the treatment of AIDS is being coordinated with the Biochemical Pharmacology Section of CPB and the Office of the Associate Directory, Clinical Oncology.

(2) Blood Level Working Group

Due to reduced manpower within the Pharmacokinetics Section and the allocation of resources to the suramin study, work on this project has been restricted to collaborations with the extramural DCT staff at CTEP and DTP. Over the last year, there has been significant progress in the integration of continuous infusion testing between preclinical and clinical testing. This coordination has resulted in a 3-fold acceleration in reaching target clinical levels of HMBA, a differentiation inducer, based upon a more appropriate starting dose. Similar savings are anticipated over the next 6 months for merbarone and deoxyspergualin. Work continues on the establishment of a relationship between species differences in toxicology and in metabolism.

(3) Iododeoxyuridine

Collaboration with the Radiation Oncology Branch continues in the development of halogenated pyrimidines as radiosensitizers. This section has focused upon monitoring of the biochemical modulation of endogenous pyrimidine pools as a result of iododeoxyuridine infusion. For the next year, we will be extending these studies towards strategies which can manipulate intracellular thymidine pools and enhance the rate of incorporation of iododeoxyuridine into DNA.

(4) Thio-TEPA

As part of a continuing effort to improve our understanding of the clinical pharmacology of established anticancer agents, we explored the pharmacokinetics of Thio-TEPA in collaboration with Dr. Poplack in the Pediatric Branch. We have defined the plasma and CSF levels of Thio-TEPA and its cytotoxic metabolite (TEPA) in monkeys following intravenous and intrathecal administration. Confirmatory studies were undertaken in two patients.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06519 02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Non-Invasive Studies of Metabolism Using Nuclear Magnetic Resonance Methods

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jack S. Cohen, Ph.D.	Research Chemist	CPB, COP, DCT, NCI
Robbe Lyon, Ph.D.	Sr. Staff Fellow	CPB, COP, DCT, NCI
Patrick Faustino, M.Sc.	Chemist	CPB, COP, DCT, NCI
Gerald Batist, M.D.	Visiting Associate	CPB, COP, DCT, NCI
Mildred Shoemaker	Bio Lab Technician	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biophysical Pharmacology Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	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.)*

A true understanding of cellular biology, and of therapeutic effects on pathological conditions under a variety of experimental conditions, requires the noninvasive monitoring of metabolism. Nuclear magnetic resonance (NMR) methods enable metabolism to be studied noninvasively. We have developed a cell perfusion technique allowing the effective application of NMR methods to cell lines grown in culture. This technique consists of embedding cells in a neutral agarose gel thread (0.5 mm) which allows continuous perfusion and rapid diffusion of metabolites into the cells. We are currently applying ^{31}P ^{1}H and ^{13}C NMR to study the metabolism of human lymphocytes and breast cancer (MCF-7) cell lines and the effects of perturbants, such as heat, pH and drugs, upon them. We are also developing sensitive surface coils using the same multi-nuclear NMR approach. This will enable us to carry out investigations on subcutaneous tumors in rodents in vivo, in order to correlate findings with the well-controlled in vitro studies.

Objectives:

To compare details of metabolism noninvasively in normal, diseased, and cancerous cells, particularly comparison of drug sensitive and resistant cell lines. To quantitate metabolic rates and changes in internal pH, and to follow drug metabolism, both in vitro and in vivo.

Methods Employed:

High resolution ^{31}P, ^{1}H and ^{13}C NMR and double resonance methods utilizing the new Varian XL-400 spectrometer. Cell perfusion method developed by this laboratory (see below).

Major Findings:

A. Differences in Phosphocreatine Levels in Drug Sensitive and Resistant Cell Lines by ^{31}P NMR Spectroscopy

^{31}P spectra were recorded numerous times for sensitive (7 times) and resistant (15 times) cell lines over a period of one year on two spectrometers using the gel perfusion method (see below). The wild type cells showed the usual peaks corresponding to phosphate monoesters, Pi, ADP/ATP, and diphosphodiesters. The spectra of the resistant cell lines contained an additional peak at -3.7 ppm corresponding to phosphocreatine (PCr) (spectrum B). Such a peak was never observed in spectra of the sensitive control cells, while it was observed in almost every case with the resistant cell lines. The relative area of the PCr peak compared to ATP (using the β-ATP peak; ATP was ca. 2 mM), varied from up to 20% for the ColR and its corresponding subcloned lines, and up to 50% for AdrR cells. The PCr peak remained constant throughout changes in temperature and pH (7.5/5.5/7.5), and samples stored overnight at 4° regenerated the PCr peak on perfusion.

Whether this observation results from increased levels of creatine kinase in the resistant cell lines, or expression of PCr due to a change in metabolic control, is under investigation. It should be noted that the level of diphosphodiesters (e.g. UDPG) were generally higher for the sensitive compared to the resistant cells. These results are consistent with previous results of studies of lung cancer cell lines, where increased PCr peaks were observed only for the clinically more drug resistant (small cell variant) cell lines.

B. Development of a perfusion system for ^{13}C NMR studies of metabolites and drugs.

The perfusion system developed previously on the basis of cells embedded in gel threads has been improved and extended for ^{13}C NMR studies of ^{13}C-enriched metabolites and drugs. The use of ^{13}C-labeled materials requires a small, closed pefusion system in which the gas composition can also be controlled. We have built such a system and are monitoring uptake of ^{13}C-acetate and ^{13}C-glucose into the gel threads containing cells. Our results show that, a) the presence of the cells does not prevent rapid diffusion and equilibration of metabolites into the cells; b) the use of a small volume results in a mixing artifact, which nevertheless, allows equilibration in ca

2 mins. The rate of glucose metabolism has been measured in human lymphocytes using ^{13}C-glucose over a period of hours. These studies will be extended to cancer cells monitored under different conditions, and to the use of $^{1}H[^{13}C]$ NMR observation, which should enable faster metabolic processes to be observed.

C. In Vivo Studies

NMR spectroscopy of small animals in vivo requires specialized probes which are not routinely commercially available although several are described in the literature. In general an in vivo probe consists of the following components: 1) RF coils, with appropriate electronic circuitry, each specific for the nuclei of interest, designed and positioned to examine the tissues or organs of interest; 2) a cradle to restrain and position each type of animal; 3) a probe head which accommodates the first two components in proper alignment; and 4) the stack which positions the probe head in the magnet.

At the present time we have designed a stack which will be universal to our operations and two probe heads. The first probe head contains a plastic cradle designed to restrain a small rat and a surface coil tuned for phosphorous (162 MHz) which slides vertically into position on the surface of the animal. This probe head is best suited for ^{31}P studies of muscle, brain, or liver. The second probe head was designed to study subcutaneous tumors in mice. A copper cradle restrains the mouse and serves as a shield which blocks NMR signals from the host tissue. The tumor protrudes through a hole in the shield into a area enclosed by two coaxial surface coils. The outer coil is tuned for ^{1}H at 400 MHz and the resonance frequency of the inner coil ranges from 90-165 MHz to include ^{13}C (100 MHz) and ^{31}P (162 MHz). This probe is designed for ^{1}H observation with either ^{13}C or ^{31}P decoupling or ^{13}C or ^{31}P observation with ^{1}H decoupling.

Construction of the stack, the first probe head and the coaxial surface coils have been completed. The second probe head is currently under construction at the NIH instrument shop.

Significance to biomedical research and the program of the Institute:

Studies of metabolism using noninvasive NMR methods should provide insight into fundamental aspects of metabolic pathways, elucidate differences between normal and cancerous states, and allow drug metabolism to be monitored in situ

Proposed course:

Work will actively continue applying non-invasive NMR techniques to in vitro studies of cell metabolism, particularly using the perfusion system we have developed. Rates of ^{13}C-glucose metabolism will be compared for drug sensitive and resistant cell lines under aerobic and anaerobic conditions. Priority will be given to studies having potential clinical significance, particularly drug effects on human cancer cell metabolism. Parallel experiments will be

carried out in vivo using surface coils to obtain optimal signal for ^{1}H. Mice and rats will be used to detect metabolic activity using the ^{1}H [^{31}P] and ^{1}H [^{31}C] double resonance methods. Initial experiments will concentrate on subcutaneous and brain tumors including the use of ^{13}C-2-deoxyglucose. This in vivo work will become a major effort when an in vivo NMR spectrometer is purchased as proposed.

PUBLICATIONS:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CP-06520-02-CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Magnetic Resonance Imaging Applied to Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jack S. Cohen, Ph.D.	Research Chemist	CPB, COP, DCT, NCI
Robbe Lyon, Ph.D.	Sr. Staff Fellow	CPB, COP, DCT, NCI
Charles E. Myers, M.D.	Chief	CPB, COP, DCT, NCI
David Colcher, M.D.	Chemist	DCBD, NCI
Francoise Mornex, M.D.	Visiting Fellow	DCBD, NCI
Arthur Katz, Ph.D.	Guest Researcher	CPB, COP, DCT, NCI
Patrick Faustino, M.Sc.	Chemist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Peter Hambright	Professor	Howard University
Robert Bryant	Professor	Rochester University
Seymour Koenig	Staff Scientist	IBM Research Center

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biophysical Pharmacology Branch

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

To develop strategies to facilitate the application of magnetic resonance imaging (MRI) to the detection of malignant growths. Specifically to develop contrast agents to enhance the vizualization of tumors. These agents are considered to be valuable both for the increase in intrinsic contrast relative to surrounding soft tissue, as well as the discrimination between benign and malignant growths by MRI. Also, by the use of MRI to attempt to localize the in vivo distribution of anti-cancer drugs.

GPO 914-918

Objectives:

Magnetic resonance imaging is becoming an important tool in diagnostic radiology. In particular, it is possible to discriminate between a growth and surrounding soft tissue on the basis of differences in the overall relaxation times (T_1 and T_2) of the bulk water in the respective tissues. A substance which alters the relaxation rate of the water must be paramagnetic, i.e. it must contain one or more unpaired electron spins. Our initial objective was to identify a class of paramagnetic substances which would be selectively retained by a tumor, but which would not be toxic.

We have chosen to test water-soluble metalloporphyrins as NMR contrast agents for several reasons. Porphyrins are ubiquitous natural products, and many derivatives of hematoporphyrin are well known to be selectively retained by tumors. Water-soluble porphyrins such as the tetraphenylsulfonyl porphyrins (TPPS) have also been shown to be localized in tumors and in cells. These porphyrins form complexes with a wide variety of paramagnetic metal ions and the metalloporphyrins formed from them are stable in vivo. Metalloporphyrins have been used as contrast agents in radiological imaging, and a great deal is known about their selective retention by different tissues, including tumors, in live animals. Particularly, it was found that the radioactive ^{109}Pd derivative of TPPS, showed the greatest degree of selective retention in cancerous vs normal tissue in mice. Consequently, we have evaluated several of these water-soluble metallo-TPPS complexes as potential contrast agents in NMR imaging by measuring their effects on the spin lattice relaxation rate ($1/T_1$) of water.

Methods Employed:

Relaxation times (T_1 and T_2) of bulk water are measured at 10 MHz with a Praxis II pulse spectrometer. All measurements were performed at a temperature of 37°C, determined with a glass-encased thermocouple immersed in the sample. The effectiveness of different metalloporphyrins in altering the relaxation rate ($1/T_1$) of water on a molar basis (the relaxivity) was compared.

The bulk water relaxation rates of samples of excised tissues and whole tumors from athymic mice (musmusculus) were measured in the same way, both before and several hours after intravenous injection with the metalloporphyrins. The mice had subcutaneously implanted human colon carcinoma (LS-174T) cells, which had produced malignancies of ca. 1-2 cm.

Magnetic resonance imaging was carried out on a Picker Corp. whole body superconducting system operating at 0.26 T (10.08 MHz) using a 30 cm transmitter coil. Maximum contrast of the colon carcinoma implants was obtained with a spin echo image sequence (TE=26 msec, TR=250 msec) and with an inversion recovery image sequence (TI=400-600 msec, TR=1500 msec). The mice were sedated with prior injection of pentobarbital, and the metalloporphyrins were injected intravenously into the tail.

Major Findings

A. Tissue Distribution and Stability of Metalloporphyrin NMRI Contrast Agents

Water-soluble paramagnetic metalloporphyrins (MPs) exhibit efficient relaxivity, and the MnIII complexes of tetra-(phenylsulphonato)porphyrin (TPPS) and tetra-(N-methyl-4-pyridyl) porphyrin (TMPyP) have been shown to provide enhanced contrast in NMR images of human colon carcinoma in mice. The present study was undertaken to elucidate: 1) the in vitro and in vivo stability of the MPs, 2) the in vivo distribution and dose-response relationship of the relaxation enhancement, and 3) the relaxation processes operating for the MPs. It is hoped that these results will provide a basis upon which the utility of MPs as NMRI contrast agents can be further evaluated.

(i) Stability of MPs

For in vitro stability studies MPs were dissolved in human plasma, alone and in mixture with the parent porphyrin (10 and 20%). Optical spectra clearly showed the differences between the porphyrin and its MP derivative upon which an estimate of the degree of free porphyrin could be based. Spectra were collected as a function of time following incubation at 37°. In the case of MnTPPS no detectable free porphyrin was observed. There was a gradual loss of intensity in all peaks, which we attributed to increased turbidity. Preliminary optical spectroscopic results with extracts of tissues taken after timed intervals also indicate the absence of free TPPS.

(ii) In Vivo Distribution and Dose-Response Relationship of MPs

Although distribution studies of radioactively labeled MPs have been reported, these were done at much lower concentrations than are required for paramagnetic relaxation effects. Therefore, we studied the distribution of MnTPPS in mouse tissues using comparative relaxation measurements, optical spectroscopy, and metal ion determination on extracts. We found in general that MPs are most concentrated in the kidney, tumor, and liver, in that order, with very little in the muscle; however, the precise relaxation rates for all tissues were initially enhanced and then decreased with time. But, at a dose of 667 mg/kg, both single frequency and NMRD measurements showed a significant increase in the relaxation rate for the tumor with time, while the values for the kidney and liver continued to decrease. This high dose level provided enhanced contrast in NMR images of tumors in mice. We have no simple explanation for this unusual dose relationship of the relaxation effect in tumors, but it does appear that the MP is being re-distributed at the higher concentration, and then subsequently retained in the tumor.

(iii) Relaxation processes of MPs

An unexpected maximum at 10 MHz has been observed in the NMRD curve for MnTPPS. This has not yet been satisfactorily explained, but it does emphasize that the relative values of relaxivity of any series of compounds do depend on their

relative dispersion characteristics, and that 10 MHz happens to be a good choice of field at which to observe water relaxation with MnTPPS. Similar studies are underway with other MPs, including GdTPPS and a CoII analog.

B. Relaxivity of MP Contrast Agents

In order to investigate why the Fe(III) MP complexes are less efficient relaxation agents than the Mn(III) complexes we have compared the relaxivity of several "picket fence" porphyrins (PFP) as a function of pH. The standard Fe-porphyrin complexes dimerize, while the bulky picket fence groups on the periphery of the porphyrin ring sterically prevent this occurring. We have found that the relaxivity of Fe-TPPS decreases by a factor of a 50 in going from pH 1 to 7, where dimerization occurs, while the FePFP decreases by only ca 1.5. By contrast the Mn III FPF shows no pH dependence and its relaxivity is twice as large as Mn TPPS, indicating that it may be a promising contrast reagent.

Proposed Course:

Preliminary results indicate that improved selective contrast enhancement of a tumor can be obtained with certain water-soluble paramagnetic metalloporphyrins. Currently we are testing similar water soluble porphyrin complexes of other paramagnetic metal ions (Gd(III)) and Mn(III) complexes with various porphyrins. We are comparing the effectiveness of different dosages and time courses of the Mn complexes. It is hoped that non-toxic doses of such metalloporphyrins may, in the future, be applicable as MRI contrast agents for tumors in humans. To carry out studies of dose vs. image contrast in animals, it is proposed to purchase an animal MR imager with 30 cm bore horizontal magnet operating at 2.3T. This would also be used for spectroscopy and ultimately would be used to obtain spatially localized spectral information.

PUBLICATIONS:

Patronas, N.J., Cohen, J.S., Knop, R.H., Dwyer, A.J., Colcher, D., Lundy, J., Monex, F., Hambright, P., Sohn, M. and Myers, C.E.: Metalloporphyrin Contrast Agents for Magnetic Resonance Imaging of Human Tumors in Mice. Cancer Treatment Reports, in press.

Lyon, R., Faustino, P., Monex, F., Cohen, J.S., Colcher, D., Koenig, S., Baglin, C. and Bryant, R.: Tissue distribution and stability of metalloporphyrin contrast agents. Manuscript in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06521 02 CP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the bordars.)*
Conformations and Interactions of Nucleic Acids, Proteins and Drugs in Solution

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jack S. Cohen, Ph.D.	Research Chemist,	CPB, COP, DCT, NCI
Babul Borah, Ph.D.	Vis. Assoc.	CPB, COP, DCT, NCI
Charles E. Myers, M.D.	Chief	CPB, COP, DCT, NCI
Siddhartha Roy, Ph.D.	Vis. Assoc.	CPB, COP, DCT, NCI
Patrick Faustino, M.Sc.	Chemist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Dennis A. Torchia, NIDR; Gerald Zon, BB/FDA; Ettore Appella, BCBD/NCI;
Heisaburo Shindo, Tokyo College of Pharmacy, Tokyo, Japan;
Todd Miles, Senior Investigator, LMB, NIADDK

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Biophysical Pharmacology Section

INSTITUTE AND LOCATION
NIH, National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	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.)*

Details of the interactions of biological macromolecules (proteins and nucleic acids) in solution with each other and with small molecules, such as drugs, are being probed at the molecular level. Generally such interactions are mediated by conformational alterations in the macromolecule. The method of choice to investigate the conformations of proteins and nucleic acids in solution is nuclear magnetic resonance (NMR) spectroscopy. We use ^{1}H, ^{2}H, ^{13}C and ^{31}P NMR in combination with selective stable isotopic enrichment (^{2}H and ^{13}C) to study the conformations and solution properties of proteins and DNA. Currently we are focusing on the effects of base sequence on the conformational transitions of selected synthetic poly and oligodeoxy-nucleotides and the effects of cytotoxic drugs on those transitions. We are applying 2-dimensional proton NMR to obtain relative atomic distances and hence are able to define DNA conformations and drug complexes in solution in molecular detail.

Objectives:

1. To elucidate the detailed conformations of DNA in solution, and to investigate their relationships to genetic function, interactions with protein, and drug binding. To specify the molecular structure of the complex between DNA fragments and adriamycin and its analogs. 3) To estimate the mobility of deuterated sugar residues in DNA and RNA and to use them as monitors of binding properties. 4) To extend the study of protein functional groups (such as Met, Glu, Asp, Thr, etc.) which are not amenable to study by methods other than NMR spectroscopy, in order to study conformations, transitions and binding properties of biologically active peptides and proteins in solution.

Methods Employed:

High resolution NMR spectroscopy is the method of choice to study molecular conformation and interactions in solution. In FY 1984 we purchased a Varian XL-400 NMR spectrometer. We have spent much time learning how to use this sophisticated computer-controlled system. Much of our current work utilizes two main kinds of two-dimensional NMR. (In these methods two orthogonal proton frequency scales are produced from a series of spectra, which differ systematically in the state of the spin system by varying a time between two pulses). We have utilized 2D correlated spectroscopy (2D-COSY), in which the cross-peaks give information about through bond couplings of protons, and 2D-nuclear Overhausar effect spectroscopy (2D-NOESY), in which cross-peaks are derived from through-space interactions of protons, which are closer than Ca.4A. We have also used ^{31}P NMR of oligo and polynucleotides, and ^{13}C NMR of drug molecules, as well as the ^{1}H-^{13}C 2D heterocorrelation method as a valuable aid in resonance assignments.

The relaxation properties of NMR observable nuclei can be related to molecular motions. Relaxation of nuclei with spin 1/2 (e.g. ^{1}H and ^{31}P) are related to molecular motions through internuclear (dipolar) and intranuclear (chemical shift anisotropy) mechanisms. If these two interactions are comparable in strength, it is difficult to analyze relaxation data to obtain information on motion, although in favorable cases motional characteristics can be deduced. Relaxation of nuclei with spin > 1/2, such as ^{2}H with spin = 1, are usually straightforward to interpret in terms of motion, because the dominant relaxation mechanism is the intranuclear electric quadrupole coupling. Consequently we have developed a novel method of synthesis of selectively deuterated nucleosides from which we will prepare polynucleotides. We will study the mobility of the sugar rings using solution and solid state deuterium NMR methods. We also routinely use optical spectroscopy (UV) and circular dichroism (CD) to study conformational transitions.

Major Findings:

A. Polynucleotide conformation - The proton 2D-NOE NMR method is perhaps the best technique for elucidating the conformations of polydeoxynucleotides in solution. In particular the patterns of cross peaks observed for the B and Z forms of DNA are entirely distinct. The $2NH_2A$ base is of interest

because it forms 3 H-bonds (with T) like a GC base pair. Poly(d2NH_2A.dT) exhibits a sharp conformational transition in high salt (mid point 1.5 M NaCl), and from the CD spectrum, it was presumed that the high salt form was a Z-form. However, 2D-NOE spectra gave a pattern of cross peaks that corresponded to neither the B nor Z forms. The presence of an AH8,TH_6-H3' cross peak indicates the presence of a 3' endo sugar conformation, which corresponds only to an A-form. This is the first time that an A-form has been described in salt solution for the deoxy series. However, ribopolymers are known to exist, predominantly in the A-form. Due to the shift of sugar resonances in the ribose series, the resolution between H2', H2'', H3' and H_2O signals is very poor. However, preliminary evidence indicates that we can resolve a similar H3' cross peak in the case of poly(r2NH_2A-rU). We are also comparing hybrid r.d and alternating r/d copolymers.

B. Oligonucletide Conformation and Drug Binding - Of the four bases present in DNA only cytosine contains two adjacent aromatic protons (at C5 and C6) that are strongly spin-coupled and that give rise to cross-peaks in the aromatic region of 2D-COSY spectra of oligodeoxynucleotides. We have observed these cross-peaks in oligomers of different base sequences. Although the C5 and C6 proton resonances of individual bases are not well resolved in 1D spectra because of overlapping resonances, the cross-peaks in the 2D-COSY provide superior resolution. In d(GC)5 up to four such cross peaks can be resolved, and the pattern is essentially independent of temperature in the range 6-37 degrees. Ths indicates that the resolution observed does not arise from fraying of chain ends, but is an intrinsic difference reflecting chemical shift end-effects and perhaps also local conformational effects. In the duplex d(ATATGGCCCAATT/TATACCGGTTAA) four 2D-COSY cross-peaks are also resolved. We are now using these cross-peaks to monitor the environments of individual C bases in oligonucleotides in the B and Z forms. These base sequences were also chosen in order to investigate the base sequence specificity of the anti-cancer drug daunomycin, and we have preliminary evidence that the binding of daunomycin affects certain C bases preferentially. A similar analysis applies to the 2D-COSY cross-peaks of oligoribonucleotides, with the additional feature that uridine (U) as well as C give cross-peaks. This approach should be useful in examining the environments of individual pyrimidine bases in t-RNA.

Preliminary evidence from observation of imino (Watson-Crick) hydrogen bonded protons shows that daunomycin does not bind to d(A_6T_6) duplex, which contains only one alternating AT sequence, while it does bind to $d(AT)_{12}$ and is reported from other methods to show a preference for this alternating sequence. Since it is known that poly(dA).poly(dT) has a different conformation than the standard B-form, we presume this difference in drug binding may arise from the local DNA conformation, and that a two base pair sequence alone is not enough to define the daunomycin binding site. We are examining the conformation of these AT oligomers and poly(dA).poly(dT) to clarify this phenomenon.

We have carried out Fe^{3+} binding studies with adriamycin and daunomycin in various solvents. For both drugs very broad NH and OH resonances are observed in non-aqueous solvents (d_6-DMSO,d_4-methanol) unless the solution is acidified.

This arises from exchange processes with the residual water. Upon addition of Fe^{3+} to daunomycin in acidified d_6-DMSO or d_4-methanol no selective line-broadening effects on the NH, OH or aromatic resonances have yet been observed, indicating an exchange process of Fe^{3+} between several sites. Studies are being conducted at different "pH" values to detect selective binding. However, quantitation of this process, and average distance information from specific protons have not yet been estimated. ^{13}C NMR observation should also provide structural information on the Fe^{3+}-drug complexes.

D. Synthesis of 2', 2''-Deuterated 2'-Deoxyguanosine and Sugar Ring Flexibility by Solid State Deuterium NMR - Information on the mobility of sugars in general is not available for polynucleotides, because of the lack of resolution of the sugar proton resonances, and the difficulty of labeling sugars with ^2H and ^{13}C. Deuterium NMR has been used to study motion of deuterium labeled substances. Information of sugar mobility may throw new light on conformational states and mechanisms of conformational changes in DNA and drug binding properties.

We have developed a new enzymatic synthesis of a selectively deuterated deoxyribonucleoside, 2',2''-deuterated 2'-deoxyguanosine. We have deuterated 2-deoxyribose 5-phosphate from unlabeled material by enzyme catalyzed exchange with solvent D_2O. The enzyme used was 2-deoxyribose 5-phosphate aldolase. Usually equilibrium in the aldolase reaction lies well towards the synthesis side and hence the yield of labeled material will be high. The next step involves conversion of labeled 2-deoxyribose 5-phosphate to 2-deoxyribose 1-phosphate by phosphopentomutase, followed by attachment of the base guanine by purine nucleoside phosphorylase. The equilibrium of the phosphopentomutase reaction lies well towards the 2-deoxyribose 5-phosphate, but the equilibrium of the nucleoside phosphorylase reaction lies well towards the nucleoside product. In order to obtain a reasonable yield of nucleoside from 2-deoxyribose 5-phosphate it is necessary to couple these reactions.

This method has the potential to introduce both ^{13}C and ^2H at any position of the deoxyribose ring of guanosine or hypoxanthine. Although nucleoside phosphorylase from calf thymus has strict specificity for hypoxanthine and guanosine, the E. coli enzyme accepts adenine as a substrate. Thymidine phosphorylase is commercially available and we have used it to synthesize deuterated thymidine. Since the reaction synthesizes the deoxyribose ring from smaller molecules, acetaldehyde and glyceraldehyde 3-phosphate, this method has potential to become a general method of synthesizing nucleosides with labeled deoxyribose sugars.

We have also made a preliminary examination of the mobility of the sugar ring by solid state deuterium NMR spectroscopy. The spectrum shows a component with a quadrupolar splitting of ca. 120 kHz which is approximately the same as that of rigid polymers. However, it was estimated by observing peak intensity at different recycle delay times that the average T_1 of the deuterium was less than ca. 1 sec. This is considerably shorter than the T_1 (ca 20 sec) of the more rigid deuteriomethylene group of crystalline polyethylene. This

indicates the presence of small amplitude motions on a time scale of $< 10^{-6}$ sec.

E. Lambda Repressor-DNA Interaction in Solution

Lambda repressor C1 is a protein of MW 2x22000. It binds to a 17 base pair deoxynucleotide fragment of known sequence. The repressor has been cloned and its primary sequence and crystal structure determined. Thus, this is an ideal system in which to study protein-nucleic acid interaction.

We have been interested in determining the origin of specificity of protein-nucleic acid interactions. We would like to introduce labels of NMR observable nucleic into the protein as well as the 17bp nucleic acid fragment at residues thought to play important roles in the interactions. Then NMR could be used to monitor the loci of interaction of the protein and the 17bp fragment.

Initially, our plan is to introduce deuterium label at the N-terminal residue. This part of the protein has been hypothesized to be flexible but becomes immobilized upon addition of the 17bp DNA. These few N-terminal residues are thought to provide some of the specificity of the repressor operator interaction. The deuterium label would give us a direct handle to determine its flexibility and would be useful in verifying this model. Later other labels can be introduced to probe interactions more specifically. The 17bp operator has already been synthesized. We are in the process of purifying the protein from an overproducing strain.

F. Miscellaneous

As part of our function to provide other labs, particularly within NCI, with a service, we have run NMR spectra for two groups. a) ^{1}H NMR spectra of a splice peptide in D_2O and a helix-forming solvent (CF_3CD_2OD) in order to detect loci of secondary structure. b) ^{31}P NMR of liposomes, including effects of Mg^{2+}, in order to confirm average size of liposomes from resolved inside/outside phospholipid signals.

Significance to Biomedical Research and the Program of the Institute

The results of this work are of fundamental significance in understanding the detailed structure of DNA in solution and its relationship to function. Continuing NMR studies of poly and oligonucleotide conformations and of protein DNA complexes and their interactions with drugs in solution should reveal further insights of important physico-chemical processes in molecular biology of direct relevance to cancer pharmacology.

Proposed Course:

Studies are continuing on the conformational variants of DNA using the method of 2-dimensional proton NMR. Attempts will be made to observe the

linear-cruciform transition in short palindromic sequences. We have received several oligonucleotides which form loops of different sizes (from Dr. Appella). Since the Fe-adriamycin complex cleaves SV40 DNA at a few distinct sites, it seems unlikely that simple intercalation could be operative. I have proposed that the drug complex may be selectively interacting and cleaving sites on DNA with loop structures. To test this hypothesis we plan to compare the binding properties of daunomycin and the Fe-daunomycin complex with these oligonucleotide loops. Preliminary evidence indicates that the phosphate groups in the loop may provide a locus for selective metal-drug complex interaction.

Deuterium NMR will be applied to selectively ^{2}H-enriched synthetic oligo and polynucleotides to obtain information on the mobility of the deoxyribose ring. ^{2}H and ^{13}C NMR will be used to study protein-DNA and drug-DNA interactions with selectively enriched molecules. ^{13}C-Glu derivatives of methotrexate will be synthesized in order to investigate selective interactions with thymidylate synthetase.

PUBLICATIONS:

Hughes, L.T., Cohen, J.S., Szabo, A., Niu, C. and Matsuura, S.: ^{13}C NMR studies of the molecular dynamics of selectively ^{13}C enriched ribonuclease complexes. Biochemistry 23: 4390-4394, 1984.

Borah, B., Cohen, J.S. and Bax, A.: Conformation of double-stranded polydeoxynucleotides in solution by proton two-dimensional nuclear overhauser enhancement spectroscopy. Biopolymers 24: 747-765, 1985.

Borah, B., Chen, C.-W., Egan, W., Miller, M., Wlodawer, A. and Cohen, J.S.: NMR and neutron diffraction studies of the complex of RNase A with uridine-vanadate, a trimation-state analogue. Biochemistry 24: 2058-2067, 1985.

Borah, B., Cohen, J.S., Howard, F.B. and Miles, M.T.: Poly(dNH_2A-dT): conformation of the high salt form by two-dimensional NMR spectroscopy. Biochemistry, in press.

Roy, S., Torchia, D.A. and Cohen, J.S.: Synthesis of 2',2''-deuterated 2'-deoxyguanosine and sugar ring flexibility by solid state deuterium NMR, submitted for publication.

Cohen, J.S., Borah, B. and Roy, S.: Unique pyrimidine 2D-COSY cross-peaks as monitors of pyrimidine environments in oligo- and polynucleotides. Manuscript in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06522 02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Enzymatic Mechanisms Protecting Cells Against Free Radical Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Charles E. Myers, M.D.	Chief	CPB, COP, DCT, NCI
Kenneth Cowan, M.D., Ph.D.	Sr. Staff Fellow	CPB, COP, DCT, NCI
Aspandiar Katki, Ph.D.,	Guest Researcher	CPB, COP, DCT, NCI
Gurpreet Dhillon, Ph.D.	Guest Researcher	CPB, COP, DCT, NCI
Gerald Batist, M.D.	Visiting Associate	CPB, COP, DCT, NCI
Grace W. Yeh, Ph.D.	IAG	USUHS
Birandra K. Sinha, Ph.D.	Cancer Expert	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Victor Ferrans, M.D., Chief of Ultrastructure, NHLRI
Robert Ozols, M.D., MB, COP, DCT, NCI

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	6	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.)*

1. We have been able to show that human GSH peroxidase is selenium dependent and that a dose of 200 µg/day of selenium.

2. Selenium and tocopherol deficiency do not alter radiation sensitivity of normal tissue.

3. Pleiotrophic drug resistance in human breast cancer cells can be associated with increased levels of glutathiones transferase.

We have continued to expand the work in this area.

1. Human glutathione peroxidase is selenium dependent

We have completed a randomized controlled clinical trial of hyperalimentation plus or minus selenium. We have shown that human erythrocyte and cardiac glutathione peroxidase are: 1) dependent upon dietary selenium; 2) that it takes 6-8 weeks in a selenium deficient diet to reach a nadir and 3) 200 µg of selenium per day is sufficient to maintain normal enzyme levels. This study should be of value to those planning to use selenium as an anticarcinogen.

2. Selenium and Vitamin E deficiency do not cause major changes in the radiation sensitivity of normal tissues

We were able to show that severe selenium and vitamin E deficiency only have harginal effects upon the radiation sensitivity of gutmucosa and bone marrow, predominantly because there is a compensatory increase in enzymes like glutathione transferase. This "built in" compensation suggests the sophisti cated nature of these defenses.

3. Pleiotrophic Drug Resistance

This has been attributed to diminished drug uptake in the resistance cells, is associated with alterations in membrane glycoproteins, and can be reversed by verapamil. We have found that, in fact, in human cell lines, altered drug uptake is of only minor importance and verapamil has little activity (Biochem. Pharm., in press). We have chosen to focus in detail on the human breast cancer cell line MCF-7 and an adriamycin resistant clone selected by Dr. Ken Cowan. We find that adriamycin resistance is associated with increased activity of glutathione transferase, glutathione peroxidase, glucouramyl transferase and sulfotransferase. These changes are not only associated with adriamycin resistance, but also VP-16, actinomycin-D and vincristine. The glutathione transferase enzyme expressed in this case is able to use organic peroxides and epoxides as substrates. Thus, we have been able to show that this line is more resistant to toxic peroxides (cumene hydroperoxide) and epoxides (the yellow raintoxin, DAS).

We plan in the coming year to study the biochemistry and genetics of drug resistance in this model.

PUBLICATIONS:

Batist, G., Carney, D.N., Cowan, K.H., Veach, S.R., Gilliom, M., Bunn, P.A. and Ihde, D.C.: Etoposide (VP-16) and Cisplatin in Previously Treated Small Cell Lung Cancer: Clinical Trial and In Vitro Correlates. Cancer Research, in press, 1985

Batist, G., Reynaud, A., Katki, A.G., Travis, E.L., Shoemaker, M.C., Green, R.F. and Myers, C.E.: Enzymatic Defense Against Radiation Damage in Mice: Effect of Selenium and Vitamin E Depletion. Biochemical Pharmacology, in press, 1985.

Batist, B., Norton, J., Katki, A.G., Wagman, L., Ferrans, V.J., Maher, M. and Myers, C.E.: Selenium Dependence of Human Glutathione Peroxidase: Results of a Prospective Randomized Trial in Patients on TPN. Cancer Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06523 01 CP

PERIOD COVERED

October 1, 1985 to September 30, 1985

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

Metabolism, Irreversible Binding and Mechanism of Action of Etoposide (VP-16, 213) to Cellular Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Birandra K. Sinha, Ph.D.	Cancer Expert	CPB, COP, DCT, NCI
Charles E. Myers, M.D.	Chief	CPB, COP, DCT, NCI
Nissim Haim, M.D.	Guest Researcher	CPB, COP, DCT, NCI
Aspandiar Katki, Ph.D.	Guest Researcher	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Dr. J. Nemec, Frederick Cancer Center; Dr. K. Kennedy, Dept. of Pharmacology, George Washington Univ., Wash., D.C.; Dr. B. Kalyanaraman, Medical College of Wisconsin, Milwaukee, WI

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

VP-1-16 undergoes demethylation to generate active intermediates that binds to protein and DNA. The demethylation is P450 dependent. The generation of the reactive intermediates from VP-16 may be related to its biological activities.

GPO 914-918

The semisynthetic podopyllotoxin derivative, etoposide (VP-16) has shown activity against a number of human tumors. Although the mechanism of action of this drug is not clear, DNA damage induced by VP-16 has been suggested for its cytotoxicity. Recently, we have proposed that the cellular damage induced by VP-16 may result from the formation of a reactive intermediate during bioactivation of the drug. We have studied the metabolism of VP-16 by mouse hepatic microsomes. Using HPLC analysis of the chloroform extracts of the microsomal incubation it was shown that VP-16 formed the 3'-4'dihydroxyl derivative (DHVP). The formation of this metabolite (2% of the parent drug) was NADPH-, protein-, VP-16 and time-dependent suggesting that the activation was enzymatic. Moreover, DHVP formation was inhibited by SKF-525A and piperonylbutoxide suggesting that the O-demethylation was P-450 dependent. Incubation of [^{3}H] VP-16 with microsomes containing NADPH and DNA resulted in irreversible binding of the drug to DNA and proteins. These events i.e., the formation and irreversible binding of the reactive intermediates of VP-16 to cellular macromolecules may be related to the cytotoxicity of VP-16.

PUBLICATIONS:

Sinha, B.K. and Myers, C.E.: Irreversible Binding of Etoposide (VP-16,213) to Deoxyribonucleic Acid and Proteins. Biochem. Pharmacol. 22: 3725-3728, 1984.

Sinha, B.K., Trush, M.A. and Kalyanaraman, B.: Microsomal Interactions and Inhibition of Lipid Peroxidation by Etoposide (VP-16, 213): Implication for Mode of Action. Biochem. Pharmacol. 34: 2036-2040, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03403-20 M

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 chrerecters or less. Title must fit on one line between the borders.)*: Clinical Trials and Miscellaneous Clinical Investigations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert C. Young	Chief	M	NCI
Other:	Charles Myers	Chief	CP	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

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 MAN-YEARS:	PROFESSIONAL:	OTHER:
30	21.5	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, pheochromocytoma and melanoma. Phase I-II clinical trials have been completed this year on the following new experimental agents: Tiazofurin, DHAC Phase I-II trials continue on CBDCA, Trimetrexate, and intraperitoneal chemoth erapy of aclacinomycin. In 1984-1985 the Medicine Branch staff published 128 papers, articles, or book chapters and has accepted or has in press 32 additional publications. This is the largest number of scientific publications in the history of the Branch and represents a 28% increase over last year. Twenty-five active protocols are maintained primarily by the Medicine Branch and over 1070 patients are on clinical trials, 993 (93%) at the Medicine Branch, 61 (6%) at the Navy-MOB and 16 (1%) at the University of Maryland. Details of the clinical and laboratory studies will be reviewed in the subsequent sections. 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 Malignant Lymphomas, Mechanisms of Hormone Dependence of Human Malignancy, Genetic Regulation of the Immune Response, and Retroviruses and Transforming Genes in Malignancy.

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
Robert Makuch	Head	BR	NCI
Steven Rosenberg	Chief	S	NCI
Eli Glatstein	Chief	RO	NCI
Allen Zabell	Sr Investigator	RO	NCI
Daniel Ihde	Sr Investigator	Navy-MOB	NCI
Edward Sausville	Sr Investigator	Navy-MOB	NCI
Elaine Jaffe	Sr Investigator	LP	NCI
Gregory Curt	Sr Investigator	OD,DCT	NCI
Susan Hubbard	Chief	SI	NCI
Ami Ostchega	Chemo Res Nurse	M	NCI
Joan Jacob	Chemo Res Nurse	M	NCI
Caroline Bagley	Chemo Res Nurse	M	NCI
Pat Duffey	Chemo Res Nurse	M	NCI
First and Second Year Associates		M	NCI

Major Accomplishments in 1984-1985

Non-Hodgkin's Lymphoma: Established:

1) Advanced Diffuse Aggressive Lymphomas: ProMACE-MOPP vs. ProMACE-CytaBOM: One hundred and eleven patients with advanced diffuse aggressive lymphoma have been treated with either ProMACE-MOPP or ProMACE-CytaBOM. Complete remissions have been achieved in 35/48 (73%) of those treated with ProMACE-MOPP and 41/46 (89%) of those with ProMACE-CytaBOM. Median follow-up of the complete remission now exceeds 16 months and only 16% of patients have relapsed. Only 25% of all patients on study have died. There were only two granulocytopenic deaths (2%). However, there was a highly significant increase in cases of pneumocystis carinii pneumonia in the patients treated with ProMACE-CytaBOM. Both the modified ProMACE-MOPP regimen and the ProMACE-CytaBOM have very high complete remission rates and both have less granulocytopenic deaths than ProMACE-MOPP flexitherapy. ProMACE-CytaBOM had an initially unacceptable incidence of pneumocystis carinii pneumonitis. Since then all patients with ProMACE-CytaBOM have been treated with prophylactic trimethoprim and sulfa. Since this addition, 108 cycles of ProMACE-CytaBOM have been administered without a single incidence of pneumocystis carinii pneumonia. Both of these new regimens appear to have a very high complete remission rate, have reduced the incidence of septic deaths, and have eliminated the requirement for in-patient treatment necessary in the ProMACE-MOPP flexitherapy regimen.

2) Stage I Diffuse Aggressive Lymphoma: Twenty-five patients with clinically staged early (Stage I) diffuse aggressive lymphoma have been treated with a modified ProMACE-MOPP regimen at 75% doses on a monthly basis for 4 months followed by involved field radiation therapy. This treatment is carried on entirely as an outpatient. There have been no treatment related deaths, few hospitalizations for leukopenia and 22/23 (96%) of patients have entered a complete remission. To date none of the complete remissions has relapsed. This regimen appears to eliminate the necessity for surgical

staging in these early disease patients and has produced an outpatient regimen with modest toxicity producing a complete remission rate in excess of 96%.

3) Nodular Lymphoma Trial: Eighty patients with advanced stage favorable prognosis non-Hodgkin's lymphomas have been randomized to receive either "watch and wait" therapy or intensive chemotherapy with ProMACE-MOPP and total nodal irradiation. Of those randomized to aggressive combination chemotherapy and evaluable, 90% of patients have entered a complete remission and only 2 patients have relapsed in unirradiated, extranodal sites. All others remain in continuous complete remission with a median of 22+ months (range 3+ - 43+ months). Of those patients randomized to "watch and wait", 60% remain off therapy or with limited radiation treatment with a median of 24+ months. Forty percent of patients have been crossed over to the intensive treatment arm and, of those, 36% have entered a complete remission with intensive treatment. Of interest, 3 patients have undergone histologic progression without exposure to any chemotherapy. Several preliminary conclusions can already be derived from this study. (1) Approximately half of patients with minimal therapy will remain relatively asymptomatic for periods of time in excess of two years. (2) Initial aggressive chemotherapy and radiation treatment will produce a high complete remission rate and appears to have reduced the incidence of relapse after complete remission. (3) Histologic progression occurs in some patients without exposure to cytotoxic therapy and is therefore a part of the intrinsic natural history of disease.

4) Confirmed Activity of VP-16 in Diffuse Aggressive Lymphomas: Long-term follow-up of three trials of diffuse lymphoma has confirmed the activity of this drug. Initial trials with VP-16 as a single agent in relapsed diffuse lymphoma confirmed initial activity (40% response rate). This was followed by the ProMACE-MOPP flexitherapy trial and the ProMACE-MOPP vs. ProMACE-CytaBOM trial described in detail in (1).

5) Documented Activity of Interferon in Advanced Non-Hodgkin's Lymphoma and Cutaneous T-Cell Lymphomas: Two studies published this year documented the activity of recombinant leukocyte A interferon in the treatment of both T and B cell lymphomas. Clinical trials were carried out jointly by the Medicine Branch, Navy-MOB and the BRMP in Frederick.

Non-Hodgkin's Lymphoma: Published

1. Longo, D.L., Gelmann, E.P., Cossman, J., Young, R.A., Gallo, R.C., O'Brian, S.J., Matis, L.A.: The isolation of a human T cell leukemia/lymphoma virus (HTLV) transformed B lymphocytic clone from a patient with HTLV-associated adult T cell leukemia. Nature 310: 505-506, 1984.

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Testicular Carcinoma: Established

1) Advanced Poor Prognosis Testicular Cancer - PVBV vs PVB: A new four-drug combination, PVBV, composed of high dose cisplatin (40 mg/M^2 qd x 5), velban, bleomycin, and VP-16, in initial trials produced a high (89%) complete remission rate in patients with poor prognosis advanced non-seminomatous testicular cancer. In a subsequent randomized comparison between PVBV and PVB, 46 poor prognosis testicular cancer patients have been treated. Newly established hydration techniques prevented renal toxicity with high dose cisplatin. The complete remission rate for PVBV was 25/29 (86%) compared to 10/16 (62%). Currently, 5/16 (31%) of patients on PVB are alive and continuously disease-free after induction compared to 20/29 (69%) for PVBV, p = 0.034. These results indicate that PVBV treatment of poor prognosis non-seminomatous testicular patients results in more patients alive and continuously disease-free than previously published standard regimens.

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/M^2 had been dose limiting because of nephrotoxicity. Studies published 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 furosemide. This new approach to therapy allows higher doses of cis-platinum to be safely used in both testicular and ovarian carcinoma.

Testicular Carcinoma: Published

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AIDS and Kaposi's Sarcoma: Established

1) AIDS and Kaposi's Sarcoma: The Medicine Branch has continued its intramural clinical research effort in AIDS-Kaposi's sarcoma and has supplied substantial quantities of bone marrow, lymph node, blood, and semen specimens to Dr. Gallo. These specimens have been contributory to the isolation and characterization of HTLV-III as the etiologic agent of AIDS. In addition, 52 patients with AIDS-Kaposi's sarcoma have been entered on a series of clinical trials. First, we have documented the relatively modest activity of interferon in inducing Kaposi's sarcoma regressions and have documented the high frequency of infectious complications even in the interferon-treated patients. Subsequent trials randomizing patients to interferon on an alternating non-crossresistant combination chemotherapy regimen have now entered 18 patients. Five of nine patients treated with chemotherapy have achieved complete remissions of short duration. Only 3 of 9 patients treated with interferon have achieved a partial remission. Infectious complications were similar in both arms of the trial. In addition, trials of recombinant alpha interferon and DFMO in Kaposi's sarcoma and AIDS have been initiated with 4 patients thus far entered on

trial. Finally, important continued study of inhibitors of reverse transcriptase such as suramin have been carried out in collaboration with the Clinical Pharmacology Branch and the Office of the Associated Director, COP. Trials with the new azidodeoxythymidine compound have just been initiated. 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.

AIDS and Kaposi's Sarcoma: Published

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Hodgkin's Disease: Established:

1) Early Hodgkin's disease: Radiation therapy vs. combination chemotherapy: While radiation therapy is generally successful in the management of early stage Hodgkin's disease, as many as 25% of patients relapse from radiation-induced complete remissions and, although many can be salvaged by chemotherapy, this is accomplished at some risk of induced second malignancy. Furthermore, successful radiotherapeutic management of early stage disease demands considerable technical expertise and access to sophisticated equipment not always available to all patients. Because combination chemotherapy is curative in advanced disease and can salvage many patients who relapse after radiation therapy and because small trials with MOPP chemotherapy in early stage Hodgkin's disease appeared successful, the Medicine Branch and Radiation Oncology Branch are performing a randomized comparison between these treatments. Thus far, 41 patients have been randomized to chemotherapy and 37 to radiation therapy. The complete remission rates are 100% for combination chemotherapy, and 94% for radiation therapy. With a median follow-up in excess of 32 months, 8% of the MOPP treated patients have relapsed compared to 36% for those treated with radiation therapy. Disease-free survival in randomized patients is significantly different ($p = .007$), and overall survival in randomized patients is now significantly different ($p = 0.03$) in favor of the MOPP chemotherapy treatment. Initial results of this trial establish uniformly high complete remission rates with both modalities and generally equivalent results regarding overall survival. If further follow-up substantiates initial observations, this trial will establish MOPP chemotherapy as an excellent alternative to radiation therapy in the management of early Hodgkin's disease.

2) Advanced Hodgkin's disease: MOPP vs. alternating non-crossresistant combinations (MOPP-CABS): A randomized trial of MOPP vs. MOPP-CABS in advanced Hodgkin's disease is one of the few trials of an alternating non-cross-resistant combination other than MOPP-ABVD currently under study. Ninety-nine patients have been randomized. Initial complete remission rates are

88% for MOPP and 86% for MOPP-CABS. At 4 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.

3) Massive Mediastinal Hodgkin's Disease: Massive mediastinal Hodgkin's disease represents one of the most difficult remaining problems in Hodgkin's disease management. Combined modality approaches appear to be the most successful in this group and we are studying MOPP-ABVD followed by aggressive simulator-designed radiation therapy. Thirty-seven patients have been entered on study. Eighty-three percent of patients have entered a complete remission and only one of the complete remissions has relapsed (4%) with a median duration in excess of 18+ months. Overall survival at two years exceeds 95% and disease-free survival for all patients exceeds 75%.

Hodgkin's Disease: Published

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Ovarian Carcinoma: Established:

1) Early Ovarian Cancer: Two hundred and five patients have now been randomized to two separate clinical trials in early ovarian cancer. The first includes 78 patients with Stage IA and IB disease and compares adjuvant melphalan to no additional therapy after comprehensive surgical staging.

The second trial includes 127 patients with minimal residual disease and compares melphalan to intraperitoneal P^{32}. This clinical trial performed in conjunction with the Ovarian Cancer Study Group and GOG remains the only randomized trial in early ovarian cancer treatment in the United States. Initial conclusions are: a) that accurate staging is a crucial prerequisite to decisions regarding appropriate adjuvant therapy, b) carefully staged patients with Stage IA_1 and IB_1 disease with well- or moderately well-differentiated histology have an extremely good prognosis (4 year survival in excess of 95%) and may not require any adjuvant therapy, c) other patients with Stage IA-IIC disease, even after careful surgical staging experience a 20% recurrence and 12% death rate in the first two years after surgery. Such patients are appropriate for adjuvant therapy and this trial indicates that both melphalan and intraperitoneal P^{32} are of similar effectiveness (survival in excess of 80% at 4 years). A replacement protocol comparing P^{32} to a short course of combination chemotherapy with cyclophosphamide-cisplatin has been initiated.

2) Advanced Ovarian Cancer: High Dose Cis-platinum: The activity of high dose (40 mg/M^2 qd x 5) cis-platinum in refractory ovarian cancer has been established. Nineteen 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). Overall response rate in these heavily pre-treated patients was 32% with 16% CR. Median duration of survival was in excess of one year. This represents the best salvage chemotherapy yet reported in ovarian cancer and led to our new advanced disease trial.

3) CPR for Advanced Ovarian Cancer: Cyclophosphamide, high dose cisplatin and total abdominal irradiation are now being used in advanced previously untreated patients. Twenty-two patients have been entered on trial and 18 are evaluable. After only 3-4 months of induction chemotherapy, clinical reevaluation was undertaken and patients clinically free of disease were restaged. Sixty-seven percent of patients achieved a clinical complete remission and 78% of those have been pathologically free of disease at second look surgery. The majority of patients free of disease at second look had significant residual disease prior to chemotherapy. No patient free of disease at second look surgery has died. This short term aggressive regimen has substantial toxicity, particularly hematologic and peripheral neuropathy.

4) High-Dose CBDCA in Refractory Ovarian Cancer Patients: Because of the activity of high dose cisplatin in the treatment of refractory ovarian cancer, we initiated a trial of the carboplatin analogue (CBDCA) in patients who had failed primary induction therapy. CBDCA 400 mg/M^2 x 2 days (total of 800 mg/M^2) x 4 cycles was utilized. Fourteen patients have now entered clinical trial. Thirteen of 14 patients had previously failed cisplatin therapy. In spite of previous therapy, objective responses were seen in 36% of patients. Dose limiting toxicity was thrombocytopenia and leukopenia. There was no peripheral neuropathy, ototoxicity and no renal toxicity. This platinum analogue is very active in ovarian cancer and has a different spectrum of dose-limiting toxicity than the parent drug.

Ovarian Carcinoma: Published

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Breast Carcinoma:

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

Miscellaneous:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06119-16 M

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Cytogenetic Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jacqueline Whang-Peng,	Senior Investigator	MB	NCI
Other:	Turid Knutsen	Med Tech.	MB	NCI
	Elaine Lee	Med Tech.	MB	NCI
	Chien-Song Kao-Shan	Visiting Assoc.	MB	NCI
	John Minna	Branch Chief	MOB-NNMC	NCI
	Kenneth Cowan	Sr. Staff Fellow	CPB	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. Virol, Bureau Biologics, FDA

LAB/BRANCH

Medicine Branch

SECTION

Cytogenetic Oncology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	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 aquired 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 c-oncogenes)c-myc, c-sis, c-fms, etc.) in the neoplastic cells (direct or tissue culture) of Burkitt's lymphoma (including /AIDs), CML, Ewing's sarcoma, 5q-syndrome, etc.

   b. Localization of pseudogenes c-Ki-ras-i and c-Ha-ras-Z in normal human lymphocytes.

   c. Localization of HTLV gene in patients with HTLV positive diseases; one patient with HTLV leukemia has been studied thus far.

   d. Localization of the genes for DHFR in various HSR and double minute bearing tissue culture lines.

Other (cont'd):	Mark Israel	Sr. Investigator	POB	NCI
	Ian Magrath	Sr. Investigator	POB	NCI
	Susan Sieber	Deputy Director	DCCP	NCI
	Neal Young	Sr. Investigator	CHB	NHLBI
	Robert Fine	Clinical Assoc.	CPB	NCI
	Esther Chang	Sr. Investigator		USUHS
	Yves Pommier	Visiting Assoc.	LMP,DCT	NCI
	Ann Dean	Chemist	LCB	NIADDK
	George Morstyn	Clinical Assoc.	ROB	NCI
	Arthur Nienhuis	Branch Chief	CHB	NHLBI
	Gary Armstrong	Biologist	Div. Virol, BB	FDA
	Timothy Triche	Sr. Investigator	LP	NCI

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, cutaneous T cell lymphoma, Ewing's sarcoma, Askin's tumor, peripheral neuroepithelioma, and esophogeal cancer.

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. In vitro studies of sister chromatid exchanges in the bone marrow and peripheral blood of heavy cigarette smokers and in normal controls.

8. In vitro studies of fragile sites in peripheral blood chromosomes from normal individuals of different ages and in heavy cigarette smokers.

Projects Completed:

1. Localization of c-fms in patients with 5q-syndrome: In situ hybridization studies with bone marrow chromosomal preparations from a patient with 5q- and 3H-labeled nick-translated probe of c-fms showed that the c-fms remains on the 5q-chromosome.

2. In vitro study of sister chromatid exhanges (SCE) in cigarette smokers: cigarette smokers were found to have a signficantly higher SCE frequency in both peripheral blood and bone marrow cells than did non-smokers.
   9.654 ± 0.143 vs. 4.614 ± 0.141 (peripheral blood)
   7.656 ± 3.64 vs. 3.981 ± 0.133 (bone marrow)

3. Cytogenetic studies of 27 cases of Ewing's sarcoma, Askin's tumor, and peripheral neuroepithelioma: All direct tumors and tissue culture lines were shown to carry the specific chromosomal translocation t(11;22)(q24;q12).

4. Localization of c-Ha-ras-2 and c-Ki-ras-1 genes: In situ hybridization studies with normal lymphocyte chromosomal preparations and 3H-labeled nick translated probes of c-Ha-ras-2 and c-Ki-ras-1 showed that c-Ha-ras-2 is located on Xq25 and c-Ki-ras-1 is located on 6p22-24.

5. In vitro studies of fragile sites: Increased expression of breakpoints in general was observed with increasing age although no change with age was noted in breakage at oncogene, c-fra, h-fra, and X-chromosomal sites. The most common site of chromosome breakage was 3p14.2, followed by 1q21.3, 7q32.3, and 11q13.3; these four sites are at or near known cancer break sites.

Publications:

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3. Third International Workshop on Chromosomes in Leukemia: Chromosomal abnormalities and their clinical significance in acute lymphoblastic leukemia in childhood. Cancer Res. 43: 868-873, 1983.

4. Whang-Peng, J. and Lee, E.C.: Cytogenetics of human small cell lung cancer. In: Recent Results in Cancer Research, Vol. 97, S. Seeber (Ed.): Springer-Verlag, Berlin, 1985, pp. 37-46.

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10. Bakhshi, A., Minowada, J., Arnold, A., Cossman, J., Jensen, J.P., Whang-Peng, J., Waldmann, T.A., Korsmeyer, S.J.: Lymphoid blast crises of chronic meylogenous leukemia represent stages in the development of B-cell precursors. N. Eng. J. Med. 309: 826-831, 1983.

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12. Israel, M.A., Thiele, C., Whang-Peng, J., Kao-Shan, C-S., Triche, T.J., Miser, J.: Peripheral neuroepithelioma: genetic analysis of tumor derived cell lines. In: Advance in Neuroblastoma Research, Alan R. Liss, 1985, p. 161-170.

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15. Johnson, B.E., Ihde, D.C., Bunn, P.A., Becker, B., Walsh, T., Weinstein, A., Matthews, M.J., Whang-Peng, J., Makuch, R.W., Johnston-Early, A., Cohen, M.H., Lichter, A.S., Carney, D.N., Glatstein, E.J., Minna, J.D.: Small cell lung cancer patients treated with combination chemotherapy with or without irradiation; data on potential cures, chronic toxicities, and late relapses after five to eleven year follow-up. Ann. Int. Med. (In Press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06700-12 M

PERIOD COVERED: October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marc E. Lippman	Senior Investigator	M	NCI
Other:	Caroline Bagley	Nurse	M	NCI
	Margaret Wesley	Biostatistician	BR	NCI
	Peggie Findlay	Physician	ROB	NCI
	Helene Smith	Collaborator	Peralta	CA

COOPERATING UNITS *(if any)*: Biometric Research Branch, NCI; Radiation Oncology Branch, NCI; Surgery Branch, NCI; Peralta Cancer Research Institute, CA.

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 MAN-YEARS:	PROFESSIONAL:	OTHER:
3 1/2	2 1/2	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 stimulating 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. Concurrent cytokinetic data are being collected. An advanced disease hormonal therapy trial comparing tamoxifen plus fluoxymesterone to tamoxifen plus danazol, and a Phase II trial of CBDCA. We have developed a successful treatment program for Stage III-Stage IV Mo breast cancer (objective response rate 41/45)(51% CR rate). We are attempting to further refine these techniques. We have initiated a randomized trial to explore the usefulness of an in vitro chemosensitivity assay system in collaboration with Helene Smith, Ph.D. (Peralta Cancer Research Institute). 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; 200 patients are on study.

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

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. 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. Twenty-five patients are on study.

2. We have initiated a trial using "synchronization" techniques for patients with Stage III locally advanced breast cancer. 48 patients are on study. Objective response rate is 91%; 51% of patients have complete responses to chemotherapy alone.

3. We have begun a randomized trial using a novel in vitro culture system to assess drug sensitivity. We have achieved nearly a 2/3 success rate in getting drug sensitivity information using this assay.

4. A 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. Approximately 60 patients are on study.

5. A psychological study of how patient attitudes influence survival is ongoing.

6. 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 is also underway. 22 patients are on study. 5 patients have relapsed. Actuarial 5 year disease free survival is 80%.

7. A randomized trial of radical radiation therapy versus simple mastectomy is underway with 200 patients on study. Thus far, DF survival and overall survival are identical.

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 progestin receptors.

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 Clinical Professor of Medicine and Pharmacology at the USUHS Medical School.

C. Dr. Lippman is NCI coordinator for the Consensus Development meeting on the Adjuvant Therapy of Breast Cancer.

D. Dr. Lippman is chairman of the 1985 Gordon Conference on Hormone Action.

Publications:

1. Lippman, M.E.: Steroid Malignant diseases: progress in patient selection. Hospital Practice p. 93-106, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06702-10 M

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marc E. Lippman	Senior Investigator	M	NCI
Other:	Edward Gelmann	Senior Investigator	M	NCI
	Attan Kasid	Visiting Scientist	M	NCI
	Diane Bronzert	Technician	M	NCI
	Karen Huff	Technician	M	NCI
	Susan Aitken	Technician	M	NCI
	Robert Dickson	Senior Staff Fellow	M	NCI

(continued on next page)

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 MAN-YEARS:	PROFESSIONAL:	OTHER:
10	10	

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. We are studying the molecular mechanisms by which estrogens and antiestrogens specifically alter growth of human breast cancer.

1. We have introduced viral onc genes (ras and myc) into human breast cancer cells. These retroviruses are stably integrated and viral mRNA is expressed at high levels. Ras induces a hormone independent phenotype. This occurs through increased secretion of several specific growth factors in an autonomous fashion.

2. We are using the technique of differential hybridization to identify specific estrogen regulated genes for cloning and subsequent analysis. We have identified several unique genes induced within 6 hours by estradiol. We have also identified a gene which is specifically induced by antiestrogens and de-induced by estrogen stimulation.

3. We have identified and partially purified several estrogen induced growth factors which are secreted by breast cancer cells into the medium. Some of the activities cross react with EGF receptor and are candidate novel transforming growth factors. Purification and cDNA studies suggest that this TGFα-like activity is not true TGFα but a novel transforming action. Breast cancer cell lines are also secreting physiologically relevant concentrations of TGFβ, IGF-1, a PDGF like competency factor and an epithelial growth factor. All of these activities are under intensive investigation.

Nancy Davidson	Medical Staff Fellow	M	NCI
Dwight Kaufman	Medical Staff Fellow	M	NCI
Sandra Swain	Medical Staff Fellow	M	NCI
George Wilding	Medical Staff Fellow	M	NCI
Susan Bates	Guest Worker	M	NCI
Cornelius Knabbe	Guest Worker	M	NCI

4. We have examined the regulation of thymidine kinase on estrogen regulated enzyme, activity by using a cDNA for human thymidine kinase. Study of transcriptional regulation of this gene is underway.

5. We have demonstrated a novel 39K secreted glycoprotein whose secretion is induced by antiestrogens. This represents the first documented induction by an antiestrogen and may represent an important component of antiestrogen induced inhibition of human breast cancer.

B. We are studying nuclear sites within breast cancer cells which are putative targets for estrogen receptor binding. We have developed an assay for these acceptor sites which are present in limited numbers in estrogen target tissues. We plan to assess their value in clinical receptorology in the coming year.

C. We are using iodinated R2858 (a synthetic estrogen) to develop a useful compound for tumor imaging and potentially for therapy as well.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06708-06 M

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

The Genetic Regulation of the Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	Dan L. Longo	Senior Investigator	M, NCI
Others:	Ada Kruisbeek	Cancer Expert	M, NCI
	Ronald Steis	Clinical Associate	M, NCI
	Tai-Chi Shan	Guest Researcher	M, NCI
	Margaret Weston	Biologist	M, NCI
	Danny Dean	Biologist	M, NCI
	Walter J. Urba	Medical Staff Fellow	M, NCI
	Sandra Bridges	Cancer Expert	M, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Medicine Branch

SECTION

Experimental Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

1. The effects on the development of the T-cell repertoire of anti-Ia antibody administration.

2. The effects of anti-Ia antibodies on normal and tumor bearing adult mice.

3. The mechanism of thymic determination of MHC restriction of T lymphocyte antigen recognition.

4. The physiology of HTLV-1 transformed B lymphocytes.

5. The development and characterization of monoclonal antibodies to Reed-Sternberg cells.

PHS 6040 (Rev. 1/84) GPO 914-918

Selected Highlights of Work Completed this Year:

1. We have been studying the effects of monoclonal anti-Ia antibodies on the development of the T-cell repertoire. In our previous work we have demonstrated that administration of anti-Ia antibodies to neonatal mice results in the deficiency of helper-type T lymphocytes while cytotoxic T lymphocytes seem to function normally in such animals. This year we have studied the mechanism of the effects of the neonatal anti-Ia antibody treatment. We have defined that the subsets of T cells affected by the antibody treatment include: class I MHC specific, class II MHC specific, and Mls-reactive proliferating T cell subsets. When one examines the thymus of animals treated with anti-Ia antibodies, one detects a lack of development of the L3T4 positive, LYT2$^-$ T cell subset. The absence of this subset of T cells intrathymically correlates with the absence of thymic Ia bearing antigen presenting cells. In fact upon withdrawal of anti-Ia antibody treatment, first Ia-bearing cells appear at about the same time in the thymus and in the periphery, then one can detect the L3T4 positive LYT2$^-$ T cell subset in the thymus, and only subsequently does this subset appear to repopulate the periphery. This sequence of events suggests that the intrathymic Ia-bearing antigen presenting cell is the critical cell in determining the development of the T cell subsets that are missing in anti-Ia treated mice.

2. We are interested in using monoclonal anti-Ia antibodies to treat Ia-bearing tumors in man. This would include all B cell lymphomas and a number of leukemias. As preliminary work in the development of a preclinical model, we have tested the effects of anti-Ia antibody administration on the normal immune function of an adult animal. Quite surprisingly we have discovered that a single injection of monoclonal anti-Ia antibodies results in the disappearance of all splenic antigen presenting cell function concomitant with a modulation of splenic cell surface class II antigens. It appears that the antigen-presenting cell itself is not eliminated by such treatment. However, an anti-Ia antibody specific for a single allele is capable of modulating not only the allele for which the antibody is specific but also all other class II molecules on the cell surface even though the antibody does not recognize the other class II molecules. Anti-Ia antibodies have been proposed for use in patients with a variety of autoimmune diseases and, in fact, have been used to treat experimental allergic autoimmune enchephalitis. Our work suggested that the effects of the Anti-Ia antibodies cannot be expected to be haplotype specfic because of the nonspecific effects of the anti-Ia antibodies on Ia-bearing cells. In other words, one can expect a paralysis of the immune system even with monospecific anti-Ia antibodies. We have also been exploring the use of monoclonal anti-Ia antibodies in tumor-bearing mice. We have seen dramatic anti-tumor effects by the administration of anti-Ia antibodies in vivo. An interesting preliminary observation is that it appears that anti-Ia treated mice who are cured of their lymphoma by anti-Ia antibody treatment are protected against subsequent challenge with Ia-bearing cells suggesting that some tumor specific immunity has resulted from the anti-Ia treatment. Subsequent studies are underway to characterize the nature of this response.

3. We have been studying the mechanism by which the thymus affects the development of class II MHC restricted T lymphocytes. We have developed a model whereby adult thymuses can be transplanted into F_1 nude mice to assay their capacity to affect the developing T cell repertoire. Using such a model one can use thymuses from adult bone marrow chimeras whose thymic epithelium is of one genotype and whose thymic Ia-bearing dendritic cells are of a distinct genotype. Transplanting such chimeric thymuses into F_1 nude mice reveals that it is the bone marrow derived thymic antigen presenting cell that is of critical importance in determining the MHC restriction repertoire of developing T lymphocytes. The thymic epithelium may play a role in the development of the repertoire as well. However, if it does so, it acts in concert with the thymic antigen presenting cells. Work is continuing to attempt to recreate the thymic environment in vitro in an effort to dissect the factors responsible for T cell development in the thymus.

4. We isolated an HTLV-1 transformed cytogenetically normal EB virus$^-$ monoclonal B cell from a patient with adult T cell leukemia. This B cell line expresses the receptor for T cell growth fact but does not produce or require T cell growth factor for its own growth. Coculture experiments have demonstrated that the HTLV-1 virus is capable of infecting and transforming both B cells and T cells. Preliminary studies suggest that antibody to the T cell growth factor receptor can prevent the transmission of HTLV-1 to other cells. In addition, some of the B cell lines that we have isolated that are HTLV-1 transformed can be induced to differentiate to immunoglobulin secreting cells by the addition of T cell growth factor. Thus, although their proliferation is independent of IL-2 effects, their differentiation can be affected by this growth factor. The mechanism of these differentiating effects of IL-2 is under investigation.

5. We have developed a monoclonal antibody specific for a Hodgkin's disease cell line. This monoclonal antibody identifies a cell surface structure with limited distribution on normal hemopoietic cells. A rare cell in a normal lymph node in the pericortical region bears this antigen, and occasional T cell lymphomas bear this antigen. In addition, certain EBV-infected B cell lines can be induced to express this antigen. The function of the antigen is not clear, but we have managed to isolate a variant of the Reed-Sternberg cell line which does not express the antigen recognized by the monoclonal antibody. Interestingly, the morphology of the antibody negative variant is less dendritic than the typical Hodgkin's disease cell line. The cells of the variant line grow as smooth cells instead of having the projections that are characteristic of the dendritic cell. We are now examining the phenotypic differences between the antibody reactive and nonreactive variants and are moving to get the monoclonal antibody into clinical trials.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06709-05 M

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 chereacters or less. Title must fit on one line between the borders.)*: Mechanisms of Drug Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Neme, title, leboretory, end institute effiliation)*

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
	Thomas C. Hamilton	Staff Fellow	M	NCI
	Brent Behrens	Medical Staff Fellow	M	NCI
	Karen Louie	Medical Staff Fellow	M	NCI
	Takashi Tsuruo	Visiting Scientist	M	NCI

COOPERATING UNITS *(if any)*

Laboratory of Molecular Biology, DCBD
Clinical Pharmacology Branch

LAB/BRANCH: Medicine Branch

SECTION: Experimental Therapeutics

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

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

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

We are studying the biology of ovarian cancer, the mechanisms of antineoplastic drug resistance in ovarian cancer, and the pharmacologic reversal of the drug resistant phenotype. This work required the development of appropriate model systems of human ovarian cancer including in vitro cell lines and a nude mouse xenograft bearing a transplantable intraperitoneal human ovarian carcinoma. We have characterized 5 new ovarian cancer cell lines including a line which has steriod hormone receptors. Drug resistant variant cell lines have been developed by stepwise incubation of sensitive cell lines with progressively increasing concentrations of melphalan, adriamycin, and cisplatin. The drug resistant variants are 6-100 more resistant to chemotherapy than the sensitive parental cell lines. The intraperitoneal model of human ovarian cancer produces ascites, pulmonary metastases, and death from intraabdominal carcinomatosis. Using these model systems we have demonstrated that resistance to melphalan, cisplatin, and adriamycin is linked, in part, to glutathione levels. We have shown that buthionine sulfoximine, a synthetic amino acid which inhibits the synthesis of glutathione, leads to a decrease in glutathione levels in the drug resistant cell lines and increases the cytotoxicity of melphalan, cisplatin and adriamycin. In addition, we have demonstrated that some drug resistant human ovarian cancer cell lines have a decreased accumulation of adriamycin which can be reversed by exposure of the cells to verapamil. These studies led to a trial of verapamil plus adriamycin in refractory ovarian cancer patients and the results with buthionine sulfoximine have led to the preclinical evaluation of buthionine sulfoximine by the Decision Network of the NCI.

Ongoing Studies:

1. Ovarian Cancer Cell Lines. We have established and characterized 5 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 functionality of the estrogen receptor has been demonstrated by induction of the progesterone receptor in nude mouse xenografts.

2. Intraperitoneal Model of Human Ovarian Cancer: By various in vitro and in vivo selection procedures we have established a transplantable human ovarian cancer in nude mice which produces ascites, intraabdominal carcinomatosis and pulmonary metastases, and reproducibly leads to death. The tumor cells secrete the ovarian cancer antigen OC-125 and retain their receptor status. This model is being used for in vivo studies or the pharmacologic reversal of drug resistance.

3. 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, or cisplatin we have developed variant cell lines which are 6-100 fold more resistant to these drugs than the parent cell lines. The drug resistant variants have been characterized as to their glutathione levels, karyotype, and patterns of cross resistance.

4. 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. In addition, we have identified a series of compounds initially selected by the human tumor stem cell assay which may have specific anticancer activity against ovarian cancer.

5. Mechanisms Of Drug Resistance. We are examining the mechanisms of drug resistance to melphalan, adriamycin, and cisplatin in experimental models of human ovarian cancer. Our studies have demonstrated the absence of a uniform transport defect for melphalan and adriamycin in the drug resistant cell lines. The pleiotropic drug resistant phenotype expressed in these cell lines, however, does appear to be linked, in part, to elevated intracellular levels of glutathione (GSH). Studies are in progress to evaluate the role of GSH in the formation of DNA-cisplatin adducts, and in the repair of DNA damage in drug resistant cell lines. In addition, we have also demonstrated that the cell lines which developed resistance to melphalan and cisplatin developed cross resistance to irradiation and that this cross resistance could be eliminated by exposure of the cells to buthionine sulfoximine.

6. Alterations 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 some adriamycin-resistant variant cell lines and that manipulations of glutathione will alter sensitivity to melphalan, cisplatin, and adriamycin. Pharmacologic studies have demonstrated that adriamycin resistance in some ovarian cancer cells may be the result of decreased accumulation due to an increased efflux mechanism which can be reversed with verapamil. This led to a clinical trial of verapamil plus adriamycin in refractory ovarian cancer patients. In addition, we have demonstrated that buthionine sulfoximine plus melphalan prolongs survival in the nude mouse model compared to treatment with melphalan alone providing further evidence that GSH modulations may be clinically feasible.

7. Immunotherapy of Ovarian Cancer. In collaboration with Dr. Ira Pastan, Laboratory of Molecular Biology, DCBD we have demonstrated that monoclonal antibodies linked to toxins (pseudomonas, ricin) are cytotoxic to human ovarian cancer cell lines. Preclinical studies have been initiated based on these observations.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06710-03 M

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Transforming Genes and Retroviruses in Human Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward Gelmann	Senior Investigator	M	NCI
	Nancy Davidson	Res. Asst. Professor	Pharm	USUHS
	George Wilding	Medical Staff Fellow	M	NCI
	Attan Kasid	Senior Staff Fellow	M	NCI

COOPERATING UNITS *(if any)*

Pediatrics Branch, COP, DCT, NCI
Dept. of Pharmacology, USUHS

LAB/BRANCH: Medicine Branch

SECTION: Medical Breast Cancer Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	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.)*

A. Isolation of hormone regulated genes in the human MCF-7 breast cancer cell line. The purpose of this project is to clone, sequence, and identify genes which are important in the estrogen induction of the tumorigenic phenotype in MCF-7 human breast cancer cells. We have constructed a cDNA library from estrogen treated human breast cancer cells and have isolated from the library several cDNA clones which are regulated by estrogen treatment of the cells. Several of the clones represent genes whose transcription is stimulated shortly after exposure of the cells to estrogen. Estrogen treatment for approximately 24 hr results in growth stimulation in vitro and the continuous presence of estrogen required for tumor formation in the nude mouse. It is our goal to characterize the genes we have cloned and study their relevance to the tumorigenic phenotype. We have also isolated the gene whose expression is inhibited by estrogen treatment of the cells. Moreover, antiestrogens, which reversibly stops cell growth, causes the expression of this gene to be enhanced. Since this phenomenon does not result from mere arrest of cell growth but seems to be hormonal dependent, we are actively investigating the hypothesis that antiestrogens may operate through induced genes which are inhibitory to cell growth. We are also asking the question if the clone we have isolated represents the gene for one of these growth inhibitory factors.

B. Molecular cloning of human fibroblast growth factor. The purpose of this project to isolate a full length cDNA clone of the growth stimulatory protein known as fibroblast growth factor. FGF is a protein produced by certain human carcinoma cells including the human breast cancer cell

line MDA-MB-231. This growth factor can be assayed by specific in vitro colony formation of the SW-13 human adenocarcinoma cell line. This cell line contains receptors for FGF but not for other known tumor growth factors. We have constructed a cDNA library from MDA-MB-231 mammary carcinoma cells and plan to screen it to try to isolate genome cDNA clone of FGF message. Simultaneous efforts by Dr. Marc Lippman and coworkers in trying to concentrate large amounts of FGF with the production of antisera will allow the screening of a cDNA expression library with such antisera. Together these studies will help to delineate the role of FGF in human tumor cell growth.

C. Molecular characterization of a mutant human c-myc gene. The purpose of this study is describe the mutations in a human c-myc gene involved in a case of Burkitt lymphoma. In this particular case, PA682, the Burkitt cells have a 8;22 chromosomal translocation and the human c-myc gene is neither translocated nor rearranged. We have succeeded in cloning the c-myc gene from the abnormal chromosome and characterized it as having at least a 50-100 nucleotide deletion between the first and second exons of the myc gene. Currently experiments are underway to sequence the entire abnormal myc gene to characterize mutations that can occur independent of myc gene rearrangement and to try to define more clearly the regions of the myc gene that are essential for oncogenic activation.

D. Search for a human mammary tumor virus. The purpose of these studies are to investigate the possibility that there is a human homolog to the mouse mammary tumor virus. Breast cancer is a common malignancy in the United States and is a collection of several diverse malignant disorders of the breast. One of the more rare subclasses is inflammatory carcinoma of the breast. Inflammatory carcinoma of the breast is quite common in Tunisia where it represents the greatest portion of breast cancer that occurs and can be characterized as being a highly aggressive tumor that is largely unresponsive to therapy. We have chosen inflammatory breast cancer as a model for a malignancy which is both endemic in some areas of the world and sporadic in others and may represent a link between a tumor and infectious agent. Experiments are underway to isolate from Tunisia inflammatory breast carcinoma tissues particular fractions which may contain retrovirus-related activities.

E. Onc gene transformation of human breast cancer cell lines. The purpose of this study is to characterize the action individual mutated onc genes may have in influencing different aspects of the malignant phenotype. As a model system we have transfected the hormonally responsive MCF-7 human breast cancer cell line with the v-ras^{H} onc gene. This stable progeny transfectant is no longer responsive to hormones and was found to secrete growth factor activities into the media which were able to stimulate the sustained tumor growth of other breast cancer cells. These growth factor activities were not present in supernatant from cultured cells. However, parental MCF-7 cells, when stimulated with estrogen secrete growth factors into the media. There is some overlap between estrogen-stimulated growth factors and those produced by the v-ras^{H}-transfected cells. These data are consistent with data published from experiments in other systems

which suggests that onc gene activation may result into secondary production of secreted growth factors. Further experiments will pursue the influence of other known oncogenes on the phenotype of different breast cancer cells.

F. A unique property of malignant cells is the ability to develop in vivo chemotherapy resistant variants whereas normal cells such as hematopoietic stem cells have not been observed to do so working with an adriamycin resistant ovarian cancer cell line which is the single reason that gene amplification and chromosome 7, we have constructed a cDNA library and are cloning mRNA specific for the resistant cell line. Since this cell line selected to be resistant to adriamycin possesses pleiotropic resistance by virtue of its growth in the presence of other chemotherapeutic agents, this work may result in the cloning of the gene responsible for pleiotropic drug resistance.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06711-01 M

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Functional and Molecular Biology of T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Matis	Senior Staff Fellow	M	NCI
Other:	Susan Heckford	Visiting Associate	M	NCI
	Michael Bookman	Medical Staff Fellow	M	NCI
	Eric Groves	Medical Staff Fellow	M	NCI
	Tai-Chi Shan	Guest Researcher	M	NCI
	Danny Dean	Biologist	M	NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI
Laboratory of Genetics, NCI

LAB/BRANCH

Medicine Branch

SECTION

Experimental Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	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.)*

1. Immune response to tumors: in vitro and in vivo.
2. Gene activation in T lymphocyte clones.
3. Molecular biology of T lymphocyte receptor genes.
4. Acquisition of the T cell receptor repertoire.
5. Mechanisms of T-cell B-cell collaboration.
6. Control of major histocompatibility gene expression.

Selected Highlights of Work Completed this Year:

I. We have characterized T cell clones specific for retrovirus induced syngeneic leukemias. Proliferative T cell clones specific for the FBL-3 leukemia and restricted to both class I and class II self major histocompatibility antigens have been propagated in vitro. The former have the Lyt 2^+ surface phenotype and are cytotoxic, and appear to recognize a tumor specific non-viral cell surface antigen. The latter are $T4^+$ positive, noncytotoxic and proliferate in response to the major viral envelope glycoprotein gp 70. In addition, syngeneic T cell clones have been characterized which are specific for a non-viral tumor cell surface glycoprotein gp 175 purified from the Rauscher virus-induced T cell leukemia RBL-5. Prior studies had shown that pre-immunization of mice with as little as 1 ug of this protein protected mice against subsequent subcutaneous challenge with the RBL-5 tumor.

The in vivo activity of these tumor-specific T cell clones has been investigated. We have been able to demonstrate significant in vivo activity of the proliferative cytotoxic T cell clones specific for the FBL-3 leukemia. In particular, we can reproducibly cure more than 50% of mice bearing established tumor by the intravenous administration of 2×10^7 cloned T lymphocytes followed by twice daily i.p. injections of recombinant interleukin 2 (IL-2) for 1 week. IL-2 administered alone was shown to have no effect on tumor growth or survival.

Of major importance was the discovery that the in vivo anti-tumor activity of the T cell clones was markedly enhanced by pre-activation of the clones with antigen in vitro prior to in vivo administration. This was shown to be due to the fact that such antigen activation markedly increased the clone's expression of IL-2 receptors and dramatically enhanced the proliferative response of the clone to IL-2 both in vitro and in vivo. Ongoing studies are investigating the mechanism of tumor elimination in vivo by T cell clones and the in vivo activity of T cell clones of distinct function and phenotype.

II. Using T cell clones, we have assessed the signals required for the induction T cell proliferation and lymphokine production. We have found, for example, that resting (GO) T cell clones may be induced to proliferate when stimulated by recombinant IL-2 but secrete no immune interferon (IFN-γ). Such clones produce IFN-γ when activated specifically by antigen or non-specifically by the lectin concanavilan A (con A). Con A-induced lymphokine production is further amplified by co-stimulation with the phorbol ester PMA. Initial experiments indicate that these responses are controlled at the level of mRNA transcription. Thus, no mRNA for IFN-γ is induced following IL-2 stimulation of the T cell clones. As noted, IL-2 does induce clonal proliferation, and preliminary evidence indicates that this proliferation is associated with a rapid induction of the c-myc gene expression. In contrast to IL-2, antigen, con A, and con A + PMA activation of the T cell clones resulted in rapid induction of IFN-γ mRNA synthesis. Thus, qualitatively distinct signals are induced by

engaging the IL-2 versus the antigen receptor on T lymphocytes, with only the latter resulting in the tissue-specific transcriptional activation of lymphokine genes.

III. In collaboration with Dr. Steven M. Hedrick at the University of California at San Diego, we are studying the relationship of functional recognition specificity of antigen-specific T lymphocyte clones to the sequence of the genes encoding the β and α chains of the T cell receptor. Work completed to date has focused on a series of murine T cell clones specific for the well-defined globular protein antigen pigeon cytochrome c. A family of T cell clones has been derived from the B10.A strain of mice, all of which recognize an epitope in the carboxy-terminal fragment of the antigen in association with the same class II MHC Ia molecule ($E^k_\beta E^k_\alpha$). However, fine specificity differences between these clones can be discerned by assessing the clonal response to related species variant cytochrome-c peptides and concomitant reactivity (MHC-restricted or alloreactivity) to other allelic Ia molecules. For example, we have shown thus far that all the pigeon cytochrome-c T cell clones which exhibit a strong cross-reactivity to the cytochrome-c peptide from the species Tobacco hornworm moth (THWM) share the same beta chain variable region and display a similar pattern of T cell receptor beta chain-gene rearrangement on Southern blot hybridization analysis. In addition, several cytochrome-c specific clones which display the same concomitant alloreactivity also have identical B chain gene rearrangements. These structure-function analyses should ultimately define the molecular basis of T cell antigen recognition, including the phenomenon of MHC-restriction.

IV. We have been able to characterize long term proliferative T cell lines in vitro from congenitally athymic (nu) mice. These lines are exclusively of the L3T4$^-$ Lyt 2$^+$ phenotype and have cytotoxic effector function. They produce both IL-2 and IFN-γ when activated by the appropriate stimulator cells. The generation of such long-term T cell lines from such athymic mice should allow a direct comparison of their T cell receptor repertoire with the repertoire expressed by MHC compatible euthymic animals. In this way the influence of the thymus on the development of cytotoxic T cells can be directly assessed.

V. In collaboration with Dr. James Mond at the Uniformed Services University of Health Sciences, we have been exploring the mechanism by which T cells activate B lymphocytes to proliferate and secrete immunoglobulin. T cell clones specific for the protein antigen TNP-ovalbumin were derived. B cell proliferation in induced non-specifically at high concentrations of antigen, but only in an MHC-restricted fashion at low concentrations of antigen. B cells from a strain of mice exhibiting a synergistic B cell defect (both the xid and nu/nu genes in the same strain) were able to respond only in an MHC-restricted fashion to antigen-activated T cell clones. The nature of both the specific and non-specific T cell stimuli is currently under investigation.

VI. A murine ovarian teratocarcinoma cell line has been studied for the expression of major histocompatibility class I genes. Markedly diminished expression of mRNA for class I MHC genes was noted. Both mRNA and cell surface MHC protein expression were induced in the presence of the differentiating agent cis-retinoic acid. However, class I MHC mRNA expression peaked at 72 hours and thereafter steadily declined even in the continuous presence of cis-retinoic acid. Treatment of the cells with the protein synthesis inhibitor cycloheximide induced a rapid re-expression of class I MHC mRNA, a process which was shown to depend on de novo mRNA synthesis. The effect of cycloheximide was observed only in the simultaneous presence of cis-retinoic acid. Therefore expression of class I MHC genes (which are normally constitutively expressed) in this cell line requires induction by cis-retinoic acid and also appears to be regulated by a labile repressor protein. The nature of the protein repressor is now being studied.

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October 1, 1984 to September 30, 1985

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

The NCI-Medical Oncology Branch is conducting a series of clinical investigations into the treatment and biology of human lung cancer. Dr. Ihde and Dr. Mulshine lead the clinical trial effort, while Dr. Gazdar leads the in vitro chemotherapy sensitivity testing effort for the clinical trials. In extensive stage small cell lung cancer, we have demonstrated that tumor can be safetly obtained from patients and have in vitro chemotherapy testing performed. In nonsmall cell lung cancer this has also been possible, but the results in growing the lines have, as yet, not been as successful as in small cell lung cancer. However, new techniques for growing nonsmall cell lung cancer in serum free hormone supplemented media have been worked out, and the ability to grow nonsmall cell lung cancer successfully and perform these tests remains a major objective of the Branch. A panel of well-defined lung cancer cell lines of both small cell and nonsmall cell lung cancer has been made available to the drug therapy evaluation program to use for in vitro screening for new agents. The NCI-Navy Branch is cooperating with these efforts and is anxious to participate in the clinical trial of any newly discovered agents. In addition to in vitro chemotherapy sensitivity testing of patient samples, a prospective study of biochemical and immunohistochemical markers is being undertaken in work led by Dr. Linnoila. These potentially could be of widespread clinical diagnostic use in the typing of lung cancer.

In adult cutaneous T-cell lymphomas (mycosis fungoides), combined modality therapy studies have shown a significant fraction of long-term disease free survivors, up to five to seven years after receiving combined modality therapy. In addition, radiolabeled monoclonal antibody scanning with T101 given both intravenously and subcutaneously has showed impressive uptake in tumor bearing lymph nodes.

Dr. Johnson has correlated the presence of C and N myc amplification in small cell lung cancer with impaired survival. This could represent some of the first data in an adult tumor correlating oncogene status with the clinical course.

In the tumor cell biology laboratory, Dr. Gazdar leads the work on supporting of clinical trials of lung cancer by in vitro drug sensitivity testing. In addition to establishing techniques for growing both small cell and nonsmall cell lung cancer in serum free hormone supplemented media, he has discovered great heterogeneity of the nonsmall cell lung cancer lines. In nude mouse xenografts, he has found that some nonsmall cell lung cancers are widely metastatic directly upon transplantation from the patient. These studies, in collaboration with the DTEP program of Dr. Mayo, provide an entirely new model of metastatic human cancer in xenografts. The molecular biology of this phenomenon specifically dealing with oncogenes and their amplification is under study.

Dr. Linnoila immunohistochemically, besides supporting the clinical lung cancer trials with the prospective studies of markers, has demonstrated the greatly depressed or absent expression of beta 2 microglobulin in a series of

human small cell lung cancers and carcinoid tumors. In collaboration with a group in Finland, she has demonstrated that beta 2 microglobulin dramatically increases after Interferon therapy of the patient. This would be the first demonstration in a patient of induction of beta 2 microglobulin in a previously negative tumor. It has potential treatment implications using Interferon.

Dr. Gazdar has recently established a human IgA kappa secreting human myeloma line that is of potential tremendous significance. This tumor line grows well in culture and makes enormous amounts of immunoglobulin which it secretes into the culture fluid. He has also been able to demonstrate that it has a rearranged c-myc oncogene and expresses this oncogene. This line should be of use in the preparation of human hybridomas, the study of human B-cell development (which Dr. Michael Kuehl of our laboratory is beginning), and the cloning of a new rearranged gene adjacent to the c-myc gene (being undertaken by Drs. Hollis and Kirsch of our laboratory).

In the molecular genetics and immunology laboratory, Dr. Minna has led work on the isolation and characterization of a new proto-oncogene in human lung cancer. This gene is part of the myc family of oncogenes and has been named L-myc. In several small cell lung cancers, it has been found to be amplified and or tremendously over expressed. The structure and role of this gene in the biology of lung cancer is being explored, as well as its developmental expression. In addition, the method used to isolate the gene was based on the finding of a small homologous region between the various myc genes. Using the same strategy, Dr. Minna's group has been able to identify even further additional members of this myc family, and they are beginning the cloning and isolation of these genes.

Dr. Minna has also led the work, conducted by Dr. Cuttitta, of the role of bombesin (gastrin releasing peptide) in the physiology of small cell lung cancer. A monoclonal antibody against bombesin has been established, and this antibody will inhibit the clonal growth of these cells in vitro and the growth of small cell lung cancer xenografts in nude mice. This suggests the exciting possibility of using the antibody in a clinical therapeutic trial. Such a trial is being planned right now, and appropriate preparations of the antibody instituted to obtain approval from the FDA. In addition, an anti-idiotype antibody against the antibombesin antibody has been prepared. This anti-idiotype antibody appears to react with the bombesin receptor on the surface of tumor cells, and will actually stimulate their growth. It should provide a new reagent for isolating and characterizing the bombesin receptor, and also could be of potential therapeutic value.

Dr. Battey and Dr. Sausville have done elegant work on the structure and expression of peptide hormone genes in human lung cancer cells. They have cloned and sequenced the genes for arginine, vasopressin and oxytocin, and shown that they are tightly linked, within 50 killobase of each other, but in opposite orientations. In addition, they have cloned and sequenced, using cDNA clones, the bombesin (GRP) from human small cell lung cancer. Using these probes, they have established S1 nuclease assays that allow the detection of GRP messenger RNA in tumor samples. These studies should lead to a molecular genetic analysis of the expression of this important peptide hormone gene. In addition, plans using the antiserum against the bombesin receptor are being made to clone the bombesin receptor in an expression vector.

Drs. Hollis and Kirsch have recently isolated and cloned the gene for galactosyl transferase. This should allow a molecular genetic study of an important multigene family in cancer cells. These molecules determine the cell surface structure of cancer cells. In addition, they have identified a rearranged c-myc gene in the human myeloma cell line described above, and have cloned across the break point to identify a new piece of DNA involved in the rearrangement. They have also documented the regulation of myc and myb oncogenes in mouse erythroleukemia cells during differentiation. They have cloned and sequenced new members of the lambda family of genes, and have found a completely new set of genes that are not linked to the previously known genes on human chromosome 22. This will allow a molecular evolutionary picture of this lambda family of genes, and provide potential new targets for finding gene rearrangements in human lymphomas.

Dr. Kuehl's laboratory has studied the structure and function of the myb gene during the development of mouse lymphomas in B-cell development. This is one of the first detailed studies of the structure and expression of this gene being carried out. Dr. Segal, with Dr. Kuehl, has established transvection techniques for introducing genes into a variety of differentiated cell types, including lung cancer cells and mouse erythroleukemia cells. She is defining the controlling and enhancing regions of genes needed for the gene expression after transvection. This should provide powerful new tools for studying the expression of genes in a variety of different cell types, and the influence of genes on the behavior of their cellular biology. An excellent example of this is the effect of transvecting the c-myc oncogene into lung cancer cells that do not express this oncogene. The presence of the gene dramatically changes the phenotype and growth characteristics of human small cell lung cancer.

In summary, the NCI-Navy Medical Oncology Branch has integrated its clinical and laboratory research. The clinical protocols are built around the discoveries in the cell biology and molecular genetics labs. In addition, both cell biology and molecular genetics are being used to establish new ways to diagnose, treat and study the biology of human malignancy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03024-16 NMOB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel C. Ihde, M.D., Chief, Clinical Investigations Section, NCI-NMOB

COOPERATING UNITS *(if any)*
See attached sheets

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, Maryland 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
56	16	40

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-Navy Medical Oncology Branch studies new methods of evaluating and treating patients with malignant disease and provides general medical oncology consultations for the Naval Hospital Bethesda. 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, and breast and testicular cancer. 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 general medical oncology consultations per month are seen in the NHBETH and outpatient care (200 visits/week) provided for patients requiring chemotherapy who are not eligible for any protocol studies.

Senior Staff Oncolgists, PI:

John D. Minna, M.D., Chief	NCI-NAVY MOB NCI/NHBETH (USPHS)
Daniel C. Ihde, M.D. (Deputy Chief, Clinical)	NCI-NAVY MOB NCI/NHBETH (USPHS)
James L. Mulshine, M.D.	NCI-NAVY MOB NCI/NHBETH (USPHS)
Bruce E. Johnson, M.D.	NCI-NAVY MOB NCI/NHBETH (USPHS)
Edward A. Sausville, M.D.	NCI-NAVY MOB NCI/NHBETH (USPHS)

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

CAPT Stephen R. Veach, M.D.
CDR John C. Phares, M.D.
CDR John Nanfro, M.D.

Senior Staff Other, PI:

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

Clinical Associates and Medical Staff Fellows:

Third and Fourth Year

Mark Browning, M.D.	Heme/Onc Branch/NHBETH
Vincent Shen, M.D.	Heme/Onc Branch/NHBETH
Gerald Batist, M.D.	NCI-Navy MOB NCI/CPB/NCI

Second Year

Burke Brooks, M.D.	NCI-Navy MOB NCI/BETH
Ethan Dmitrovsky, M.D.	NCI-Navy MOB NCI/BETH
Eric Seifter, M.D.	NCI-Navy MOB NCI/BETH
LT Walter Morley, M.D.	Heme/Onc Branch/NHBETH

First Year

Mark Levitt, M.D.	NCI-NAVY MOB NCI/NHBETH
Allison Martin, M.D.	NCI-NAVY MOB NCI/NHBETH
Stacey Nerenstone, M.D.	NCI-NAVY MOB NCI/NHBETH
Pam Sholar, M.D.	NCI-NAVY MOB NCI/NHBETH
Joseph Frank, M.D.	NCI-NAVY MOB NCI/NHBETH
James O'Brien, M.D.	NCI-NAVY MOB NCI/NHBETH
Carl Freter, M.D.	NCI-NAVY MOB NCI/NHBETH
Sanford Markowitz, M.D.	NCI-NAVY MOB NCI/NHBETH
Fred Kaye, M.D.	NCI-NAVY MOB NCI/NHBETH
LCDR Dennis Azuma, M.D.	Heme/Onc Branch/NHBETH
CDR Randy Howard, 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
Margaret Edison, RN	Clinical Research Nurse	NCI-Navy MOB NCI/NHBETH
Ann Bastian, RN	Clinical Research Nurse	NCI-Navy MOB NCI/NHBETH
Maria Poblet, RN	Clinical Nurse	NCI-Navy MOB NCI/NHBETH
Delphine Knop, R.Ph.	Clinical Pharmacist	NCI-Navy MOB NCI/NHBETH
Herbert Holmes, R.Ph.	Clinical Pharmacist	NCI-Navy MOB NCI/NHBETH
Rebecca Puffenbarger, RN	Clinical Nurse	Heme/Onc Branch/NHBETH

Cooperating Units

NHBETH

Judith Bader, M.D.	Radiation Oncologist	ROB-NCI/NHBETH
Jane Grayson, M.D.	Radiation Oncologist	ROB-NCI/NHBETH
Gilles McKenna, M.D.	Radiation Oncologist	ROB-NCI/NHBETH
CDR Charles Lee, M.D.	Chief, Thoracic Surgery Service	
CAPT Kevin O'Connell, M.D.	Chief, Urology Service	
CDR Patrick James, M.D.	Pathology Service	
CAPT David Garvin, M.D.	Pathology Service	
CAPT Thomas Walsh, M.D.	Pulmonary Branch	
CAPT H. Parlette, M.D.	Dermatology Branch	
CAPT May Lesar, M.D.	Radiology Branch	

Cooperating Units

National Institutes of Health

W. Blattner, M.D.	Senior Investigator	EEB/NCI
S. Broder, M.D.	Assoc. Director for COP	DCT/NCI
K. Foon, M.D.	Senior Investigator	BRMP/DCT/NCI
R. Ozols, 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
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
T. Waldman, M.D.	Chief	MET/DCBD/NCI
J. Whang-Peng, M.D.	Senior Staff (Cytogenetics)	MB/DCT/NCI
S. Larson, M.D.	Chief	NM/CC/NIH
C. Myers, M.D.	Chief	CPB/ DCT/NCI
R. Schroff, M.D.	Senior Investigator	BRMP/DCT/NCI
L. Matis, M.D.	Senior Staff Fellow	MB/DCT/NCI
K. Cowan, M.D.	Senior Staff Fellow	CPB/DCT/NCI
R. Gallo, M.D.	Chief	LTCB/DCT/NCI
M. Robert-Guroff, M.D.	Senior Investigator	LTCB/DCT/NCI
R. Young, M.D.	Chief	MB/DCT/NCI
M. Zwieg, M.D.	Laboratories	CC/NIH

Cooperating Units, Other

G. Schechter, M.D.	Hematology Section, VAMC, Washington, DC
M. Cohen, M.D.	Oncology Section, VAMC, Washington, DC
B. Fischmann, M.D.	Dermatology Section, VAMC, Washington, DC

Small Cell Lung Cancer

Limited Stage Clinical Trial: Over 85 patients have been randomized on a clinical trial comparing the administration of 6 weeks of aggressive induction combination chemotherapy alone with identical chemotherapy plus chest irradiation (4000 rad/15 fractions/3 weeks) beginning on Day 1 of chemotherapy. All patients receive an additional 42 weeks of combination chemotherapy in the outpatient clinic, and prophylactic cranial irradiation is administered to complete responders at Week 13. The complete response rate with combined modality therapy is significantly higher (79% vs. 48%), as is disease-free survival. Despite an increased number of treatment-related deaths on the combined modality arm (6 vs. 2), there is a strong trend in overall survival favoring the administration of chemotherapy plus chest irradiation ($p = 0.07$). Actuarial 2 to 4 year survival is 32% with combined modality treatment vs. 12% with chemotherapy alone. Survival on the chemotherapy alone arm is virtually identical to our historical experience in which patients did not receive chest irradiation. This trial is continuing, with planned accrual of 100 patients. In contrast to several other randomized trials, this study appears to demonstrate benefit for adjuvant chest irradiation. We believe this is due to the aggressive manner in which both forms of therapy are simultaneously administered. A new clinical trial should be initiated in 6 to 12 months.

Extensive Stage Clinical Trial: This recently opened study has several objectives: a) to determine if very high doses of etoposide (VP-16) plus cisplatin induction chemotherapy is superior to standard doses; b) to determine how frequently viable tumor specimens from extensive stage patients can be transported to the cell biology laboratory; c) to determine in a prospective series of specimens the frequency with which drug sensitivity testing information can be obtained; and d) to determine the frequency of new complete remissions which can be obtained with a 3-drug regimen dictated by in vitro drug testing results in patients not in complete remission after 12 weeks of etoposide plus cisplatin. Patients on whom no such information is available at 12 weeks will receive a standard new 3-drug regimen. Thirty patients have been entered thus far. There have been 8 complete remissions with etoposide plus cisplatin, 16 partial remissions, 3 early deaths (including 2 toxic deaths on the high-dose arm with minimal residual tumor at autopsy), and three patients did not respond. There are no major differences in response between the high-dose and low-dose arms. Tumor specimens reached the cell biology laboratory in 24/30 cases. Cell lines have been established from 11/24 patients. Two of 3 patients have achieved a new complete response after week 12 on the "best in vitro regimen", compared with 2/9 on the new standard 3-drug regimen. Etoposide plus cisplatin appears to be an active induction regimen in extensive stage small cell lung cancer. The original doses on the high-dose arm have been reduced because of 2 toxic deaths and no toxic deaths have occurred since. Tumor specimens have reached the cell biology laboratory in the great majority of patients. This trial, which we believe our Branch is uniquely equipped to conduct, is continuing.

Long-Term Survivors of Small Cell Lung Cancer: As reported last year, long-term follow-up evaluation was conducted on all 252 patients entering NCI therapeutic trials for small cell lung cancer from 1973 to 1978. Twenty-eight (11%) of patients were 30-month disease-free survivors. With 6 to 11 years of total follow-up, 14 (5.5% of all patients) remain alive and free of cancer. This is the first large series of patients with minimum 5-year follow-up demonstrating the curative potential of current therapy for this disease. More recently, we have examined the clinical characteristics of the 16 patients who developed late lung cancer among a group of 40 two-year cancer-free survivors treated for small cell lung cancer. Ten of the 16 late lung cancers were pathologically confirmed small cell carcinoma, while six were proven to be non-small cell lung cancer. After three years, the number of non-small cell cases exceeded the number of small cell tumors. Based upon sites of recurrent tumor and smoking history, most non-small cancers appear to be new metachronous tumors associated with continued cigarette smoking, while late small cell cancers are usually true recurrences of the original tumor. Detailed neurologic, neuropsychologic, and cranial CT studies have been performed on 20 small cell lung cancer patients alive and free of cancer 2 1/2 to 10 1/2 years (median 6) from start of therapy. Sixty-five percent had abnormal neurologic and neuropsychologic tests, and 75% had abnormal cranial CT findings. Patients receiving therapeutic cranial irradiation (2) or prophylactic cranial irradiation with large (400 rad) fractions or during induction chemotherapy (6) were much more likely to have abnormal tests. This suggests that prophylactic irradiation delivered after induction chemotherapy with low (200-300 rad) doses per fraction may be associated with fewer long-term side effects. A randomized study comparing low-dose, late prophylactic irradiation with no irradiation in complete responders is being initiated.

Staging and Prognostic Studies: In our prospective randomized study of combination chemotherapy with or without chest irradiation in limited stage small cell lung cancer, we noted life-threatening pulmonary toxicity, defined as bilateral pulmonary infiltrates extending beyond the radiation portals and requiring hospital admission, in 28% of patients receiving combined modality therapy, compared with 5% in patients receiving chemotherapy alone ($p = 0.02$). Eight patients died from pulmonary toxicity, five of whom were in complete remission. Biopsy material failed to reveal any evidence of an infectious agent in all 11 cases examined. Pre-treatment pulmonary function tests revealed significantly lower VC and FEV_1 in patients who later developed pulmonary toxicity. Toxic patients also tended to be older, but this was not significant. Despite the increased incidence of pulmonary toxicity with combined modality therapy, the combined modality arm had significantly higher complete response rates and disease-free survival, and a trend toward improved overall survival. Improved methods of integrating chemotherapy and radiotherapy which retain better anti-tumor efficacy while reducing pulmonary toxicity are needed.

A study evaluating whether the addition of chest CT scans to chest radiographs and fiberoptic bronchoscopy improves the definition of complete response in small cell lung cancer patients is in progress.

Non-Small Cell Lung Cancer

Clinical Trial for All Stages: This is a unique nonrandomized trial involving the use of in vitro drug sensitivity assays to select appropriate therapy for patients with NSCLC. If SCLC "markers" are present in the tumor specimen, patients are assigned to treatment with the standard CMC/VAP small cell regimen. It is conducted in collaboration with the Thoracic Surgery, Pulmonary Medicine, and Radiation Oncology groups, and the cell biology laboratory. Preliminary results suggest that the assays can be successfully performed in more than one-third of patients. In this study we are also prospectively comparing NMR and CT scanning in the preoperative evaluation of the mediastinum in patients undergoing primary surgical therapy. As of March, 1985, 42 patients have been entered. Ten have received chemotherapy, and 3 have responded, including 1/1 with "small cell markers" who was given CMC/VAP.

Monoclonal Antibodies in Lung Cancer Therapy: As described elsewhere in this report, the mammalian analog of the amphibian peptide bombesin appears to function as an "autocrine" growth factor for small cell lung cancer in vitro. In nude mice in vivo, the monoclonal antibody 2A11 raised against bombesin inhibits the growth of subcutaneously implanted small cell lung cancer cell lines. Manufacture of clinical quantities of 2A11 should begin shortly, and we hope to begin a Phase I clinical trial, including radiolabeled imaging studies, in small cell cancer patients within six months.

CUTANEOUS T-CELL LYMPHOMAS

Clinical Trials in the Cutaneous T-Cell Lymphomas: Primary Treatment of CTCL Patients: The primary objective of our ongoing Phase III randomized trial of combined modality therapy with total skin election beam irradiation plus combination chemotherapy versus conservative therapy with topical nitrogen mustard (followed by PUVA, total skin election beam RT, and chemotherapy) is to determine the optimal treatment strategy for CTCL patients by stage. We are also evaluating: 1) which, if any, patients can be cured; 2) the frequency and significance of cytologic transformation and whether its development is influenced by initial treatment; and 3) the long term toxicities of each treatment approach. To date 91 patients have entered this trial which was designed to accrue 100 patients. To date, there are no survival differences between the 2 arms, although there is a suggestion of improved disease-free survival with combined modality treatment in patients with limited skin disease. There have been no unexpected toxicities and no deaths from toxicity, however, 3 patients have developed congestive heart failure possibly related to adriamycin.

We plan to complete the trial during the next year and perform an interim analysis during this period. A new study design will be based on the interim analysis.

Follow-up is also continuing on our pilot combined modality trial. Of 13 early stage patients, seven remain alive and disease free beyond 6 years.

Studies of the Natural History, Staging and Cliniopathologic Correlations of CTCL: The objectives of these studies are: 1) to gain an understanding of the natural history of the cutaneous T cell lymphomas; 2) to determine the most important prognostic features; 3) to evaluate the utility of a series of pre and post therapy staging procedures; 4) to establish criteria for the morphologic diagnosis of skin, lymph node, and visceral involvement; and 5) to evaluate biologic properties of the malignant cell with respect to DNA content, cytogenetics, cell surface antigens, oncogenes, etc., and to assess the prognostic significance of these factors.

We have performed a series of pretherapy staging procedures including routine evaluation of peripheral blood, lymph nodes, and visceral organs by x-rays, scans, and light microscopic evaluation of biopsy material. In addition, cytogenic analysis, electron microscopy, DNA content analysis, monoclonal antibody binding by flow cytometry and immunohistochemistry were performed on clinical samples. This prospective material has been collected on over 150 consecutive patients and is ein e a uated to assess factors of staging classification. To date, the type of skin lesion and the pathologic evaluation of lymph node biopsy have been shown to have independent prognostic significance. Descriptions of lymph node histologic classification and multivariate analyses are being prepared or have been accepted for publication.

Clinically we have observed that cytologic transformation occurs in about 5% of patients. This is associated with a change from small convoluted cells which have near-diploid chromosome numbers and DNA content with normal expression of mature helper T cell antigens to large blastic cells with loss of some mature T cell antigens, and changes in DNA content and chromosome number, but with retention of the ability to form E-rosettes. Patients with evidence of new cytologic transformation on a second lymph node biopsy (n=6) have markedly reduced survival compared with patients whose second biopsies show absence of cytologic transformation (n=19) or Hodgkin's disease (n=3).

Secondary Treatments of T Cell Lymphomas: We have shown that to rIFNdA from Hoffman-LaRoche has considerable activity (45% response rate, 95% CI 20%-70%) in advanced refractory CTCL. Since there were no complete responders, there was considerable toxicity, and tachyphalyxis to these toxicities was noted, we are studying a new higher dose, intermittent schedule of rIFNdA in these patients. Thus far, 2/10 have responded.

Clinical Serotherapy Trials:

We have completed Phase I-II studies of the monoclonal antibody T101 in patients with CTCL and CLL. There was minimal therapeutic activity. Subsequently, radionuclide conjugate T101 studies have been performed. We have performed serial scans on 13 patients after i.v. injection of ^{111}In labeled T101 which have shown dramatic tumor localization. Subcutaneous injection of ^{111}In T101 in 8 patients revealed even greater localization in regional lymph nodes. We plan to institute therapeutic trials with high dose radiation via radiolabeled T101 within the next year.

PHASE I-II STUDIES

Trimetrexate: A new Phase I trial of trimetrexate, a lipid-soluble analog of methotrexate, has begun in collaboration with the Clinical Pharmacology Branch. Three patients have been entered.

Phase II Studies: Phase II studies of carboplatinum (CBDCA) have been implemented for either treated or untreated patients with non-small cell lung cancer, and for patients with small cell lung cancer and breast cancer who have failed standard therapies. These studies are in collaboration with the Clinical Pharmacology Branch and Medicine Branch. Twenty patients have been entered. No responses have been seen.

HODGKIN'S DISEASE AND NON-HODGKIN'S LYMPHOMA

Diffuse Aggressive Lymphoma: Twenty-five patients have been entered on these studies originated by the Medicine Branch and the Radiation Oncology Branch. Patients with Stage II-IV disease are randomized to receive ProMACE-MOPP or ProMACE-CytaBOM, while those with Stage I diesase receive reduced doses of ProMACE-MOPP followed by involved field irradiation.

Nodular Lymphoma Favorable Histologies: This randomized trial of "watch and wait" vs aggressive therapy developed by the Medicine Branch and Radiation Oncology Branch at the Clinical Center has been implemented. Seven patients have been entered.

Hodgkin's Disease Massively Involving the Mediastinum: This single arm trial developed by the MB and ROB at the Clinical Center employing alternating non-cross resistant chemotherapy followed by radiation therapy for the mediastinum has been approved at the Naval Hospital. One patient has been entered.

Advanced Stage Hodgkin's Disease: This prospective randomized trial comparing alternating non-cross resistant chemotherapy vs MOPP chemotherapy in Stage III and IV Hodgkin's disease has been implemented. Thirteen patients have been entered.

TESTICULAR CARCINOMA

Advanced Nonseminomatous Germ Cell Carcinoma: Eight patients have been entered on this study developed by the Medicine Branch. Patients are randomized to receive standard PVB (cisplatin, vinblastine, bleomycin) or PVBV (a doubled dose of cisplatin, plus added etoposide [VP-16]).

BREAST CANCER

Clinical Trials in Carcinoma of the Breast: Three trials originated at the Medicine Branch or the Medicine Branch and Radiation Oncology Branch have been approved at Naval Hospital Bethesda. Nine patients have been entered.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06575-10 NMOB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Investigation of Tumor Cell Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

NCI-Navy MOB Senior Staff

John D. Minna, M.D.	Chief (USPHS)
Adi F. Gazdar, M.D.	Deputy Chief (Lab)
W. Michael Kuehl, M.D.	Senior Investigator

COOPERATING UNITS *(if any)*
See attached sheet

LAB/BRANCH
NCI/Navy Medical Oncology Branch

SECTION
Human Tumor Cell Biology and Molecular Genetics and Immunology Sections

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
20	14	6

CHECK APPROPRIATE BOX(ES)

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

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, 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. During the past two years we have established a molecular genetics group which complements the cell biological efforts cited above. Major areas of interest of this group include: (a) Analysis of chromosomal translocations; (b) characterization of growth factors elaborated by tumors, and (c) studies attempting to clarify the relationships between tumors and the genetic control of differentiation.

PHS 6040 (Rev. 1/84)

GPO 914-918

NCI-Navy MOB Senior Staff:

John D. Minna, M.D.	Chief
Adi F. Gazdar, M.D.	Deputy Chief (Lab)
W. Michael Keuhl, M.D.	Senior Investigator
Edward A. Sausville, M.D.	Senior Investigator
Bruce E. Johnson, M.D.	Senior Investigator
James L. Mulshine, M.D.	Senior Investigator

Clinical Associates:

Ethan Dmitrovsky, M.D.	NCI-Navy MOB NHBETH
Eric Seifter, M.D.	NCI-Navy MOB NHBETH
Christopher T. Denny, M.D.	NCI-Navy MOB NHBETH

Other Professionals:

Greg Hollis, Ph.D.	NCI-Navy MOB NHBETH
Shoshana Segal, Ph.D.	NCI-Navy MOB NHBETH
Ilona Linnoila, M.D.	NCI-Navy MOB NHBETH
Kieko Funa, M.D.	NCI-Navy MOB NHBETH
Frank Cuttitta, Ph.D.	NCI-Navy MOB NHBETH
Herbert Oie, Ph.D.	NCI-Navy MOB NHBETH
Jiang Gu, M.D.	NCI-Navy MOB NHBETH

Cooperating Units

Timothy Bender, Ph.D.	NCI-Navy MOB NHBETH
Richard Currie, Ph.D.	NCI-Navy MOB NHBETH
James Mitchell, M.D.	NCI-Navy MOB NHBETH
Nancy Shaper, Ph.D.	Visiting Investigator
Henry Chang, Ph.D.	Visiting Investigator
Ann-Marie LeBacq, M.D.	Visiting Investigator

NCI and Other NIH:

J. Whang-Peng, M.D.	C MD	G. Curt	C DCT
H. Schuller	C DTP	J. Mitchell	C RD

Others Cooperating

K. Becker, M.D.	VAMC, Washington, D.C.
S. Baylin, M.D.	Johns Hopkins Hospital, Baltimore, MD
T. Moody, Ph.D.	George Washington Univ., Washington, DC
A. Wolfsen, M.D.	UCLA, Los Angeles, CA
L. Deftos, M.D.	VAMC, San Diego, CA

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06578-02-NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Structure, Expression of Peptide Hormone Genes in Human Small Cell Lung Carcinoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Battey	Senior Staff Fellow	NCI-NMOB
E. Sausville	Medical Staff Fellow	NCI-NMOB
E. Seifter	Medical Staff Fellow	NCI-NMOB
A.M. LeBacq	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Laboratory of Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	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.)*

Many human small cell lung cancer cells selectively express peptide hormones both in the patient and in tissue culture cell lines derived from biopsy specimens. In patients, these hormones are known to mediate a constellation of endocrine and neurologic symptoms referred to as paraneoplastic syndromes. Two of these peptides, arginine vasopressin (AVP) and gastrin releasing peptide (GRP) are demonstrated mitogens for Swiss 3T3 cells under certain in vitro growth conditions. In addition, monoclonal antisera recognizing the five COOH amino acid residues of the twenty-seven amino acid GRP hormone have been shown to slow the growth of SCLC cells growing in culture and in nude mouse xenografts. Taken together, these results implicate GRP as an autocrine growth regulator of small cell tumors. To better understand the molecular mechanisms governing the selective expression of peptide hormone genes and their biological role in growth regulation, our group is deriving recombinant DNA clones for relevant polyprotein genes and using these clones to detail their gene structure and expression in SCLC.

Human AVP Locus

Within the last year, we have obtained human genomic clones for AVP and oxytocin (OT) genes and determined their structure and nucleotide sequence. These two structurally similar genes are linked in human genomic DNA, residing within 10 Kb of each other and in opposite transcriptional orientation. The parallels in structure between these two genes strongly suggests that they both evolved from a common ancestral polyprotein gene by duplication and inversion. Despite the similar structure and location of the AVP and OT genes, a human SCLC cell line (H378) selectively transcibes AVP and not OT. The molecular basis for

this selective expression is currently under investigaton.

We analyzed the chromatin structure of these two genes for DNase I sensitive sites and hypomethylation of CpG dinuclectides. Both of these chromatin structural features correlate with enhanced transcriptional activity in some gene families (i.e. globin and immunoglobulin genes). AVP and OT genes in H378 showed hypomethylation relative to their counterparts in H209, a SCLC cell line which does not express detectable quantities of either AVP or OT mRNA. No DNase I sensitive sites were noted around either the AVP or OT gene in either H378 or H209. We plan to further explore the basis for selective expression of AVP by introducing normal and modified recombinant clones into small cells using DNA transformation techniques. Cis-acting sequences in and around the genes that are critical for both normal and aberrant expression will hopefully be identified.

Human GRP Locus

During the past year, our group has derived twenty human GRP cDNA clones from two human SCLC cDNA libraries. DNA sequence analysis of these cDNA clones shows that they are identical in structure to cDNA clones derived from a human pulmonary carcinoid tumor. At least two forms of the mRNA for the human GRP gene have been identified in SCLC cells. These two forms differ by a 19bp insertion in the carboxy terminal peptide coding sequences, and probably result from alternative processing of the primary GRP transcript. Both forms would

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06579-02 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I.R. Kirsch	Senior Investigator	NCI-NMOB
PI:	G.F. Hollis	Senior Staff Fellow	NCI-NMOB
	C. T. Denny	Medical Staff Fellow	NCI-NMOB
	V. Bertness	Biol. Lab Technician	NCI-NMOB

COOPERATING UNITS *(if any)*

I. Magrath, PB, COP, DCT; T. Mak, Ontario Cancer Research Center; S. Smith, Children's Hospital, Stanford

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Laboratory of Genetics, Molecular Biology & Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	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.)*

Background: Chromosomal abnormalities specifically associated with tumors of specific cell types often involve chromosomal regions in which reside genes encoding important differentiated functions of the involved cell type. We have previously demonstrated this fact for erythroleukemias in which translocations can often be found to involve the chromosomal bands on which are located the globin gene families. We have developed a long term research program involved in discovering the importance of the consistent involvement of the immunoglobulin gene encoding regions in the translocations observed in Burkitt lymphoma cells and other B-cell tumors. Over the past year, using this concept as a predictive and testable hypothesis, we have and are continuing to investigate the relationship of specific chromosomal abnormalities to certain tumors. Our focus at present is on diseases of the hematopoietic system. This research program requires expertise in a number of distinct biological techniques. We have established this technical expertise which includes 1) our capability to grow and maintain a wide array of primary cells and cell lines 2) our facility in doing basic cytogenetic analyses, as well as the more involved procedure of chromosome in situ hybridization and, 3) our constantly updated ability to utilize the very newest of molecular biological techniques to clone, map, sequence and perform expression studies on DNA segments of interest.

Mapping Studies: In collaboration with S. Korsmeyer, we have mapped to a specific band (18q21) on chromosome 18 a DNA segment that appears to define one constant region of the reciprocal exchange seen in patients with nodular lymphoma.

Our hypothesis had predicted that important T cell specific functions would map to the regions involved in chromosomal abnormalities in T cell diseases (i.e. chromosome 7 band q35, chromosome 14 band q 112). The beta chain of the T cell receptor has been localized by others to 7q35. In collaboration with T. Mak, we mapped the alpha chain of the T cell receptor to 14q112 (J. Exp. Med. May 1985).

A newly described potential oncogene related to c-myc has been found to be amplified in a certain subset of tumors from patients with small cell lung cancer. In collaboration with J. Minna and W. McBride, we have identified the chromosomal region encoding this gene. Interestingly, it is a region implicated in translocations seen in other tumors of neuroectodemal as well as lymphoid origin (manuscript in preparation).

Cloning Studies:

We have completed an analysis (submitted Mol. Cell Biol) in collaboration with I. Magrath of a particularly revealing tumor cell line derived from a patient with Burkitt Lymphoma. This tumor carries a so-called "variant translocation" t(8,22). We have completely ascertained the DNA sequence of both rearranged as well as both germline counterparts to the translocation. We have demonstrated by S1 nuclease analysis that only the c-myc oncogene associated with the translocated chromosome is expressed in this cell. We have shown for the first time a case where a translocation event has created new DNA at the site of chromosome breakage and rejoining. As a fortuitous by-product of this study, we have also clearly demonstrated an apparent violation of the "rule" of ordered hierarchy of immunoglobulin gene rearrangement in B cells.

Our interest in aberrations of B cell tumors has continued into a collaboration with A. Gazdar on a newly derived human myeloma cell line. Such lines are extremely rare. The cytogenetic pattern of this line is extremely complex and provocative and our early molecular analysis is focusing on those regions that appear karyotypically abnormal (manuscript in preparation).

Our work on T cell tumors is also progressing. Anticipating the mapping of the alpha chain of the T cell receptor to band 14q112, we started a collaboration with S. Smith who supplied us with two cell lines derived from two children, one with a T cell lymphoma, the other with a CALLA + ALL, both tumors carrying a highly suggestive inv. 14 (q112, q32). Our analysis of the T cell line shows two T cell alpha chain rearrangements which are now both being cloned and characterized.

We will shortly be receiving the orthogonal field electrophoresis system designed by Schwartz and Cantor which should enable us to analyze stretches of DNA in the 50-1000 kb range and thus dramatically increase our ability to pinpoint chromosomal aberrations to specific chromosome segments.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06580-02-NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Control of Differentiation by Oncogene Expression in Erythroleukemia Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: I. R. Kirsch	Senior Investigator	NCI-NMOB
G. F. Hollis	Senior Staff Fellow	NCI-NMOB
W. M. Kuehl	Medical Officer	NCI-NMOB
S. Segal	Cancer Expert	NCI-NMOB
E. Dmitrovsky	Medical Staff Fellow	NCI-NMOB
T. Bender	Staff Fellow	NCI-NMOB

COOPERATING UNITS *(if any)*

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Laboratory of Genetics, Molecular Biology & Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) Minors

☐ (a2) Interviews

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

We have shown (manuscript submitted to Blood) that there is a correlation between the expression of the oncogenes c-myc and c-myb and the stage of development of cells of the erythroid lineage. Less mature cells make more oncogene transcript. Upon induction of differentiation by chemical agents, there is an abrupt fall (25 fold) in the level of both c-myb and c-myc transcript followed by a transient rebound. The levels of these messages tapers off until at the time of maximal globin production c-myb transcript is again 25 fold reduced while c-myc transcript is only 2-5 fold reduced compared to control culture. To test whether the alterations in oncogene transcript level is a necessary factor in the induction of differentiation, we are transfecting constitutive or regulatable contructs of these oncogenes into our murine erythroleukemia inducible cell lines. We hope to be able to control the level of oncogene message during the induction procedure and test the effect of varying transcript level on the progression of these cells toward terminal differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06581-02 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Molecular Genetics of B-Lymphocyte Development and Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: W. Michael Kuehl	Senior Investigator	NCI-NMOB
Richard Currie	Staff Fellow	NCI-NMOB
Timothy P. Bender	Staff Fellow	NCI-NMOB
Shoshana Segal	Cancer Expert	NCI-NMOB

COOPERATING UNITS *(if any)*

Laboratory of Microbiology Immunology, NIAID, (H.C. Morse); NMOB, NCI (J. Battey, G. Hollis, I. Kirsch)

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Laboratory of Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	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.)*

A. Overall objectives:

1. To identify B cell line models representing different stages of B cell development which can differentiate or dedifferentiate in appropriate in vivo or in vitro microenvironments

2. To clarify the cellular and molecular bases for B lymphocyte development

3. To clarify the relationship between B lymphocyte transformation and B-lymphocyte development

B. Species studied: mice and humans

C. Specific Studies:

1. DIFFERENTIAL EXPRESSION OF c-myb PROTO-ONCOGENE mRNA IN MURINE B-CELL TUMORS.

Three kinds of approaches have been used (i.e. tumor cell lines, induction of differentiation in a cloned cell line, and somatic cell hybrids). First, expres sion of the c-myb proto-oncogene has been examined in a series of murine B-cell tumor cell lines. These tumors included examples representative of pre B-cells (4), early and late B-cells (8), and plasma cells (3). The pre B-cell tumors express similiar high levels of c-myb mRNA while the B-cell lymphomas and plasma-cytomas produce 5-30 fold lower levels. These results indicate that transcription of the c-myb gene decreases during B-cell developement. Second, the 70Z/3.12 cell line is a carcinogen induced pre-B-cell tumor that produces a cytoplasmic u

heavy chain. It has a productively rearranged kappa light chain gene though it is not transcribed. Upon induction with LPS the rearranged light chain gene is transcribed and >90% of the cells become surface IgM+ but do not secrete IgM. These cells were observed during 45 hours in the presence or absence of LPS. Light chain mRNA was detectable by 6 hours, reached a maximum by 12 hours and plateaued. Levels of c-myb mRNA begin to decrease at 12 hours and decreased by 80% at 45 hours. This is in the range of expression seen in the B-cell lymphomas. Finally, a series of hybrid cell lines representing pre B-cell X B-cell (70Z/3B X A20.2J) and pre B-cell X myeloma (70Z/3B X 45.6TG) fusions were constructed and examined. All 10 70Z/3B pre B-cell (u+,L-,Ia-,J-chain- Ig secretion-) X A20.2J B-cell (X2a+,L+,Ia+,J-chain+ Ig secretion+) hybrids examined express the heavy and light chains encoded by each parent but are Ia-, do not express J-chain and do not secrete immunoglobulin. Each hybrid also expresses c-myb mRNA at the same level as the pre B-cell parent. Thus, 70Z/3B X A20.2J hybrids are phenotypically similiar to the lesser differentiated pre B-cell parent. The pre-B-cell X myeloma hybrids all (5/5) secreted high levels of of immunoglobulin and express the same low levels of c-myb mRNA as the myeloma parent. Therefore, in contrast to the pre B-cell X B-cell hybrids the pre B-cell X myeloma hybrid phenotype is like that of the more differentiated myeloma. To summarize, in murine B-cell tumor lines and hybrids, the level of c-myb mRNA expression appears to reflect the level of B-cell differentiation.

2. STRUCTURE OF MURINE Cmyb PROTO-ONCOGENE

We are determining the DNA sequence of murine Cmyb. Starting with an avian Vmyb probe we initially isolated genomic clones covering 40 kb, incuding sequences at each end of this span which hybridize to Cmyb mRNA but not to a Vmyb probe. We have recently prepared a murine pre-B cell cDNA library, and have isolated ten Cmyb cDNA clones. Together two of our cDNA clones include approximately 3.4 kb of Cmyb mRNA sequence. Since the two Cmyb mRNA species observed in B cells are 3.8 kb (major) and 4.2 kb (minor), completion of the DNA sequences of these two cDNA clones should enable us to deduce the protein structure of Cmyb, which is thought to require only 2kb of coding sequence.

This information, together with the cDNA and genomic clones we have isolated, should lead to definition of the murine Cmyb transcription unit(s). We also plan to: 1) express Cmyb in bacteria in order to prepare antisera and to provide large amounts of Cymb protein for study and 2) to prepare Cmyb vectors which can be transfected into and expressed in various eukaryotic cells. Our long term goal is to determine the role of the Cmyb protooncogenes in hematopoietic differentiation and tumor induction.

3. SOMATIC MUTATION IN BURKITT'S LYMPHOMA CELLS (in collaboration with J. Battey)

We have recently studied the Daudi Burkitt lymphoma cell line to examine ongoing somatic mutation in the first exon of its translocated (8:14) and selectively expressed c-myc gene. Daudi retains all three exons of the translocated gene but has suffered at least one S1 nuclease detectable alteration in the translocated first exon. With a probe specific for the first exon we have used an S1 nuclease

assay to look for further mismatches in subclones of the parent Daudi line. In an initial series of 30 subclones 3 such mutations were identified. We are presently developing a formamide gradient gel technique in conjunction with the

S1 assay as a more sensitive method to identify more subtle somatic changes. These efforts will be extended to the rearranged Ig V-region genes. DAUDI may represent a cell line that is actively undergoing somatic mutation of Ig DNA with the adjacent translocated c-myc gene a novel target.

4. ANALYSIS OF TRANSCRIPTION RATES AND RNA PROCESSING OF K L CHAIN IN CELL LINES REPRESENTING VARIOUS STAGES OF B CELL DEVELOPMENT.

Kappa L chain mRNA expression per cell increases 10-100 fold during B cell development. We are analyzing B cell lines representing early and late stages of development to determine if the level of K mRNA expression is regulated only by rates of transcription or if RNA processing is also involved.

We have used a murine pre-B cell line (702/3.12), a B cell line (A202J), and a myeloma cell line (S107), the latter two lines containing approximately 5 and 25 times as much Kappa mRNA, respectively, as the pre-B cell. Preliminary results indicate that the level of Kappa mRNA is determined mostly-if not entirely-by the relative rates of transcription (determined by nuclear run-on experiments).

5. REGULATION OF KAPPA L CHAIN GENE FORMATION IN A PRE-B CELL LINE (in collaboration with Shoshana Segal, Cancer Expert).

We have constructed a plasmid containing a germline Vk gene segment separated from the germline J_k-C_k gene segment by selectable markers (TK or GPT). Our 18-81 pre-B cell line (H+ L-) can spontaneously form functional kappa L chain genes. We have stably co-transformed our gpt version of this vector and pSV2 neo into a gpt^- derivative of 18-81 which has deleted its endogenous CK gene. We are now in the process of determining whether these cells can functionally rearrange our construct by counterselecting the cells in 6-thioguanine. If our preliminary results (i.e. that functional rearrangements of the exogenous gene occur) can be confirmed we plan to transfect a functional L chain gene into these cells to determine if the rearrangement process is inhibited, and thereby determine whether allelic exclusion is an active mechanism.

6. ROLE OF Cmyc AND Cmyb IN MURINE ERYTHROLEUKEMIC DIFFERENTIATION (in collaboration with S. Segal, G. Hollis, I. Kirsch).

Endogenous Cmyc and Cmyb mRNA levels decrease rapidly when murine erythroleukemic (MEL) cells are induced to differentiate with DMSO. We are presently introducing various myc constructs into MEL cells by stable transformation in order to determine whether constitutive expression of myc mRNA blocks differentiation or inhibits expression of endogenous myc mRNA. Similar studies will be attempted for Cmyb when we are able to prepare vectors containing a functional Cmyb gene that can be expressed in eukaryotic cells (See #2).

PUBLICATIONS (Laboratory)

Hollis, G., Mitchell, K., Battey, J., Potter, H., Taub, R., Lenoir, G., and Leder, P.: A variant translocation places the immunoglobulin genes 3' to the c-myc oncogene in Burkitt's lymphoma. Nature 307: 752-755, 1984.

Mitchell, K., Battey, J., Hollis, G., Moulding, C., Taub, R., and Leder, P.: The Effect of Translocations on the Cellular myc Gene in Burkitt Lymphoma. Journal of Cellular Physiology, Supplement 3: 171-177, 1984.

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Karawya, E., Swack J., Albert W., Fedorko J., Minna J.D., Wilson S.H.: Identification of a higher molecular weight DNA polymerase catalytic polypeptide in monkey cells by monoclonal antibody. Proc. Natl. Acad. Sci. USA 81: 7777-7781, Dec 1984.

McMahon, J.B., Schuller, H.M., Gazdar, A.F., and Becke, K.L.: Influence of priming with 5-hydroxytryptophan and APUD characteristics in human small cell lung cancer cell lines. Lung 162: 261-269, 1984.

Nau, M.M., Carney, D.N., Battey, J., Johnson, B., Little, C., Gazdar, A., and Minna, J.D.: Amplification, expression and rearrangement of c-myc and N-myc oncogenes in human lung cancer. Curr. Top Microbiol. Immunol. 113: 172-177, 1984.

Carney, D.N., Zweig, M.H., Ihde, D.C., Cohen, M.H., Makuch, R.W., and Gazdar, A.F.: Elevated serum creatine kinase BB levels in patients with small cell lung cancer. Cancer Research 44: 5399-5403, 1984.

Green, W.R., Linnoila, R.I., and Triche, T.J.: Neuroendocrine carcinoma of skin with simultaneous cytokeratin expression. Ultrastruct. Pathol. 6: 141-152, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06582-01 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Growth factors for human lung cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Frank Cuttitta, Ph.D.,	Senior Staff Fellow	NCI-NMOB
John Minna, M.D.,	Chief	NCI-NMOB
Joseph Fedorko, M.D.,	Microbiologist	NCI-NMOB
James Mulshine, Ph.D.,	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	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.)*

Our group has previously shown that bombesin/gastrin releasing peptide (BN/GRP) functions as an "autocrine growth factor" for human small cell lung cancer (SCLC). We have produced a monoclonal antibody (2A11) to this peptide which inhibits the clonal growth of SCLC in vitro and in vivo in nude mouse xenografts. In addition, we have shown that rabbit anti-idiotypic antiserum to 2A11 blocks bombesin/SCLC receptor interaction and stimulates the clonal growth of SCLC in vitro. The rabbit antiserum represents a heterologous mixture of many different types of anti-idiotypic antibodies some having agonistic and some showing antagonistic properties. In an effort to dissect such function we have generated syngenic monoclonal anti-idiotypic antibodies against 2A11 with the hopes of finding an antagonistic reagent capable of blocking "autocrine growth" at the receptor level.

The cDNA gene for BN/GRP has recently been cloned by Eliot Spindel and Joel Habner at Massachusetts General Hospital in Boston. The BN/GRP mRNA encodes a precursor of 148 amino acids containing a typical signal sequence, human BN/GRP consisting of 27 or 28 amino acids and a carboxy-terminal extension peptide. Using these reported amino acid sequences we have synthesized fragmentary peptides homologous to the amino-terminus of human BN/GRP and the mid portion and carboxy-terminal region of the extension peptide. Rabbit polyclonal antiserum has been generated against these peptides and is now being used as an immunohistochemical reagent to assess the expression of gene products in SCLC cell lines.

PHS 6040 (Rev. 1/84) GPO 914-918

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06583-01 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Expression of histocompatability antigens on lung cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Keiko Funa, M.D.,	Visiting Fellow	NCI-NMOB
Ilona Linnoila, M.D.,	Senior Staff Fellow	NCI-NMOB
Adi Gazdar, M.D.,	Senior Investigator	NCI-NMOB
James Mulshine, M.D.,	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

J. Cotelingham, M.D., Naval Hospital Bethesda

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☐ (a1) Minors

☐ (a2) Interviews

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

SCLC cell lines, unlike NSCLC lines, have markedly decreased or absent class I major histocompatibility antigens. To examine whether this is valid in vivo we have studied serial paraffin -embedded tissue sections from 36 SCLC patients and 79 NSCLC. patients for expression of B_2microglobulin. Most of the tissues were completely negative or weakly positive. The opposite was true for NSCL. Thus B_2 microglobulin can be used as a diagnostic marker for SCLC vs. NCLC on tissue sections. Furthermore, we demonstrated in vivo induction of B_2-microglobulin on SCLC patients and mid gut carcinoid patients treated with interferon. Before interferon treatment, biopsies from 3 SCLC patients and 6 mid-gut carcinoid patients did not express B_2 microglobulin. In contrast, biopsies from the same patients after interferon therapy showed clear positivity for B_2-microglobulin. Lack of B_2-microglobulin expression on SCLC cells may play a role in their widely metastatic behaviour, and prevent the patient's immune surveilance system from inhibiting their growth. In addition, our findings suggest a new novel method by which interferon exerts its antitumor effects.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06584-01 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Immunohistochemical study of marker expression on lung cancers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ilona Linnoila, M.D.,	Senior Staff Fellow	NCI-NMOB
Adi Gazdar, M.D.,	Senior Investigator	NCI-NMOB
James Mulshine, M.D.,	Senior Investigator	NCI-NMOB
Daniel Ihde, M.D.,	Senior Investigator	NCI-NMOB
Jiang Gu, M.D.,	Visiting Fellow	NCI-NMOB

COOPERATING UNITS *(if any)*

J. Cotelingham, M.D., Naval Hospital Bethesda

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

There is cumulative evidence for multidirectional differentiation in lung carcinomas. Based on in vitro studies on the biology of small cell carcinoma of the lung (SCLC) good markers for this tumor type have been established. Accordingly, we investigated the occurrence of relevant neuroendocrine NE markers in 120 newly diagnosed lung cancers of major histological types by immunocytochemistry. We examined paraffin sections using avidin-biotin immunoperoxidase method with monoclonal and/or polyclonal antibodies to chromogranin A (a structural protein in endocrine granules), Leu-7 (HNK-1, an antigen shared by neurons, endocrine cells and their tumors with human natural killer cells), neuron specific enolase, the amine serotonin, and the polypeptide hormones bombesin, adrenocorticotropin, calcitonin and neurotensin. From the results we can conclude that 1) most, but not all, SCLC and carcinoids express multiple (more than 3) NE markers in a high percentage of tumor cells; 2) occassional non small cell lung cancers (NSCLC) show staining patterns indistinguishable from SCLC; 3) many NSCLC contain a small subpopulation of cells expressing NE markers. The same panel of markers will now be prospectiely used to study the tumor tissues from patients in SCLC and non small cell lung cancer protocols, and the results correlated with their prognosis and response to therapy. The patients who have non small cell lung cancer with multiple NE markers may benefit from SCLC -like treatment protocol.

It is apparent that the c-myc and myc related sequences such as N-myc and the newly discovered L-myc genes may play a role in the clinical and cellular biology of human SCLC. To further study the relationship of oncogene amplification and expression to tumor progression and morphology we are in a process to establish in situ hybridization techniques using various myc related DNA probes in our laboratory. This will be the major research effort during the coming year of Dr. Jiang Gu, who has recently joined our staff as a Fogarty Fellow.

GPO 914-918

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06585-01 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Culture and characterization of human myeloma

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)*

PI: Adi Gazdar, M.D.,	Senior Investigator	NCI-NMOB
Herbert Oie, Ph.D.,	Microbiologist	NCI-NMOB
Gregory Hollis, Ph.D.,	Senior Staff Fellow	NCI-NMOB
Ilana Kirsch, M.D.,	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

We established a plasma cell line, NCI-H929, from a malignant effusion obtained from a patient with IgAK myeloma. The cell line was cultured in ACL-3, a defined serum-free medium, and was mycoplasma free. The cultured cells had the morphologic, ultrastructural and cytochemical features of plasma cells. They synthesized and secreted very high concentrations of IgAK. The tumor and cultured cells lacked Epstein-Barr virus nuclear antigen and most B cell antigens, but expressed the plasma cell associated antigen PCA-1. These studies demonstrate that NCI-H929 is a fully differentiated, highly secretory plasma cell line. While a modest number of human 'plasmacytoid' cell lines have been established, most are lympho blastoid lines lacking plasma cell features, while others appear to be less differentiated secretory cells. Most of NCI-H929 cells were near tetraploid, with six copies of chromosome 8 having an 8q+ abnormality. Of major interest, the tumor and cultured cells had a rearrangement of the cellular c-myc proto-oncogene (located at 8q24) and expressed c-myc RNA. These studies link for the first time, at the molecular level, plasma cell tumors of two mammalian species, man and mouse. At present, we are investigating the precise nature of the DNA rearrangement in NCI-H929 cells and its relationship to the 8q+ abnormality by molecular cloning. We are drug marking the line for future use as a fusion partner for the generation of human monoclonal antibodies.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06586-01 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies of Human Immunoglobulin Gene Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Gregory F. Hollis	Senior Staff Fellow	NCI-NMOB
P.I.: Ilan R. Kirsch	Senior Investigator	NCI-NMOB
Ethan Dmitrovsky	Medical Staff Fellow	NCI-NMOB
Henry Chang	Visiting Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

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NCI-Navy Medical Oncology Branch

SECTION

Laboratory of Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	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.)*

Background: We have been interested in the molecular structural of the human immunoglobulin genes for many years. To understand these genes in fine structural detail we have examined both human heavy and light chain genes by molecular cloning, restriction map analysis and nucleotide sequence determination. Those studies have allowed us to understand the molecular events involved in the formation of a functional immunoglobulin gene. During our analysis of the human lambda light chain gene system, we discovered a class of genes that on Southern genomic blot analysis showed weak homology to the lambda constant regions.

Results: To determine the nature of these new genes, we isolated recombinant DNA clones that contained these regions of human lambda constant region homology. Fine structure analysis of these genes, including DNA sequence, revealed two genes which showed homology to lambda constant regions but differed by approximately 15% at the deduced amino acid sequence level. These two genes contained open reading frames that in size and structure could encode functional light chain proteins. Further analysis of these constant regions revealed a single J region element, including the heptamer and nonamer recombinational signal sequences, in front of each gene. Therefore, these genes could be functional immunoglobulin light chain genes, but to date have not been described at the protein level. Because they differ by greater than 15% from the described lambda protein, these genes may encode a new class of human light chain proteins, or may represent an example of immunoglobulin genes that have been recruited for a new function. At present, we are continuing our studies of these genes by looking for their rearrangement and expression in human hematopoietic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06587-01-NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

RNA-DNA in situ Hybridization of Tissue Sections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: I. R. Kirsch	Senior Investigator	NCI-NMOB
PI: G. F. Hollis	Senior Staff Fellow	NCI-NMOB
K. Funa	Visiting Fellow	NCI-NMOB

COOPERATING UNITS *(if any)*

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NCI-Navy Medical Oncology Branch

SECTION

Laboratory of Genetics, Molecular Biology & Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	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.)*

Capitalizing on our experience in chromosome in situ hybridization, we have embarked on a new procedure designed to detect the expression of particular genes in tissue sections in situ. Using DNA or RNA probes, we have begun to analyze immunoglobulin, T cell receptor, and oncogene expression in cytocentrifuge preps of characterized cell lines, as well as in normal and abnormal lymph node specimens. The research and clinical utility of this technique would, we feel, be profound if it could be as easily and systematically applied as are current histochemical and immune cytochemical techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06588-01-NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

The Role of Glycosyltransferases in Development and Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: G. F. Hollis	Senior Staff Fellow	NCI-NMOB
I. R. Kirsch	Senior Investigator	NCI-NMOB
Henry Chang	Visiting Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

Joel Shaper	Oncology Department
Nancy Shaper	Johns Hopkins University

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Laboratory of Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	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.)*

One of the major features of the architecture of the cell surface is the complex carbohydrate structures that are a part of glycoproteins and glycolipids. These complex carbohydrates, as well as the glycosyltransferases that assemble them, have been the object of detailed study, yet their biological role remains uncertain. It has been suggested that these complex carbohydrate structures are one of the major means by which cells communicate with each other. In these models of cell-cell communication, cell surface receptors recognize and bind specific carbohydrate moieties on neighboring cells and through this linkage direct the information flow between cells. The specific cell surface receptors are central to this model of cell-cell communication. Therefore, the finding that glycosyltransferases are located on the cell surface, as well as at the point of complex carbohydrate synthesis in the golgi complex, strongly implicated them as the specific receptors. These models of cell-cell communication are supported by an expanding body of literature which demonstrates that specific changes in carbohydrate structure occur through embyonic development. This has led investigators to suggest that the formation and recognition of these carbohydrate structures are required for normal embryonic development. One group of mutations that is consistent with this prediction are the developmental mutants in the mouse referred to as the T/t complex. When two T/t complex mice are mated, the dividing mouse embryos die at specific stages of development. Although the precise nature of these mutations has not been established, cell surface carbohydrates and their receptors have been implicated.

A second line of investigation that has implicated these complex carbohydrate structures in cell-cell interaction comes from detailed analysis of the surface antigens of normal and tumor cells. Malignant cells differ in their surface antigens from normal cells. Careful study of these antigens by monoclonal antibodies has shown that these antigenic variations are due to carbohydrate structure differences in the malignant cells.

While much research has been done to examine the structure of these carbohydrates, nothing is known about the genes that encode the glycosyltransferases. Clearly understanding the role of glycosyltransferases in development and malignancy is necessary to understand these processes. Therefore, we have undertaken a project whose goal is to clone one of these glycosyltransferases, galactosyltransferase, and study its genetic structure and the regulation of its expression in normal and malignant cells as well as during embryonic development.

Results: To clone the gene encoding galactosyltransferase we have constructed both a human and bovine cDNA library that has been cloned into the lambda phage expression vector GT11. In this phage vector system, we can control the expression of an inducible fusion gene product where part of the protein is phage encoded and the other part encoded by the cDNA insert. These plaques can be screened with antibody directed against galactosyltransferase. At present we have constructed and screened these libraries. While we are only in the initial stages of the cloning, we have identified several antibody positive clones and are in the process of plaque purifying and characterizing these clones.

These clones will allow us to do a detailed study of the structure of this gene as well as its primary sequences. Further, these clones will provide probes that will permit us to analyze its expression in various cell types. The long range goals of this study include answering the following questions: 1) what is the molecular basis for the two cellular locations, golgi complex and cell surface, of galactosyltransferase 2) what is expression pattern of this and related genes in normal and malignant cells 3) when is galactosyltransferase expressed in the developing embryo and 4) how does its expression relate to cell-cell interactions? These studies will be made possible by the molecular cloning of the gene for galactosytransferase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06589-01 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Establishment and characterization of human lung cancer cell lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Adi Gazdar, M.D.,	Senior Investigator	NCI-NMOB
Herbert Oie, Ph.D.,	Microbiologist	NCI-NMOB
Edward Russell, Ph.D.	Chemist	NCI-NMOB
Ilona Linnoila, M.D.,	Senior Staff Fellow	NCI-NMOB
James Mulshine, M.D.,	Senior Investigator	NCI-NMOB
James Carmichael, Ch.B.,	Visiting Fellow	NCI-NMOB

COOPERATING UNITS *(if any)*

Hildegarde Schuller	C	DTP
Robert Shoemaker	C	DTP

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0

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

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

We have established and characterized 87 human lung cancer cell lines. Resected primary tumors and aspirates or biopsies of metastatic lesions were cultured in non-selective serum supplemented medium or in partially or fully defined selective media (HITES for small cell carcinoma, SCLC, and Rheinwald's or LA3 for non-SCLC cancers). Success rates were 62/92 (69%) for SCLC and 25/55 (45%) for non-SCLC. Cell lines were characterized by histology of xenografts in athymic nude mice, by DNA content analysis, and by cytological, biochemical and ultrastructural examination. Clonogenic and dye exclusion assays were used for in vitro drug sensitivity studies. SCLC lines, from untreated and previously treated patients consisted of classic lines (45) having typical morphology and neuroendocrine markers, or variant lines (17) having atypical morphology and selective loss of markers. Non-SCLC lines, from untreated patients, consisted of: adenocarcinomas (8), well or poorly differentiated; bronchioloalveolar (3) of Clara cell or type II pneumocyte varieties; squamous cell (3), moderately or poorly differentiated; large cell (3); adeno squamous (2); mesothelioma (3); and other (4), consisting of mucoepidermoid, carcinoid and oncocytoma. In most cases, the lines retained the morphological and biochemical features of the original tumors. Drug sensitivity assays demonstrated considerable differences in the relative sensitivity of the lines, and also in the effects of individual drugs. The cell lines demonstrate the great diversity of lung cancer types and they provide a comprehensive panel that is suitable for biological and drug sensitivity studies.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06590-01 NMOB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Growth and characterization of pulmonary endocrine cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ilona Linnoila, M.D.,	Senior Staff Fellow	NCI-NMOB
Keiko Funa, Ph.D.,	Visiting Fellow	NCI-NMOB
Adi Gazdar, M.D.,	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

K. Becker, M.D., VAMC, Washington DC.
H. Schuller C DTP

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

Hamster pulmonary endocrine (PE) cells demonstrate hyperplasia following exposure to diethylnitrosamine which is a systemic carcinogen. A long term selective culture of hamster PE cells was established using defined media. Characterization of these cells show tht they share such NE markers (dense core granules, production of peptides calcitonin and bombesin, high levels of dopa decarboxylase and creatine kinase brain isoenzyme) with SCLC and human PE cells. Cultured hamster PE cells will be now used to induce and study malignant transformation of these cells in vitro, as well as the effects of transfecting them with c-myc.

THE PEDIATRIC BRANCH RESEARCH ACTIVITIES

October 1, 1984 - September 30, 1985

A. Clinical Studies

1. In our study of acute lymphoblastic leukemia, we have 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, 176 patients have been randomized on this study: 58 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 approximately 75% at two years for the entire study group. With a median duration on study of 43 months, there is no significant difference in the CNS relapse rate for either treatment group. Long-term follow-up evaluation of neurotoxicity (by CT scan, neuroendocrine evaluation and psychometric testing) is underway. These data indicate 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.

   The results of the above study have helped to identify a subset of patients at particularly high risk of extramedullary relapse. Based on these observations and their overall treatment results, two new treatment studies for patients with acute lymphoblastic leukemia have been initiated. The first, a protocol specifically designed to treat high risk patients, involves the use of an aggressive, early intensification phase of therapy and an intensified systemic maintenance therapy, together with additional CNS specific chemotherapy. The second protocol is designed to treat patients in the average risk category and involves a randomization to one of two forms of CNS preventive therapy -- either high dose systemic methotrexate infusions or intrathecal methotrexate alone.

2. Relapse during maintenance therapy remains a major reason for failure in children with ALL. Studies on the bioavailability of 6-mercaptopurine (6-MP) and methotrexate have demonstrated poor and variable bioavailability of these agents following oral administration, raising the question of whether oral maintenance chemotherapy for patients with ALL is optimal. These observations have formed the basis for a newly instituted primary ALL protocol which will attempt to correlate the results of prospective periodic pharmacokinetic bioavailability studies with relapse rate and remission duration. To date, approximately 35 patients have been entered into this planned multi-year study.

3. Our in vitro observations regarding the optimal cytotoxic concentration of 6-MP, together with our data regarding the poor bioavailability of this agent when administered orally, led us to the development of a Phase I trial of intravenous 6-MP administered by prolonged infusion. This mode of administration reduces inter-patient variability of drug levels and achieves therapeutic levels of 6-MP in the CSF. Following successful completion of this Phase I study and identification of a safe infusion dose rate, we have embarked upon three separate Phase II studies evaluating this approach to treat pediatric patients with brain tumors, with solid tumors, and with refractory acute lymphoblastic leukemia.

4. We have successfully completed a pediatric Phase I study of Tiazofurin. A total of 22 patients were entered onto this study in which the drug was given daily for five consecutive days at three-week intervals. A maximally tolerated dose of 2200 mg/m^2/day was identified. This dose has been suggested for future Phase II studies. We have instituted two additional Phase I studies. Trimetrexate, a non-classical folate antagonist, is being studied on a weekly I.V. bolus schedule in pediatric patients. To date, 8 patients have been entered on this study and no dose-limiting toxicities have as yet been identified. We are also performing a Phase I study of Spirohydantoin Mustard in pediatric malignancies. This drug is being given intravenously on a weekly x 3 schedule. To date a total of 7 patients have been entered on this protocol. No dose-limiting toxicities have been observed at the doses utilized thus far.

5. Utilizing our previously described subhuman primate model for studying CSF pharmacokinetics, we demonstrated the feasibility of administering a newly developed aziridinyl benzoquinone (AZQ) by intrathecal and intraventricular injection. We developed an ongoing Phase I-II trial of intraventricular AZQ in pediatric patients. To date, 12 patients have been entered onto this study and 3 patients have attained complete remission. The intraventricular therapy with this agent appears to offer promise for patients with refractory meningeal disease.

6. We have established a late effects team to aid in the evaluation of the long-term effects of antineoplastic therapy in children. Recent studies in this area have demonstrated that 1) CT scan abnormalities may first appear as late as 8 years from the time of initiation of CNS prophylaxis, 2) measurement of basal pulsatile growth hormone output is a sensitive indicator of hypothalamic-pituitary dysfunction in leukemic children who have received CNS irradiation, and 3) the use of a multidisciplinary approach to study late effects permits a comprehensive evalution which facilitates the rehabilitation of effected children.

7. Our primary study (77-C-145) for patients with undifferentiated lymphomas (both Burkitt's and lymphoblastic types) employs alternating cycles of a high-dose methotrexate infusions with CHOP, administered on approximately 10-day intervals without delays for neutropenia. Our analysis of 85 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, 12 of 13 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. On the basis of this information two new pilot protocols for patients with undifferentiated lymphomas have been initiated. One is a study of a new drug combination in relapsing patients (ifosfamide, VP16 and high dose ara-C). The other is an intensified version of protocol 77-C-145. If the new drug combination proves to be active in relapsed patients, a protocol incorporating this combination for very high risk, untreated patients, will be developed. Patient numbers are too small at present for further comment.

8. We have initiated 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. This protocol combines high dose chemotherapy during induction (emphasizing intensive adriamycin) in combination with cyclophosphamide and vincristine. Following induction, patients undergo high dose total body irradiation (800 rads) in conjunction with autologous bone marrow reconstitution. To date, 59 patients have been enrolled in this protocol and the results suggest that early intensive therapy is well tolerated and highly effective (93%) in achieving a successful induction. Evaluation of the bone marrow transplant component of this protocol is currently underway.

9. 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, we randomized patients to either our conventional combination of cephalothin, gentamicin, carbenicillin (KGC) versus a new third generation cephalosporin, ceftazidime (CTZ). To date, 608 granulocytopenic episodes have been randomized to either antibiotic regimen when they become febrile. This represents the largest clinical trial done in this area. The initial response during the first 72 hours was evaluated according to whether the patient had an unexplained fever (FUO) or a documented infection: For patients with FUO, 98% of patients treated with KGC or CTZ were successfully treated; for patients with documented infection, the initial response was 98% for KGC and 97% for CTZ patients. The overall response, at the resolution of the granulocytopenia was 98% for the FUOs randomized to KGC and CTZ and 91% for the patients with documented infections randomized to KGC vs. 89% for documented infectious patients randomized to CTZ. Thus, monotherapy may be as successful as combined therapy, particularly for initial period of empiric management. Overall, our results are superior to any other reported to date.

10. We have contributed 16 patients of the 36 eligible randomized patients on the Pediatric Oncology Group multi-institutional osteosarcoma study which randomized patients with totally resected, high-grade, extremity osteosarcoma between no chemotherapy and immediate adjuvant chemotherapy using first-line agents. This study has demonstrated statistical

superiority of the immediate chemotherapy group in time to first relapse, although there is no survival difference to date between the two arms. The results of this crucial study will form the basis of future trials.

11. We have initiated a series of studies looking at pediatric pain measurement techniques, and epidemiologic and therapeutic aspects of pain in children with cancer. The epidemiologic studies are detailing firstly the incidence, duration and nature of pain in newly-diagnosed cancer patients, and secondly the predictive factors and nature of phantom limb pain and sensations in amputees. The therapeutic studies have documented dosage, toxicity and kinetic data of morphine given via the continuous intravenous or subcutaneous routes in 26 patients. Ongoing therapeutic studies are examining firstly the efficacy and kinetics of continuous intravenous or transdermal infusions of fentanyl in children with cancer experiencing pain, and secondly the efficacy and feasibility of administration of nitrous oxide in children undergoing painful diagnostic or therapeutic procedures.

B. Pre-Clinical Studies

1. In our sub-human primate model for CSF pharmacokinetics studies, we have recently evaluated 3 agents of potential utility in treating meningeal malignancy. Tiazofurin (TCAR), a C-nucleoside which produces guanine nucleotide depletion by the inhibition of inosine monophosphate dehydrogenase, was demonstrated to have excellent penetration into the CSF following IV administration. This drug is currently being studied in Phase II clinical trials. Studies of Trimetrexate, another nonclassical antifol which has been demonstrated to be active in vitro against leukemic cells resistant to methotrexate, has been studied for its CSF pharmacokinetics. Its enhanced lipophilicity holds the prospect for using this agent in the treatment of meningeal leukemia or meningeal carcinomotosis. We have also studied the pharmacokinetics of intravenous thiotepa. This latter agent has been used with extremely limited success via the intra-CSF route of administration. Our studies have demonstrated for the first time that substantial amounts of its metabolite, TEPA, are present in the cerebrospinal fluid for prolonged periods of time following intravenous administration, suggesting that this route of administration may be a more optimal one to approach CNS disease with this agent. A clinical trial of this approach will be instituted shortly.

2. To study mechanisms responsible for clinical resistance to 6-MP, leukemic cells were obtained from 10 patients with ALL at diagnosis and on the same patients at the time of their initial marrow relapse. Four of these patients had biochemical evidence of 6-MP resistance in relapse. Three of four patients had a greater than 50% decrease in intracellular HPRT activity, four of four had a greater than 50% increase in intracellular PRPP, and two of four had a greater than 9-fold increase in intracellular alkaline phosphatase activity at relapse. These results indicate that clinical resistance to 6-MP may be related to alterations in HPRT, PRPP or alkaline phosphatase activity. These findings may have implications for the manner in which 6-MP is given during ALL maintenance therapy.

3. We studied the pharmacokinetics of Trimetrexate in rhesus monkeys and found that Trimetrexate is characterized by a slower clearance rate than methotrexate, by poor CSF penetration and elimination primarily by metabolism. In addition, we have identified two new active metabolites of Trimetrexate both in plasma and urine. These metabolites are currently being identified. Knowledge of their existence may be of value in the design of Phase I and Phase II studies of this agent in man.

4. We have begun to explore the association of c-myc and Cμ expression in Burkitt's lymphoma cells and lymphoblastoid cell lines treated with retinoic acid and ethanol. Preliminary results suggest that expression of these two genes is not always concordant in Burkitt's lymphoma cells, and that whereas in B-cells without a chromosomal translocation, c-myc expression is diminished during differentiation, this is not the case in Burkitt cells. These findings have implications for the regulation of the c-myc gene in Burkitt's lymphoma cells.

5. We have developed an in situ hybridization technique, using a biotinylated probe, able to demonstrate the presence of EBV genomes in individual cells. We shall be exploring the utility of this technique in detecting the presence of EBV DNA in Burkitt tumors from various parts of the world.

6. We have determined that $pp60^{c\text{-}src}$ molecules bound to the polyoma virus middle T antigen in polyoma virus transformed rodent cells are phosphorylated on tyrosine residues close to the aminoterminal end of the molecule. This post-translational modification is likely to be closely associated with the enhanced $pp60^{c\text{-}src}$ tyrosyl kinase activity which we previously demonstrated to occur during polyoma virus transformation of cells. We have also found that the level of $pp60^{c\text{-}src}$ tyrosyl kinase activity is high in neuroblastoma and dramatically altered in other human tumors. Activated $pp60^{c\text{-}src}$ tyrosyl kinase molecules found in human neuroblastoma also bear a N-terminal phosphorylation indistinguishable from that found in polyoma transformed cells. However, activated $pp60^{c\text{-}src}$ in other human tumors is not modified in this manner suggesting the possibility that this enzymatic activity may be modulated by multiple molecular mechanisms.

7. We have found that the rcp(11;22)(q24;q12) translocation of Ewing's sarcoma and peripheral neuroepithelioma also characterizes Askin's tumor of the chest wall. Subsequent analysis of this tumor has revealed it to be indistinguishable from peripheral neuroepithelioma and provides a rationale for treatment of these patients, whose clinical course also parallels that observed in patients with Ewing's sarcoma, with therapy which is effective treatment for this tumor. Molecular studies of tissue from these tumors has allowed us to place the translocation breakpoint in these tumors at a site distal to the lambda light chain gene locus but proximal to the site of the c-sis proto-oncogene on the long arm of chromosome 22. We are currently characterizing other molecular clones in order to more precisely describe the breakpoint site and initiate an evaluation of the physiologic alterations resulting from this chromosomal rearrangement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06830-15 PB

PERIOD COVERED

October 1, 1984 - September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip A. Pizzo	Head, Infectious Disease Section; Chief	PB, NCI
Other:	J. Hathorn	Senior Staff Fellow	PB, NCI
	M. Browne	Medical Staff Fellow	PB, NCI
	M. Rubin	Medical Staff Fellow	PB, NCI
	J. McKnight	Medical Staff Fellow	PB, NCI

Continued on next page

COOPERATING UNITS *(if any)*

Medicine Branch, NCI; Diagnostic Microbiology, CC; Bethesda Naval Hospital

LAB/BRANCH

Pediatric Branch

SECTION

Infectious Disease

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	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.)*

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. We are seeking to those granulocytopenic patients who are at heightened risk for infection. 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.

To prevent infections 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 preclinical 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 evaluating the effect of various opiate derivatives on leukocyte function and we are developing experimental animal models to help elucidate current clinical dilemmas -- e.g., invasive fungal infections in neutropenic patients.

PHS 6040 (Rev. 1/84) GPO 914-918

Professional Personnel (Continued):

J. Skelton	Guest Researcher	PB, NCI
M. Thaler	Visiting Fellow	PB, NCI
D. Marshall	Research Nurse	PB, NCI
J. Gress	Research Nurse (Guest Worker)	PB, NCI
D. Longo	Head, Experimental Immunology Section	MB, NCI

Objectives and Specific Aims

Our overall objective is to develop rationale guidelines for improving the diagnosis, management and prevention of infectious complications in cancer patients. Our research efforts are specifically focused in three areas:

1. To define the risk factors associated with infection and the current patterns of infection in granulocytopenic and nongranulocytopenic cancer patients. In addition, we seek to develop better methods to evaluate and diagnose infection in granulocytopenic patients.

2. To elucidate the appropriate management of fever and infection in granulocytopenic cancer patients. In particular, we seek to determine the best approach to the initial empiric antibiotic management of the granulocytopenic patient who becomes febrile; the appropriate management of the granulocytopenic patient with unexplained fever; and the management of patients who have proven infectious.

3. To develop methods to decrease the risk or incidence of fever and infection in the granulocytopenic patient. Given the etiology and pathogenesis of these infections, we seek ways to reduce the acquisition of new microbial oganisms by the patient at risk; to suppress the host's endogenous microbial flora in order to reduce the risk for infection; and to improve and replenish altered host defenses with the goal of permitting the host to better ward-off potential pathogens.

Methods and Progress Report

A. Risk Factors and the Diagnosis of Infection in Cancer Patients

1. Antibody to the Core Glycolipid of Enterobacteriaceae

It has recently been shown by Ziegler et al. (N. Engl. J. Med. 307: 1225, 1982) that passive immunization with antiserum prepared from volunteers who were vaccinated with a mutant E. coli decreased mortality in patients with gram-negative sepsis. The component assumed to be important in this "J5 antiserum" is antibody to the core glycolipid, a region shared in common by virtually all gram-negative bacteria. To evaluate this possible protection, serum titers against the core glycolipid from two different rough mutants, the Re chemotype of S. minnesota R595 (Re) and the Rc chemotype of E. coli O:111 (J5) were assayed in 74 children and young adults with malignant disease by indirect hemagglutination and complement-dependent hemolysis and compared with those in normal children.

Anti-Re titers were similary low or absent in normal children, adults and patients with malignancy. In contrast, anti-J5 hemagglutinating and hemolytic titers >1:20 were present in all normal children, did not differ with age and were similar to those in healthy adults. The reciprocal geometric mean anti-J5 titers of non-infected patients with malignancy and of those with Gram-negative rod sepsis were significantly lower ($p < 0.001$) than those of normal children. Moreover, antibody titers fell during the course of the patient's illness, presumably as a consequence of chemotherapy. The mean hemolytic titer, but not hemagglutination titer, of patients with Gram-negative rod sepsis was significantly lower ($p < 0.01$) than that of non-infected patients. The lowest mean titers occurred in those who died with Gram-negative rod sepsis. These differences suggest that circulating antibody directed against the J5, but not the Re, core glycolipid affects susceptibility to and outcome of Gram-negative rod sepsis in patients with malignancy.

2. Effect of Chemotherapy on Salivary Function

As a prelude to studies on microbial colonization and adherence alterations in the oral cavity of cancer patients, we initiated a collaborative study with the NIDR (Drs. P. Fox and B. Baum) to evaluate changes in salivary quantity and content in patients receiving chemotherapy. In this study, previously untreated patients receiving chemotherapy likely to result in oral mucositis are being studed prior to and then 7, 14 and 28 days following treatment. Saliva and crevicular contents are sampled, and measurements of flow rate, protein content, electrolytes, mucins, anionic proline-rich proteins, lactoferrin, lysozyme and total immunoglobulins are made. The cellular content in the crevicular fluid is also being measured. In 11 patients who have been serially studied, no significant effect on salivary flow has been observed. However, each had a significant reduction in the salivary protein and sodium content within a week after chemotherapy. Since certain salivary glycoproteins are important in blocking the attachment of bacteria, the nature of this reduced protein content warrants further investigation. We are correlating these findings with the degree of mucositis which develops and, in parallel studies, we are determining the oral granulocyte pools.

3. Evaluation of a New Blood Culture Methodology to Detect Isolates in Potentially Infected Patients

We have collaborated with the Diagnostic Microbiology Laboratory (Drs. Gill and Maclowry) to evaluate the ability of a lysis-centrifugation blood culture system or a lysis-filtration systems to increase the recovery of organisms over that of a conventional blood culture system. To date, 3111 blood cultures have shown that both lysis-centrifugation and lysis-filtration increases the recovery (73% and 68% respectively) of significant isolates when compared to an unvented conventional system for aerobic and faculatively anaerobic microorganisms. Although the time to detect a positive culture was not significantly different amongst the three methods for most bacteria, the lysis-centrifugation system provided positive cultures more rapidly. The lysis centrifugation and

filtration methods had comparable recovery for significant isolates while the lysis centrifugation was more sensitive for staphylococci and yeast than the conventional bottles. We are presently comparing the quantitative yield from cultures to the patient's clinical outcome.

4. Nonbacterial Pathogens in the Initial Evaluation of Febrile Granulocytopenic Patients

Whether viruses may be co-factors or antecedent agents causing fever and/or secondary bacterial infections in immunocompromised cancer patients is being prospectively studied in collaboration with Dr. R. Yolken of the John Hopkins Hospital. ELISA assays are being performed on throat washings and stool samples from patients who become febrile. During an 11 month period, throat washings obtained from 60 adult and pediatric cancer patients with fever and granulocytopenia were assayed for common respiratory viruses. Positive viral washings were found in 25% of patients (15% influenza, 8% adenovirus, 2% parainfluenza). Patients with positive washings could not be distinguished from patients with negative viral washings on the basis of presenting symptoms, intial chest x-ray findings, height of initial fever, duration of fever, intial neutrophil count, or duration of granulocytopenia. A bacterial infection was diagnosed in 15 of 44 patients with negative viral washings and 1 of 15 with positive viral washings. Although the relatively small number of episodes studied precludes a definitive conclusion, respiratory virus infection does not appear to be implicated as an antecedent event for bacterial infection in febrile-neutropenic episodes but may be a primary cause of fever in this patient population. This study is continuing.

B. The Management of Fever and Infection in Cancer Patients

1. What Constitutes Appropriate Empiric Antibiotic Therapy for Granulocytopenic Cancer Patients?

a. 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, we randomized patients to either our conventional combination of cephalothin, gentamicin, carbenicillin (KGC) versus a new third generation cephalosporin, ceftazidime (CTZ). To date, 608 granulocytopenic episodes have been randomized to either antibiotic regimen when they became febrile. This represents the largest clinical trial done in this area. The initial response during the first 72 hours was evaluated according to whether the patient had an unexplained fever (FUO) or a documented infection: For patients with FUO, 98% for CTZ patients. The overall response, at the resolution of the granulocytopenia was 98% for the FUOs randomized to KGC and CTZ and 91% for the patients with documented infections randomized to KGC vs 89% for documented infectious patients randomized to CTZ. Thus, monotherapy may be as successful as combined therapy, particularly for initial period of empiric management. Overall, our results are superior to any other reported to date.

b. Are the Pharmacokinetics of Ceftazidime Reliably Predictable in Febrile-Granulocytopenic Patients?

Ceftazidime pharmocokinetics have been studied in normal volunteers and patients with serious infection but not in febrile-neutropenic patients. In order to determine the reliability of ceftazidime we obtained blood sample 2 and 7.75 hours following a zero order 20 minute infusion of ceftazidime (90 mg/kg/day in three divided doses). Ceftazidime levels were measured by Dr. P.K. Narang (Pharmaceutical Development Service) using an HPLC assay with cephalothin as an internal standard.

Eighty patients have been studied so far. The mean half-life was longer in these patients (t 1/2 β = 3.70 hr) compared to that reported in a normal volunteer population (t 1/2 β = 1.66 hr), with a range in half-life of t 1/2 β = 1.24 to 6.27 hr. Importantly there was nearly an 80% variation in peak and trough levels of the patients (i.e., peaks from 2.2 to 57.2 µg/ml and troughs from 0.8 to 13 µg/ml). This degree of variation in many patients is surprising and suggests that pharmacokinetic monitoring and dose adjustments may be necessary. We are presently comparing the pharmacokinetic results to the microbiological and clinical outcome of these patients to further define this problem.

2. Management of Patients with Unexplained Fevers (FUO).

The necessary duration or modifications of therapy are especially perplexing when the initial evaluation of the febrile-granulocytopenic patient has failed to reveal a defined infectious cause for the fever. This is not a trivial problem, since 40 to 60 percent of patients who become febrile while granulocytopenic have such unexplained fevers.

We have tried to define rational guidelines for management by comparing the commonly (but frequently arbitrarily applied) treatment options utilized in these patients. The major question is how long to continue therapy and whether continuing therapy increases the risk of secondary infections or superinfections as well as antibiotic-related organ toxicity.

a. Overview of FUOs and their Therapeutic Modifications

Between January, 1979 through July, 1984 we have evaluated 590 consecutive episodes of unexplained fever (FUO) in patients ranging in age from 1-74 years (median age 19 years). Of these, 331 (57%) are low risk (i.e., duration of granulocytopenia was less than 7 days) and the remainder were high risk. It is notable that only 8 deaths occurred among these 590 episodes (1.4%), although modifications of the initial antimicrobial regimen were frequently required, particularly in patients with prolonged granulocytopenia. These data can be summarized as follows:

Outcome	Episodes of Unexplained Fever (FUO)		
	Low Risk (<7 days G+)	High Risk (7-14 days G+)	High Risk (>14 days G+)
Number of Episodes	331	166	93
Median time to defervescence (in days)	2	4	5
Range	(1-7)	(1-26)	(1-30)
Recurrent Fever	2 (0.6%)	7 (4%)	35 (38%)
Overall Success	329 (99%)	163 (98%)	90 (97%)
o Without Modification	315 (95%)	131 (79%)	30 (32%)
o With Modification	14 (4%)	32 (19%)	60 (65%)
Death	2 (1%)	3 (2%)	3 (3%)

Thus, while the FUO patient can be successfully treated, modifications of therapy are necessary, particularly for patients with protracted periods of granulocytopenia. Some of these modifications are made empirically (e.g., because of persistent fever or clinical deterioration without a microbiological focus) while others are required when a clinical or microbiological infection becomes defined. We have evaluated the value of some of these modifications in randomized clinical trials. An overview of the types of therapeutic modification made in FUO patients according to the duration of granulocytopenia follows:

Modifications of Initial Antibiotic Therapy [Number]	Episodes of Unexplained Fever Low Risk (< 7 days) [331]	High Risk (7-14 days) [166]	High Risk (> 14 days) [94]
Empiric Modifications	7 (2%)	19 (11.5%)	19 (20.2%)
o Add antibiotic	4	6	3
o Add antifungal	0	13	16
o Add antiviral	0	0	0
o Switch regimen	3	0	1
o Resume antibiotic	0	0	3

RANDOMIZATION ON DAY 14

Outcome	Continue Antibiotics	Discontinue Antibiotics
Evaluable Episodes	11	14
New Fever Following Randomization	4 (36%)	4 (29%)
Overall Success:	11 (100%)	14 (100%)
1. Without Modification	7 (64%)	10 (7%)
2. With Modification	4 (36%)	4 (29%)
o Addition of antibiotic	1	0
o Addition of antifungal	4	0
o Change to alternate regimen	1	0
o Resume antibiotics	0	4

Thus, although the numbers of patients are small, the outcome of these patients was excellent regardless of whether antibiotics were continued or discontinued after two weeks of treatment. Patients stopping antibiotics may become febrile again and require re-institution of antibiotics whereas new fevers in patients already receiving antibiotics result in empiric antifungal therapy.

c. The Patient with Persistent Fever and Granulocytopenia - The Role of Empiric Antifungal Therapy

To further evaluate empiric antifungal therapy in febrile-granulocytopenic patients and to assess ketoconzole in particular, we initiated a randomized clinical trial comparing ketoconazole to amphotericin B. Between June, 1981 through April, 1985, we have utilized empiric antifungal therapy in three settings: 1) Patients who were persistently febrile and neutropenic following a week of antimicrobial therapy for either unexplained fever or for the treatment of a documented infection, have been randomly assigned to empiric antifungal therapy with either amphotericin B or ketoconazole; 2) Patients who fulfilled the criteria for persistent fever and granulocytopenia but who are unable to swallow oral ketoconozale have been nonrandomly assigned to receive parenteral amphotericin B; 3) Patients who are afebrile before day 7 and who remain granulocytopenic but then become febrile again, while still on antibiotics, non-randomly start on amphotericin B.

To date 72 febrile patients have been randomized on day 7 to either empiric amphotericin B (32 patients) or to empiric oral ketoconazole (32 patients). The median duration of granulocytopenia was 11 days (range 1-64) for patients randomized to amphotericin B and 14 days (range 2-52) for patients randomized to oral ketoconazole. Results are as follows:

Outcome	Randomization: Amphotericin B (32)	Randomization: Ketoconazole (32)
Days to Defervescene (median/range)	1 (1-36)	5 (0-8)
Documented Fungal Infections	4	6
Cross-over of Therapy		
o Intolerance	3	3
o Progressive Disease	0	6
Outcome		
o Survived	25	27
o Fungal Death	3	2

These data suggest that ketoconazole is as effective as amphotericin for empiric therapy but that when fungal infections occurs, progression is likely unless the patient is switched to amphotericin B.

3. Management of Patients with Proven Infections

Within 48 to 72 hours after beginning an empiric antibiotic regimen, the plan of managment can be reassessed in light of the results of the patient's initial evaluation, pre-antibiotic culture results, and response to therapy. We have conducted several investigations to help define management problems:

a. Pathogen-Specific vs Broad-Spectrum Antibiotics for Microbiologically Defined Infections

Should the pre-antibiotic blood cultures yield positive results, the antibiotic regimen can be adjusted to maximize its efficacy and specificity.

To date, 46 episodes of microbiologically documented infection in granulocytopenic patients have been evaluable and randomized to either continue on broad-spectrum antibiotics (BRD) or to be switched to a pathogen specific (PS) antibiotic. Patients are being assessed for new fevers or second infections according to the duration of granulocytopenia following randomization:

DURATION OF GRANULOCYTOPENIA FOLLOWING RANDOMIZATION

	Less than 10 days		More than 10 days	
Outcome	BRD	PS	BRD	PS
Number of evaluable episodes	19	12	7	8
Occurrence of a new fever	1	1	4	4
Occurrance of a second infection	3	3	4	3
o Bacteremia	1	0	1	2
o Fungal Infections	1	0	1	0
o Other Infections	1	3	2	1
Change and/or addition of antibiotics	2	3	5	6
Deaths	1	0	1	0

Overall, 11/20 (55%) of the episodes randomized to pathogen-specific antibiotics vs 12/26 (40%) of those randomized to continue broad-spectrum antibiotics had a second fever or infection. Antibiotics were eventually altered in 9/20 (45%) of those randomized to pathogen-specific antibiotics vs 7/26 (27%) of those randomized to broad-spectrum antibiotics. These data do not presently support either an increased incidence of superinfection with broad spectrum therapy or an advantage of broad-spectrum over pathogen specific therapy for patients with microbiologically-defined infection, even in patients with persistent granulocytopenia. This study is continuing.

b. Approach to the Patient with a Pulmonary Infiltrate

1) Empiric Therapy vs Specific Diagnosis in the Patient With a Diffuse Pulmonary Infiltrate

A major question in the patient who presents with a diffuse pulmonary infiltrate is whether and how to establish the diagnosis. Two issues are pertinent: First, is it better to treat patients empirically, according to the most probable causes of the infiltrate or should the diagnosis be established by whatever means, so that specific therapy can be instituted at the earliest possible time? Second, if a diagnostic procedure is

to be done, which one provides the highest likelihood of establishing the diagnosis? Ancillary but important issues relate to the hazards of the diagnostic procedures versus the side effects of empirically chosen therapies. In order to assess the risks and benefits more carefully we are currently conducting a clinical trial that directly addresses this problem. Patients in whom a diffuse pulmonary infiltrate develops have been randomly assigned either to undergo immediate open lung biopsy (OLB) with therapy then dictated according to the results of the procedure, or to receive an initial trial of empiric antibiotics. The empiric antibiotics depend upon whether or not the patient is granulocytopenic or receiving antibiotics when the infiltrate appeared. Patients randomly assigned to receive empiric antibiotics who are either stable or improved after a four-day trial of therapy then receive a standard course of therapy. However, patients who have failed to improve after a four day trial of empiric therapy, then undergo an open lung biopsy. Twenty-two non-neutropenic patients have been evaluated to date. The patients' underlying malignancy includes non-Hodgkins Lymphoma (15), T-cell (HTVL) lymphoma (2), acute lymphocytic leukemia (2), Hodgkin's disease (2) and breast cancer (1). The median age was 40 years with fever (18/22) and tachypnea (13/22) the most frequent presenting signs. The median room air PO_2 in 21 hypoxic patients was 55 torr. Eight of 10 patients randomized to empiric therapy improved after 4 days. Both patients undergoing delayed OLB had P. carinii Pneumonia (PCP) with improvement in one and death from hemorrhage in the other. Time to clinical resolution in the 9 surviving patients was 14 days; 4 required prolonged ventilation (>24 hours). The findings in the 12 patients randomized to immediate OLB were PCP in 5, and non-specific pneumonitis in 7; there were 3 OLB related deaths. The time to resolution in the 8 surviving patients was 13 days for PCP, and 5 days for non-specific pneumonitis; 7 required prolonged ventilation. There was no significant differences in mortality between the two groups (p=.54), and there was substantially greater morbidity among patients randomized to immediate OLB. These data suggest that initial empiric therapy for acute respiratory compromise in this population of cancer patients may be as efficacious as immediate OLB and is associated with decreased morbidity.

2) Excess Incidence of Pneumocystis Pneumonia in Lymphoma Patients Receiving Combination Chemotherapy

Between October 1981 and December, 1983, 70 consecutive adult patients with previously untreated advanced stage diffuse or aggressive non-Hodgkin's lymphoma in the NCI Medicine and Navy Branches were randomized to receive treatment with either ProMaceMOPP (P.M.) or Promace-CYTABOM (P.C.). Both regimens included cytoxan, VP-16, adriamycin, methotrexate with leukovorin rescue, and a 14 day course of prednisone. The main differences

in the regimens were the lengths of the cycles (21 for P.C. vs 28 day cycles for P.M.) and the inclusion of bleomycin and cytosine arabinoside in the P.C. regimen (in place of nitrogen mustard and procarbazine in the P.M. regimen).

Of note, 13/37 or 35.1% of patients randomized to receive P.C. chemotherapy developed diffuse interstitial pulmonary infiltrates compared to 3/32 (9.4%) of patients receiving P.M. (p_2 =.022). The etiology of the interstitial pulmonary infiltrate was putatively Pneumocystis carinii in 12/13 of the patients receiving P.C. and 1/3 of patients receiving P.M. (p_2 = .0027). Of the 13 patients on P.C. with interstitial infiltrates, 7 had the diagnosis of P. carinii pneumonia (PCP) confirmed by open lung bx, 1 had blastomycoses and 5 were strongly suspected clinically of having PCP and were treated empirically with trimethoprim-sulfamethoxazole (T-S). One of the 3 patients with interstitial infiltrates receiving P.M. had clinically suspected PCP and was treated with 14 days of T-S; however, an open lung biopsy performed 12 hrs. after the initiation of T-S showed necrotizing granulomatous inflammation without any evidence of PCP. Neither of the other two P-M treated patients had P. carinii.

4/37 (10.5%) P.C. treated patients with biopsy proven PCP died of pneumonitis. There were no deaths from pneumonitis in P.M. treated patients.

When comparing the patients who received P.C. to those who received P.M. chemotherapy, there were no signifcant differences between the 2 groups in age, sex, stage, response to treatment, the presence of 'B' symptoms; WBC nadir, febrile-granulocytopenic episodes, concurrent or antecedent infections; history of pre-existing lung disease, history of smoking; pulmonary function test results (including the DLCO at staging); treatment with radiation therapy, or exposure to patients with the AIDS.

Of note, patients receiving P.M. had more modifications of drug dosage (66% vs 49% of patients on P.C. p_2 = 0.008) and received less of the total dosage of drugs during the first three cycles.

Within the group of patients who received P.C., there were no differences in any of the above demographic factors or in the doses of chemotherapy received in those who developed infiltrates to those who did not develop interstitial infiltrates. No patients developed an infiltrate while neutropenic. None of the infiltrates appeared before cycle 3. The reason for this significantly greater occurrence of interstitial pneumonia and PCP in patients receiving P-C is not clear but may be related to the addition of two pulmonary toxic drugs (bleomycin and cytarabine) or to alteration of local or systemic immunity consequent to the P.C. regimen. We plan to explore these possibilities in an experimental model.

c. Management of Cancer Patients with Foreign Bodies

Another tenet of traditional infectious disease teaching is that foreign bodies should be removed when the patient becomes febrile or infected. This is of particular relevance to cancer patients in view of the marked increase in the use of indwelling intravenous catheters of the Hickman-Broviac type during the past several years. Although the benefit of these cathethers in providing ready venous access (especially in children) is enormous, the frequency of catheter-associated bacteremia and other problems has become appreciable.

1) Infections Associated with Hickman/Broviac Cathethers

We are reviewing the bacteremias associated with over 150 catheters in children and adults with cancer. The incidence of bacteremia in nongranulocytopenic patients was increased nearly 40-fold in patients who had indwelling catheters compared with the incidence in patients without catheters. The incidence of bacteremia was increased fourfold in granulocytopenic patients with indwelling catheters compared with the incidence in comparably granulocytopenic patients without indwelling catheters. Gram-positive bacteria (particularly S. epidermidis) were the most frequent isolates. The question is whether the catheter should be removed when a patient becomes a bacteremic. This is not a trivial matter in a patient whose venous access is compromised. Consequently, we have attempted to treat patients who had catheter-associated bacteremia with a trial of antibiotics (generally including vancomycin) and found that the bacteremia cleared in nearly 90 percent of patients even when the catheter was left in place. An exception to this occurred in patients who had local cellulitis, a tunnel infection, or a Bacillus infection (see below).

2) Evaluation and Management of Intraventricular Reservoir Infections

To characterize the infectious complications associated with such reservoirs, we reviewed the 10 year experience of the Pediatric Branch and Children's Orthopedic Hospital with 61 patients (49 with leukemia, 8 with lymphoma, 4 with sold tumors) who had IRs placed for intraventricular chemotherapy. The criteria for insertion and the patient profiles at these two centers were exactly comparable. IRs were in place for a median of 36 weeks and were punctured a median of 29.5 times. Infectious complications occurred in 14 patients (19.7%) had 19 episodes of clinically suspected and microbiologically documented meningitis or positive IR CSF cultures without symptoms. Of the 13 episodes of proven or suspected meningitis, all were treated successfully with parenteral antibiotics, although five had their IRs removed (three because of associated infection and two because of malfunction unassociated with infection). Patients with positive

CSF cultures in the absence of symptoms or pleocytosis pose a therapeutic dilemma. Of four such patients, two received no therapy while one achieved sterilization with parenteral antibiotics and another received parenteral and intra-IR antibiotics with only temporary CSF sterilization. Importantly, two of these patients subsequently developed a clinical meningitis with the same organism found at the time of the initial asymptomatic episode (P. acnes). Thus, while asymptomatic patients with positive CSF cultures may not require immediate therapy, they should be followed carefully for the development of clinical meningitis. While infections complications occur in cancer patients who have IR's placed, the incidence of serious morbidity and mortality is surprisingly low.

d. Approach to the Patient with Perirectal Cellulitis.

We reviewed retrospectively 44 patients with 57 episodes of anorectal infection. The majority of patients had leukemia or lymphoma. Cultures obtained during surgical drainage (22 cases) or by needle aspiration (7 cases) revealed multiple organisms in 26 of the 29 samples. Anaerobic organisms were the most common isolates. Of the 22 patients treated with surgery and antibiotics, the infection resolved in 17 cases (77%). Overall, resolution of infection occurred in 54% of the episodes in which antibiotics were the sole mode of therapy. However, when the antibiotic regimen included a specific anti-anaerobic agent in addition to an aminoglycoside, resolution of the infection was observed in 14 of 16 (88%) cases. The most important prognostic indicator of outcome was the number of days of neutropenia. These results suggest that perirectal cellulitis will respond to medical therapy, particularly if an anti-anaerobic drug is included in the regimen.

e. New or Emerging Pathogens

1) Bacillus Infections

We have reviewed 16 episodes of bacillus bacteremia in cancer patients at the NCI which occurred since 1976. This review was prompted by our recent experience in being unable to treat patients successfully for a bacillus bacteremia if they had a catheter in place and it was not removed. We have observed this phenonmena in three recent patients, particularly with Bacillus pumulis. This is quite contrary to catheter associated bacteremias with S. aureus or S. epidermidis, where successful therapy is accomplished without the need for catheter removal.

2) Clostridium Tertium in Cancer Patients

The isolation of C. tertium is usually considered a contaminant. However, in the neutropenic patient it may be a true-pathogen, perhaps selected by treatment with broad spectrum antibiotics.

We identified ten cases of C. tertium bacteremia in patients with leukemia or aplastic anemia who were receiving broad spectrum antibiotics. A GI focus was associated in virtually every patient. This organism has variable resistance to common anti-anaerobic agents (e.g., clindamycin). Awareness of its possible pathogenicity and therapeutic requirements (vancomycin and clindamycin together will cover virtually all isolates) is important.

2) Clostridium difficile - Surveillance and Disease

Since March 1982, we have surveyed Pediatric Branch patients who developed diarrhea for the presence of C. difficile, with or without toxin. In addition, we have begun routine surveillance of pediatric patients being admitted to the hospital, whether they have diarrhea or not, for the presence of C. difficile or toxin. We are seeking to correlate the association of C. difficile with antibiotic or chemotherapy induced diarrhea, both with or without the presence of toxin. To date, 328 stool samples have been cultured for C. difficile and assayed for toxin. Of the 128 patients who presented with diarrhea, 30 samples have been positive for C. difficile, of which 20 also had evidence of toxin. Of the 19 patients who were cultured while asymmptomatic, 4 had C. difficile isolated from their stool culture. In one of these, toxin was also found while in a second the organism was shown to produce toxin in vitro. The remaining two isolates were non-toxigenic. We are presently correlating our findings to date with the patients clinical course, antibiotics and chemotherapy experience, in order to ascertain the significance of C. difficile in symptomatic and asymptomatic patients. We plan to continue our surveillance study and to begin biotyping positive isolates.

C. Studies to Prevent Infectious Complications in Cancer Patients

1. Intensive Therapy In or Out of Protected Isolation

Of 43 patients randomized to receive intensive therapy either in or out of protected isolation, 40 are evaluable with the following observations:

CLINICAL DATA AND OUTCOME	RANDOMIZATION: PROTECTED ENVIRONMENT	RANDOMIZATION: STANDARD HOSPITAL ROOM
Number of Evaluable Patients	19	21
Median Age (in years)	20 (0-35)	17 (9-25)

Underlying Cancer		
• Lymphoma	0	2
• Solid Tumor	19	19
WBC nadir/mm^3 following treatment	<50	<50
Duration of Granulocytopenia (days)	25 (10-36)	22 (10-46)
Diagnosis when Febrile		
• Never became Febrile	1 (6%)	0
• Unexplained Fever	15 (79%)	14 (67%)
• Documented Infection	3 (10%)	7 (33%)
o Bacteremia	1	4
o Pulmonary	1	1
o Soft tissue	0	1
o Perianal Cellulitis	0	2
o Necrotizing Gingivitis	1	1
o Other	0	1

OVERALL OUTCOME

Success without Modification	8 (42%)	7 (33%)
Success with Modification	11	13
o Addition of Antibiotic	6	9
o Addition of Antifungal	7	8
o Addition of Antiviral	0	1
o Change in Antibiotic Regimen	0	2
o Resume Antibiotic Therapy after Stopping	1	1
Death on Study	0	1*

*Due to Aspergillus Pneumonia.

Thus, although the incidence of primary and secondary infections in patients treated in a Protected Environment is less than when patients are treated in a standard hospital room, the overall success of therapy

is comparable. However, the sole mortality which occurred did so in a patient in a standard hospital room.

2. Studies to Improve Altered Host Defenses

Augmentation of host defenses has potential application for infection prevention. Areas of current interest and investigation include passive immunization, cell component therapy, stimulation of granulopoiesis, and enhancement of endogenous neutrophil function.

a. Passive Immunization Studies

1) Protocol Assessing Passive Immunization With J5 Antisera to Prevent Fever or Infection in Pediatric Cancer Patients

In this trial, pre- or post-immunization J5 antisera or albumen (as an additional control) is given to patients receiving chemotherapy expected to result in six or more days of neutropenia. The study design is double-blind with a three arm randomization. Patients continue receiving the study preparation every 7 days for as long as neutropenia persists. In addition to evaluating whether J5 antisera will reduce the incidence of fever, gram-negative bacteremia or other infectious complications, we are assessing post immunization levels of circulating antibodies (J5, P. aeruginosa, Staph A, H. Flu).

To date, 36 patients have been randomized. No undue toxicity has been observed. Because of the double-blind study design, no analysis has yet been performed.

2) Prophylaxtic Passive Immunization Utilizing Intravenous Immunoglobulin

Purified immunoglobulin for intravenous administration is now available for clinical use. Such immunoglobulin, pooled from up to 1000 donors, may contain significant titers of antibody against a variety of pathogens. It is probable that within the next few years, preparations may be tailored to contain high titers of antibody against certain desired pathogens. Thus, it may soon be possible to ensure optimal antibody levels against a variety of major pathogens throughout the period of neutropenia. As a preliminary investigation, we have initiated a trial utilizing a pooled immunoglobulin preparation manufactured by Cutter Laboratories and commercially available as IGIV. This gamma globulin preparation has measurable titers against a number of bacteria (Pseudomonas, Group B Strep, E. coli, S. pneumoniae, H. Flu, Staph enterotoxin F., Salmonella) and viruses (VZV, Hepatitis B, Coxsackie, H. Simplex). This study is being conducted in adult patients in the NCI-Navy and Medicine Branches who are receiving chemotherapy regimens rendering them neutropenic for more than six days. This protocol serves as a prelude

to future trials which might utilize selected hyperimmune antisera or monoclonal antibodies.

The objectives and study design of this protocol are similar to those for J5 antisera (see above). A double-blind randomized design is being utilized. As with the J5 study, a comprehensive analysis of preand post transfusion antibody levels will be measured (including E. coli, J5 S. minnesota, RE 595 lipopolysaccharides and lipid A) and will be correlated with clinical outcome.

This trial began in the Clinical Center on June 15, 1984.

D. Preclinical Studies

1. Antibody - White Blood Cell Interactions

We examined whether attachment of antibody to human PMNs enhanced bactericidal activity against commonly encountered bacterial pathogens (particularly Pseudomonas aeruginosa, Staph aureus, and Group B streptococcus). The "arming" procedure consisted of harvesting and purifying human neutrophils by dextram sedimentation, Ficoll-Hypaque centrifugation and hypoteonic lysis of contaminating red blood cells. The purified PMNs were incubated with either mono- or polyclonal antibody in 12% polyethylene glycol, and centrifuged through a mixture of phthalate oils (1.5:1 v/v dibutylphthalate. bis (2-ethylhexyl) phthalate. The viability of these "armed" neutrophils was >95% by Trypan blue exclusion.

a. Results with Standard Bactericidal Assay. Apparent Enhanced Bactericidal Activity

1) Armed PMNs: The Standard Assay of Bactericidal Activity

We initially utilized the standard tube method to measure bactericidal activity. This assay permits the determination of 12-15 variables at a time. As shown below, three antibody sources were used: First, murine anti-Pseudomonas monoclonal antibodies, both IgG and IgM, were supplied by Dr. J. Sadoff of Walter Reed, Second, a polyclonal pooled immunoglobulin and a "Hyperimmune" anti-Pseudomonal antibody containing antibody titres to the seven immunotypes of P. aeruginosa was used (supplied by Cutter Laboratories). Third, an IgM anti-Group B streptococcal monoclonal antibody supplied by Dr. Harry Hill (University of Utah).

Monoclonal	Polyclonal	Antibody Class	Antigenic Specificity
24H-9	-	IgM	Lipopolysaccharide
73-11-5	-	IgM	Lipopolysaccharide

11-14-1	-	IgG	LPS, Native Complex
59-11	-	IgG	Anti-pili
	Cutter pH 5.5	Reflects human serum immunoglobulins	
	Cutter pH 6.5		
	Cutter Hyperimmune	IgG	Pseudomonas aeruginosa - All seven Fisher-Devlin immunotypes
α-GBS	-	IgM	Group B streptococcus (capsular antigen)

Target bacteria included either P. aeruginosa (seven types), S. aureus, Bacillus aureus, and Group B streptococcus.

Attachment of antibody (Ab) was confirmed by indirect fluorescence. Bactericidal assays consisted of Ab, complement (C′), P. aeruginosa (Ps) and PMNs. Timed samples were collected in triplicate and PMNs lysed with d H_2O. 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, Ps	PMN,ΔC′,Ab, Ps	PMN,Ab,C′ Ps	A-PMN,C′,Ps Monoclonal	A-PMN,C′,Ps 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)

"Armed" PMNs with mono or polyclonal Ab also enhanced bactericidal ability at various bacteria: PMN ratios (1:1 to 200.1).

Initial studies also suggested that the degree of enhanced bacterial killing correlated with the specificity of the antibody used to "arm" the PMNs. We also measured the functional activity of "armed" neutrophils to determine whether they were being non-specifically activated. In these studies, "armed" neutrophils were found to be equivalent to unarmed PMNs in their chemotactic responsiveness to formyl-methionyl-leucyl-phenylalanine superoxide generation and in several quantitative or semiquantitative enzymatic assays (i.e., myeloperoxidase, alkaline phosphatase, lysozyme, β-glucuronidase).

b) The Microtiter Assay. Antibody Inhibition of Bactericidal Activity

Because the standard tube method bactericidal assay was limited by the number of controls which could be run concurrently and because we wished to further test the specificity of the "armed" neutrophils, a new microtiter phagocytosis/bactericidal assay was developed in our laboratory. This assay permits the concurrent assessment of multiple variables, reaction mixtures (i.e., PMNs, serum, antibody bacteria), and target-effector ratios in triplicate.

Application of this assay yielded an unanticipated observation: Dilutions of antibody up to 10^{-50} still showed enhanced bactericidal activity. In addition, the removal of free antibody from the reaction mixtures appeared to improve the degree of bactericidal activity by PMNs. In fact, the apparent enhanced bactericidal activity by "armed" PMNs relative to unarmed PMNs appeared to be due to an inhibition of bactericidal activity by free antibody. This inhibition could be mediated by intact IgG molecules or Fc fragments, but not by F $(ab)_2$ fragments.

The specific basis for this antibody inhibition of bactericidal activity is currently being investigated. In addition, the possibility that the specificity of arming is related to a particular antibody subclass (as has been observed with mononuclear cells in which cytotoxic function is mediated by IgG_{2a} but not IgG_{2b}) is also being explored. Pending these results, we will investigate antibody arming of neutrophils and monocytes to treat infections in neutropenic rodents.

2. Opiate - WBC Interactions

Recent studies have suggested that morphine sulfate has a suppressive effect on the immune response, including PMN fuction (Tubaro, E., et al., J Infect. Dis. 148: 656, 1983). The Pediatric Branch has initiated a number of studies evaluating pain control with various opiate compounds, including morphine. Preliminary clinical studies of PMN activity from patients receiving continuous infusion morphine showed decreased bactericidal activity and decreased ability to generate superoxide anion. We have therefore initiated a series of studies to evaluate the potential modulatory role of endogenous opiates (β-endorphin, met-enkephalins) on neutrophil function. Structurally-related endogenously occurring peptides (adrenocorticotropin, alpha interferon) are also being investigated for their potential modulatory role of human PMNs activity. These studies will utilize extensive in vitro analyses of human PMN activity -- including determinations of bactericidal, chemotactic, superoxide generation and various enzymatic capabilities (β-gluccuonidase, lysozyme). Additionally, a rabbit model will be selectively utilized to assess the effect of the infusion of various opiates on PMN function in vivo.

3. Endotoxin - White Blood Cells Interactions

We have undertaken a number of studies to more carefully characterize the interaction of endotoxin with human PMNs, in order to develop pharmacologic or therapeutic manuvers to ameliorate the devastating pathophysiology of septic shock.

Initial investigations have documented the ability of endotoxin to "prime" human PMNs to release a greater amount of superoxide anion then control PMNs. The "primary" effect of endotoxin can be prevented by the presence of polymixin B (or a nine amino acid terminus of PMB). Thus, PMNs isolated by dextran sedimentation and Ficoll-Hypaque centrifugation were incubated with or without endotoxin (Salmonella minnesota Re 595 type) at a concentration of 10 ng/cc (equivalent to that level demonstrated in endotoxemic humans) stimulated (with either phorbol myristate acetate or FMLP), and superoxide anion production was then measured by a cytochrome reduction assay. PMNs incubated with endotoxin produced significantly more superoxide (18.16 ± 2.57 nmoles $O_2/10^6$ PMNs) then control PMNs (3.20 ± 0.63, $p<0.0005$, $n=10$). The presence of polymixin B (2 µg/cc) or polymixin B nanapeptide (10 µg/cc) during the incubation with endotoxin prevented the "primary" effect, and reduced superoxide production to control levels.

These studies are currently being expanded to include further in vitro characterizations of the interaction between endotoxin and human polymorphonuclear leukocytes -- particularly with regard to bactericidal and chemotactic activity. It is the ultimate aim to apply the knowledge gained from these in vitro characterizations (and inhibition of the effects of endotoxin) to an animal model of bacteremic shock.

4. Rabbit Model for Invasive Candidiasis

One of the most significant problems in investigating new methods of diagnosing and/or treating invasive fungal diseases has been the lack of an appropriate animal model system. We have been establishing a model of systemic candidiasis in an immunosuppressed population of rabbits. Venous access is created in healthy female rabbits by means of an indwelling sialastic catheter (via the internal jugular venous system to right heart) through which rabbits can reserve medication and have blood withdrawn (for counts and culture). Rabbits are rendered neutropenic by means of cytarabine administration (15-30 mg/kg; 2-4 times per week), and are initially exposed to the Candida by means of intravenous inoculation (performed on day 5 post chemotherapy). Antibiotics (ceftazidime and ampicillin) are administered to prevent death due to supervening bacterial infection. We have been successful in establishing histopathologically documented Candida infections (lung, liver, kidney, spleen) by using this methodology. This model of systemic candidiasis will be used initially to investigate various therapeutic questions relevant to antifungal therapy (e.g., combination therapy (either amphotericin B and 5-FC or amphotericin with ketoconazole; or liposome encapsulated amphotericin B).

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 unknown 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 studies 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. Our major research target will be to develop methods for improving host defenses 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. While combining these host bolstering defenses with prophylactic antibiotics, we will also explore chemotherapeutic schedules which may have a more selective effect on tumor cells.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06840-10 PB

PERIOD COVERED

October 1, 1984, to September 30, 1985

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

Treatment of Acute Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G. Poplack	Head, Leukemia Biology Section	PB, NCI
Others:	S. Zimm	Investigator	PB, NCI
	F. Balis	Investigator	PB, NCI
	R. Wesley	Senior Staff	BR, NCI

COOPERATING UNITS *(if any)*

Laboratory of Neuropsychology, NIMH, NIH (P. Brouwers); Metabolism Branch, NCI (S. Korsmeyer); Div. of Hem/Onc, Los Angeles Children's Hospital (J. Holcenberg); Dept. of Hem/Onc, Children's Hospital National Medical Center (G. Reaman).

LAB/BRANCH

Pediatric Branch

SECTION

Leukemia Biology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	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.)*

Clinical research into the biology and treatment of acute leukemia is pursued with particular emphasis on acute lymphoblastic leukemia (ALL) of childhood. Major issues being addressed include: 1) development of therapeutic strategies aimed at improving overall prognosis of children with ALL, 2) investigation into the mechanisms of treatment failure with particular emphasis on evaluation of pharmacologic approaches to leukemic therapy, 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. Moreover, analysis of data derived from this study has identified a patient group at particular risk for CNS relapse. A new, high risk protocol has been devised in an attempt to improve the prognosis for these and other poor risk patients. Studies on the bioavailability of orally administered maintenance chemotherapy have demonstrated that many patients do not achieve adequate drug levels in the blood, raising concern over this possible mechanism of treatment failure. A major, multi-institutional pharmacologic monitoring protocol has been instituted in an attempt to study the relationship between the bioavailability of orally administered maintenance chemotherapy and relapse in children with ALL. The role of diurnal variation, concomitant food intake and inter-patient variability in intracellular drug metabolism are being explored as possible factors in treatment failure. Studies on late effects have demonstrated CT brain scan, neuroendocrine, and psychometric test abnormalities in long-term survivors of childhood ALL. These observations have stimulated the search for alternative, equally effective but less toxic methods of CNS preventive therapy.

Objectives

1. To develop effective therapeutic regimens in acute lymphoblastic leukemia which provide maximum tumor cell kill and improve the prognosis of children with acute lymphoblastic leukemia.

2. To study the pharmacologic approaches to leukemia therapy in an attempt to probe the pharmacologic reasons for treatment failure in this disease.

3. To evaluate the short- and long-term effects of antileukemic therapy on growth, development and organ function, with particular reference to the central nervous system.

Methods and Major Findings:

A. Treatment Studies of Acute Lymphoblastic Leukemia

1. NCI 77-02/CCG 191 Treatment Protocol

This ALL treatment protocol has investigated the efficacy of high-dose, protracted intravenous methotrexate infusions as CNS preventive therapy. Patients were randomized to receive CNS prophylactic therapy with cranial radiation plus intrathecal methotrexate, or with high-dose, 24-hour systemic methotrexate infusions. The hypothesis which was 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 assessed the utility of an intensified systemic maintenance schedule which alternates periodic "induction-type" chemotherapy with standard maintenance treatment. The protocol was closed to further patient entry in early 1984. A total of 176 average and high-risk patients have been randomized on this study; 58 to cranial radiation plus intrathecal methotrexate; 118 patients to receive high-dose, protracted intravenous methotrexte infusions (randomizations are weighted on 2-1 basis). The overall remission induction rate is 98%. With a median follow-up of 43 months, the actuarial disease free survival at 2 years is 76% for the entire group. 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. For the 131 average risk patients, the actuarial disease-free survival is 82%; for the 45 high risk patients it is 60% (at 2 years). When the results are broken down in terms of patient risk groups, there is also no difference in the CNS relapse rate according to the two types of CNS preventive therapy. The results to date suggest that CNS preventive therapy with high-dose methotrexate is equally as effective as that provided by cranial radiation and intrathecal methotrexate. These data indicate that excellent treatment results may be obtained in the majority of children with ALL without using cranial radiation.

B. Initiation of New ALL Treatment Protocols

Based on the results of the NCI 77-02 protocol (see above), we have initiated two new ALL treatment protocols. NCI 83-P/CCG 134-P is a protocol designed to treat patients with ALL in the high risk category. Analysis of the results of NCI 77-02/CCG-191 revealed the existence of a group of patients at particularly high risk for developing CNS relapse. Cox univariate analysis revealed that those factors significantly predictive for CNS relapse were an initial white blood count greater than 50,000 per cubic millimeter; massive splenomegaly; hepatomegaly; hemoglobin > 10 grams per dl; and lymphadenopathy. However, further analysis revealed that patients with the "lymphoma syndrome", a composite of several of these predictive clinical and laboratory features, were at the greatest risk for CNS relapse (p_2 less than .0001). One-third of such patients experienced a CNS relapse. In an attempt to deal more effectively with these and other high risk patients, NCI 83-P/CCG-134P, was instituted. This protocol builds on the experience of our previous protocol and has intensified therapy in an attempt to improve the outlook for high risk patients. This single arm, pilot protocol avoids the use of cranial radiation, using high-dose methotrexate as CNS preventive therapy. However, in addition to the latter, intrathecal chemotherapy with both methotrexate and cytosine arabinoside and highdose systemic cytosine arabinoside are employed. This protocol also utilizes an aggressive intensification period administered between consolidation and the beginning of maintenance chemotherapy. Patient entry to this protocol began in the early spring of 1984. 35 patients have been entered on study. The median duration on study is slightly less than 8 months. To date there has been only 1 relapse (bone marrow). These results are promising but require continued follow-up.

NCI 84-A/CCG-144A is a new protocol designed to treat patients with average risk ALL. Redefinition of the average risk category, such that patients with certain high risk features (e.g., the lymphoma syndrome, greater than 10% FAB lymphoblasts, etc.) are now included in the high risk category, offers the opportunity to examine whether high dose methotrexate is necessary CNS preventive therapy for patients in the average risk group. This protocol will compare CNS preventive therapy with high dose intravenous methotrexate to that offered by periodic intrathecal methotrexate alone for patients in the average risk category. This protocol was also initiated in the spring of 1984. To date 34 patients have been entered on this study. Both of these protocols are being run on a collaborative basis with 4 participating Children's Cancer Study Group institutions.

C. Pharmacologic Approaches to Leukemic Therapy: Relationship to Treatment Failure

A detailed study of the pharmacology of the major antileukemic agents is being undertaken in an attempt to examine the reasons for treatment failure in children with ALL. Particular emphasis has focused on the study of the pharmacology of orally administered 6-MP, the backbone of maintenance therapy in ALL. Studies have 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. A 5-fold variation in the area under the plasma concentration-time curve (a measure of tissue exposure to drug) following oral 6-MP administration, a 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 were observed. The peak level of 6-MP achieved by most patients following oral 6-MP was approximately one log lower than the level shown to be optimal for cytotoxicity in in vitro systems. This study raised the question as to whether oral maintenance chemotherapy is being optimally delivered and has stimulated the generation of a new protocol evaluating in a prospective fashion the clinical pharmacology of p.o. methotrexate and 6-MP in patients with ALL undergoing maintenance chemotherapy. This multi-institutional collaborative study (being performed with the CCSG) will attempt to correlate the results of prospective periodic pharmacokinetic bioavailability studies with relapse rate and remission duration in children with ALL. In addition to this major study, other protocols have been launched to study the effect of diurnal variation and food intake on the bioavailability of orally administered maintenance chemotherapy agents. In addition to these clinical pharmacologic studies, the cellular pharmacokinetics of 6-MP is being studied in human leukemia cells (see Project #Z01 CM 06880-08 PB). Accumulation, metabolism and retention of 6-MP in human leukemia cells has been studied in an attempt to evaluate the role of inter-patient variability in intracellular nucleotide metabolism as a possible cause of treatment failure.

D. 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 had 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 one 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. In an attempt to coordinate the clinical research and to provide more comprehensive attention to the needs of patients with adverse sequelae, a Late Effects Team, made up of a pediatric oncologist, pediatric psychiatrist, pediatric neurologist, psychologist, neuropsychologist, vocational therapist, teacher and primary care nurse has been established. This team will aid in the coordination of care and the study of patients with late effects.

E. Immunoglobulin Gene Rearrangement in ALL

In collaboration with Dr. Stanley Korsmeyer, Metabolism Branch, NCI, we investigated the status of immunoglobulin gene rearrangement in acute leukemic lymphoblasts. Specifically, the blast cells from patients with previously denoted non-T, non-B cell ALL were examined. Lymphoblasts from those individuals were found to have patterns of immunoglobulin gene rearrangement similar to those seen in the earlier stages of B-cell maturation. This work has helped to define a pathway of genetic maturation and has documented for the first time that non-T, non-B cell ALL is a misnomer, and that the majority of cells within this category are actually human leukemic pre-B cells. This work has subsequently been expanded. We are currently investigating the status of immunoglobulin gene rearrangement in lymphoblasts from patients who have experienced bone marrow relapse in an attempt to determine the clonality of relapse in this disease. These studies are in progress.

Significance to Biomedical Research and the Program of the Institute:

The results of our ALL treatment protocol (NCI 77-02/CCG-191) appear to be of major importance. They indicate 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 protocol, 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 and intrathecal chemotherapy) appears to be the best available therapy for patients with average risk ALL. Moreover, these data indicate that excellent treatment results may be obtained in the majority of children with ALL without using cranial radiation. In addition, pursuit of the newly instituted ALL treatment protocols for high and average risk patients should help to more clearly define the optimal form of CNS preventive therapy for patients in these risk groups.

Studies on the pharmacology of antileukemic agents have revealed problems regarding the bioavailability of oral antileukemic maintenance agents such as 6-MP. Our results raise provocative questions regarding the pharmacologic basis for treatment failure in ALL. In addition, they have stimulated the development of a multi-institutional protocol in which prospective pharmacologic monitoring of patients with ALL undergoing maintenance chemotherapy will be pursued. Hopefully, this study will more clearly define the pharmacologic basis for relapse in children with leukemia.

The studies on the late effects of antileukemic therapy have focused attention on the adverse sequelae of CNS preventive therapy and on other long-term effects of antileukemic therapy on growth, development and organ function. These studies have served to heighten the awareness of investigators in the field and have led to refinements in patient treatment.

Proposed Course:

Entry of patients into the current clinical studies for newly diagnosed patients with ALL began within the past year. It is anticipated that both of these treatment protocols will be completed within an 18 month period. For average risk patients, the high-dose intravenous methotrexate approach utilized in our previous protocol will be compared to periodic intrathecal methotrexate alone as CNS preventive therapy. Poor prognosis patients will be treated with intravenous high-dose methotrexate, intravenous high-dose Ara-C and intrathecal methotrexate and Ara-C in an attempt to offer improved prevention of CNS relapse in these patients. In addition, a comprehensive, prospective study to evaluate and compare the CNS preventive therapy regimens utilized on protocol 77-02 for possible delayed neurotoxicity, is in progress. A similar study will be undertaken in the new average risk randomized treatment protocol. Expansion of our studies of the bioavailability of orally administered antileukemic therapy to include a prospective study in which patients receiving standard maintenance chemotherapy with oral 6-MP and methotrexate are periodically studied to determine the bioavailability of these two agents is beginning. 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.

Development of the Late Effects Team provides a unique setting in which to continue research into the adverse effects of antileukemic treatment. The recent availability of Nuclear Magnetic Resonance imaging provides a potentially more sensitive means of evaluating adverse CNS sequelae.

Preliminary data from our collaborative study evaluating the status of immunoglobulin gene rearrangement in acute leukemic lymphoblasts suggests that evaluation of immunoglobulin gene rearrangement may enable earlier detection of clonal bone marrow relapse than standard morphologic evaluation of a bone marrow aspirate. A prospective study is underway in an effort to confirm our initial findings. The data to date suggests that the ability to detect clonal relapse earlier by the evaluation of immunoglobulin rearrangement may permit investigators to alter therapy appropriately. This technique may also provide a more

sensitive method of detecting persistent disease following apparently adequate induction therapy, thus enabling identification of a subset of patients at substantial risk for relapse who might benefit from more intensive chemotherapy.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06880-08 PB

PERIOD COVERED

October 1, 1984, to September 30, 1985

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

Clinical Pharmacology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G. Poplack	Head, Leukemia Biology Section	PB, NCI
Others:	S. Zimm	Investigator	PB, NCI
	F. Balis	Investigator	PB, NCI
	J. Collins	Senior Investigator	CPB, NCI
	J. Grygiel	Investigator	CPB, NCI
	P. Gormley	Senior Investigator	LCHPH, NCI

COOPERATING UNITS *(if any)*

Dept. of Pediatrics, Catholic Univ. of Rome (R. Riccardi); Dept. of Pediatrics, Children's Hospital of Los Angeles (J. Holcenberg); Pharmacy Department, CC, NIH (P. Narang).

LAB/BRANCH

Pediatric Branch

SECTION

Leukemia Biology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	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 with emphasis on the role of 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. Additional efforts to optimize 6-MP administration have been based on in vitro studies which have demonstrated a need for prolonged exposure to cytocidal concentrations of drug to maximize leukemic cell kill. A clinical protocol evaluating prolonged intravenous 6-MP infusions in a Phase I setting has been completed and Phase II studies are underway. Preclinical and clinical pharmacokinetic studies of the new agent, Tiazofurin, have been pursued and a pediatric Phase I study completed. Phase I studies of 3 new agents, 5-Fluorouracil, Spiromustine and Trimetrexate are 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 intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neuro-toxicities 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 treatment protocols. A clinical study of intrathecal AZQ is in progress. Pre-clinical studies evaluating intra-CSF drug administration via indwelling drug delivery devices is under way.

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 Agents

1. 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-06840-09 PB). Using a 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 concentraion 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; these 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/m^2 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 evaluated the toxicity and efficacy of prolonged intravenous infusion of 6-MP administered at a dose designed to provide cytocidal drug levels in the plasma. A safe 48 hour infusion schedule ws identified. Results from that study indicate that: 1) administration of 6-MP as a prolonged IV infusion markedly reduces the interpatient variability in plasma drug levels seen with conventional oral administration, 2) this mode of administration can result in biologically active steady-state levels of 6-MP for prolonged periods, and 3) cytocidal levels of 6-MP can be achieved in the CSF following systemic administration. On the basis of the results of this Phase I study we have recently initiated three separate Phase II protocols to treat patients with 1) acute lymphoblastic leukemia, 2) brain tumors, and 3) pediatric solid tumors. Results of the Phase II ALL study should provide useful information regarding the possible value of including intravenous 6-MP in future ALL treatment protocols, either as therapy during periods of intensification, or periodically during maintenance. The rationale for studying this approach in brain tumors is based on our data which demonstrates a favorable CSF:plasma ratio for intravenously administered 6-MP. In addition, HPRT, the enzyme which converts 6-MP to TIMP, its active metabolite, is found in highest amounts in the human brain. Finally, results of recent studies in a nude mouse model indicate that 6-MP is active against human brain tumors. Study of 6-MP in pediatric solid tumors seems indicated in that this anti-metabolite has never been adequately tested against these diseases.

We have also studied the cellular pharmacokinetics of 6-MP in human leukemia cells. The accumulation, metabolism and retention of 6-MP intracellularly was measured using a newly developed HPLC assay that separates the nucleotide metabolites. Differences in intracellular metabolism have been correlated with in vitro sensitivity to 6-MP.

We have investigated the mechanisms by which malignant cells become resistant to 6-MP, both in vitro and in the clinical setting. Leukemic cells were obtained from 10 patients with ALL at the time of diagnosis and from the same patients at the time of their initial marrow relapse. At the time of relapse, 4 patients had biochemical evidence of 6-MP resistance as demonstrated by decreased activity of HPRT, decreased PRPP, and/or increased alkaline phosphatase activity. These results indicate that clinical resistance to 6-MP may be related to alterations in these three enzymes and that in some patients this phenomenon may be associated with perturbations of more than one of these three biochemical parameters.

We have identified a new 6-MP metabolite. Quantitating the renal excretion of 6-MP on our recently completed Phase I study, an unknown peak was seen on HPLC analysis of patient urine samples. This peak was collected by preparative HPLC and subjected to several different methods of compound identification (co-elution with authentic 6-MP riboside,

enzymatic peak shifting with purine nucleoside phosphorylase, and gas chromatography/mass spectrometry). The compound was identified as 6-MP riboside. It is possible that this compound is an indirect marker for intracellular conversion of 6-MP to TIMP.

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. A Phase I pediatric study of this compound was recently completed. In addition to identifying a maximally tolerated dose for administration of this compound on a daily x 5 schedule, a detailed study of the pharmacokinetics of Tiazofurin in children was performed.

3. Pre-clinical and clinical studies of Trimetrexate

We have been studying the clinical pharmacology of trimetrexate, a new "non-classical" folate antagonist. Like methotrexate, trimetrexate is a potent inhibitor of dihydrofolate reductase. However, it is more lipophilic than methotrexate, entering cells more readily by passive diffusion. Preclinical laboratory studies demonstrated that human leukemia and osteosarcoma cell lines resistant to methotrexate on the basis of impaired drug transport were not cross resistant to trimetrexate. We have studied the pharmacokinetics of trimetrexate in rhesus monkeys using both HPLC and dihydrofolate reductase inhibition assays. Following an i.v. bolus trimetrexate was cleared more slowly from plasma than methotrexate at equimolar doses. Despite its greater lipophilicity, CSF penetration of trimetrexate was similar to that of methotrexate, with a CSF:plasma ratio of .034 versus .021 for methotrexate. Following oral administration, the mean bioavailability of trimetrexate was 51% with a mid-range of 34 - 68%. In contrast to methotrexate, trimetrexate was cleared primarily by metabolism. We have identified 2 previously unknown metabolites of trimetrexate in the urine which were found to interfere with dihydrofolate reductase inhibition and may also interfere with the competitive protein binding assay. The above data are important for the design of Phase I and pharmacokinetic studies in man. We have initiated a Phase I study of trimetrexate administered on a weekly schedule. That study is currently in progress.

4. Phase I Study of Spirohydantoin Mustard in Pediatric Malignancies

We have initiated a pediatric Phase I study of spirohydantoin mustard (spiromustine). This unique compound was specifically developed to treat brain tumors and is one of the few alkylating agents known to penetrate the blood-brain barrier following intravenous administration. A weekly x 3 schedule is being studied in children. Patients with solid tumors and leukemias are included. The main objectives of this study are 1) to determine the qualitative and quantitative toxicity of spiromustine administered intravenously on a weekly x 3 schedule; 2) to study the pharmacokinetics of spiromustine in children; 3) to define the anti-tumor activity of spiromustine within the confines of a Phase I study; and 4) to study the ability of physostigmine to reverse any spiromustine induced CNS toxicity.

5. L-Asparaginase Antagonism of Methotrexate Cytotoxicity: An Alternative Explanation

Previous studies have suggested that L-asparaginase pretreatment in vitro antagonized methotrexate cytotoxicity through presumed non-specific inhibition of protein synthesis and methotrexate uptake. We re-examined the mechanism of this interaction in view of recent data demonstrating the importance of methotrexate metabolism to polyglutamate derivatives in methotrexate cytotoxicity. After a 3 hour exposure to 0.5 mm methotrexate, 67% of intracellular methotrexate was in the form of methotrexate polyglutamates with a total of 2 to 5 glutamyl residues; there was only 7% cell cloning efficiency in drug free medium. After a 3 hour pretreatment with 0.1 units/ml of E. coli L-asparaginase, tridiated thymidine incorporation dropped by 29%, methotrexate polyglutamate formation during subsequent methotrexate exposure decreased by more than one-half and the cloning efficiency increased to 71% of control. Since there is significant L-glutaminase activity in E. coli L-asparaginase, our data indicate that E. coli L-asparaginase protects L5178Y cells from methotrexate cytotoxicity through L-glutamine depletion and subsequent non-specific inhibition of DNA synthesis and methotrexate polyglutamate formation.

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 anthracinedione, the combination of cytosine arabinoside and tetrahydrouridine, aclacinomycin, and interferon. Examples of recent studies 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 noeplasia. 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. To date 12 patients with refractory meningeal malignancy have been studied. An MTD has not yet been identified. However, the drug has demonstrated activity. Three patients have cleared malignant cells from their CSF.

2. In vitro Studies of Diaziquone (AZQ) Cytotoxicity

In an attempt to develop a more rational schedule of administration applicable to clinical use, we studied the relationship of concentration and time to AZQ cytoxicity in human leukemia and lymphoma lines. Our results revealed a marked increase in cytotoxicity, with increasing duration of exposure. Short (less than 6 hour) periods of exposure to AZQ in vitro did not result in appreciable cytotoxicity, whereas optimal cytotoxicity appeared only after 12 hours of drug exposure. In most clinical studies in man, AZQ has been administered as an intravenous bolus. Clinical pharmacologic studies have shown that AZQ has a short plasma half-life (approximately 30 minutes) following bolus administration. Thus, our results suggest that administration of AZQ as an infusion may result in increased drug efficacy.

3. 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.

4. Effect of Intravenous Dose and Schedule on CSF Pharmacokinetics of 5-Fluorouracil (5-FU)

5-Fluorouracil is one of the most frequently used agents in clinical oncology. It has demonstrated antineoplastic activity against a wide range of solid tumors, including cancers of the breast, colon and ovaries. Rationale for requiring information regarding the CNS pharmacology of this drug is substantial and includes both the fact that it has demonstrated efficacy against CNS metastases and on occasion has been associated with neurotoxicity. We studied the disposition of 5-FU in the CSF following 3 intravenous drug delivery schedules in monkeys, 520 mg/m^2 as an i.v. bolus, 520 mg/m^2 as a 4-hour infusion, and 180 mg/m^2 as a 4-hour infusion. Following bolus administration, the AUC for 5-FU in CSF was 48% of the plasma AUC. However, for the continuous infusions of this drug, the AUC ratio for CSF:plasma was 20% or 11% respectively for the high and lower infusion dose rates. The observation of substantial variations in CSF exposure with different patterns of plasma delivery is unusual and contrasts with the situation seen with most agents in which changes in the CSF AUC are ordinarily a passive reflection of total systemic delivery. These differences for 5-FU were felt to be due to concentration-dependent metabolism of the drug within the CNS. The differences in the CSF AUCs which were observed with the different delivery rates were found to be consistent with a saturable mechanism of 5-FU metabolism which is known to exist locally within the CNS.

Our data suggests that the variation in CSF exposure to 5-FU should be considered in the selection of treatment regimens for this compound. When the treatment goal is to maximize CNS concentrations of 5-FU, bolus delivery should be used. To minimize neurotoxicity, expecially at higher doses, a prolonged infusion would appear appropriate.

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. To better define the effects of chronic methotrexate treatment, tissue levels of methotrexate polyglutamates and folate were measured in 3 monkeys treated with weekly intramuscular methotrexate for 12 months. Methotrexate content (total methotrexate plus methotrexate polyglutamates in pmols/gm/wet weight) of liver, kidney, brain, and testes were 2817, 836, 4.9 and 44 respectively. Greater than 80% of the methotrexate was in the form of methotrexate polyglutamates. The high concentrations of methotrexate polyglutamates in liver may in part be responsible for the hepatotoxicity seen with chronic methotrexate administration. An examination of the folate content of these same tissues demonstrated a profound degree of depletion of folate in the brain (greater than 90% loss). This is of special interest, since inborn errors of folate metabolism often present with severe neurological problems similar to methotrexate neurotoxicity. Studies to evaluate the specific folate-dependent biochemical reactions are in progress in an effort to further examine the mechanism of this toxicity.

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.

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 II studies are evaluating not only the efficacy of this agent against leukemias, but also its potential against brain tumors and solid tumors. 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.

Publications:

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2. Poplack, D.G.: Editorial - Massive intrathecal overdose: Check the label twice:. N. Engl. J. Med. 311: 400-402, 1984.

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4. Zimm, S., Grygiel, J.J., Strong, J.M., Monks, T.K., and Poplack, D.G.: Identification of 6-mercaptopurine riboside in patients receiving 6-mercaptopurine as a prolonged intravenous infusion. Biochem. Pharmacol. 33: 4089-4092, 1984.

5. Jolivet, J., Cole, D.E., Holcenberg, J.S., and Poplack, D.G.: Prevention of methotrexate cytotoxicity by asparaginase inhibition of methotrexate polyglutamate formation. Cancer Res. 45: 217-220, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06890-06 PB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ian T. Magrath	Senior Investigator	PB, NCI
Others.	Jacqueline Whang-Peng	Senior Investigator	MB, NCI
	Stanley Korsmeyer	Senior Investigator	MET, NCI
	Ilan Kirsch	Senior Investigator	NMOB, NCI
	Greg Hollis	Senior Staff Fellow	NMOB, NCI

Continued on next page

COOPERATING UNITS *(if any)*
Flow Cytometry Lab., George Washington Univ. (O. Alabaster), NCI/Navy Medical Oncology Branch (L. Kirsch), Wistar Institute (C. Croce), Department of Pathology, New York University (R. Dalla Favera)

LAB/BRANCH
Pediatric Branch, NCI

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	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.)*

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 characterize, with more precision than has hitherto been achieved, differences in the spectrum of lymphoid neoplasia that occurs in different environments. The current emphasis is on molecular characterization of these tumors. In particular, we are comparing the molecular rearrangements in and EBV association of Burkitt's lymphoma from different regions.

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 and other oncogenes during differentiation of the cell lines, and after inhibition of proliferation with anti-immunoglobulin antibodies. We are interested to determine whether the expression of the translocated c-myc gene is linked in Burkitt's lymphoma cells to the expression of immunoglobulin genes.

Professional Personnel (Continued):

V. Arasi	Clinical Associate	PB, NCI
J. Sandlund	Clinical Associate	PB, NCI
J. Kiwanuka	Visiting Fellow	PB, NCI

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 and retinoids.

3. To investigate the significance of the cytogenetic abnormalities and molecular rearrangements seen in undifferentiated lymphomas.

4. To study gene expression (particularly c-myc and immunoglobulin genes) in Burkitt's lymphoma cell lines under circumstances where immunoglobulin gene expression is altered in order to determine whether expression of these genes is linked.

5. To determine the frequency and nature of the association of Epstein-Barr virus (EBV) with Burkitt's lymphoma in various geographical regions.

Methods and Major Findings:

A. Influence of Anti-immunoglobulins on Burkitt's Lymphoma Cell Proliferation and Gene Expression

We have shown that anti-immunoglobulins, specifically anti-μ or anti-light chain antibodies, will inhibit the proliferation of Burkitt's lymphoma cell lines which express the appropriate heavy and light chains on their surface. Since mature B-cells are stimulated by anti-immunoglobulins, whereas immature B-cells are inhibited from further differentiation, this information suggests that Burkitt's lymphoma is the neoplastic counterpart of an immature B-cell. During the course of these experiments, we have also shown that the expression of c-myc is similar in cells treated with anti-immunoglobulins and control cells for a period of about six hours. Thereafter, there is a rapid increase in c-myc expression in control cells, but a much slower increase in cells treated with anti-immunoglobulins such that at 48 hours there is an 8-10 fold reduction in c-myc in anti-immunoglobulin treated cells. The expression of immunoglobulin genes appears to parallel that of c-myc. This effect appears to occur regardless of the molecular rearrangement present in the cell line, or of the presence or absence of EBV. Cell lines do, however, vary markedly in their susceptibility to the inhibitory effect of anti-immunoglobulin. We have not been able to demonstrate this phenomenon in EBV transformed lymphoblastoid cell lines.

B. Gene Expression in Burkitt's Lymphoma Cells Induced to Differentiate

We have continued our earlier studies in this area by examining the expression of a broad range of genes (immunoglobulin and oncogenes) in Burkitt's lymphoma cells and EBV transformed lymphoblastoid cell lines induced to undergo differentiation by retinoic acid. There is no evidence that c-myc is down regulated in Burkitt's lymphoma cells which differentiate and continue to express immunoglobulin genes at high level, although this gene is switched off in other cell types undergoing differentiation including lymphoblastoid cell lines. Thus, this data supports the hypothesis that in Burkitt's lymphoma, c-myc gene expression is influenced by immunoglobulin gene regulatory mechanisms.

C. Double Expression of μ and γ Genes in Raji Cells

In Raji cells the c-myc gene is translocated into the γ immunoglobulin heavy chain region. We have observed, by immunological techniques that these cells express μ and γ heavy chains but no light chains. In situ staining indicates the presence of both μ and γ chains in all cells, excluding the possibility that heavy chain switching could account for this. This very unusual situation is either due to transcription of the $C\gamma$ region on the 14q+, transcription of both μ and γ from the same normal chromosome, or transcription of μ from the tranlocated gene on chromosome 8. Analysis of this situation will shed light on normal immunoglobulin rearrangements including heavy chain switching, as well as the molecular abnormalities in Burkitt's lymphoma.

D. Characterization of Lymphomas from Developing Countries

In collaboration with the International Agency for Research in Cancer and the NCI Medicine Branch, Navy, we are characterizing, at a molecular level, frozen tumor samples from various parts of the world. Studies will include immunoglobulin and T-cell receptor gene rearrangements, BCL-1 and BCL-2 rearrangements (looking for rearrangements indicating 11;14 or 14;18 translocations), c-myc gene rearrangements and the presence of EBV and HTLV I genomes. An in situ hybridization technique is being developed so that some of these studies will, in the future, be possible on formalin-fixed sections. These studies will provide information regarding the immunological sub-types of the diffuse lymphomas in developing countries and will be of potential epidemiological and etiological importance.

E. Association of Specific Molecular Abnormalities with Burkitt's Lymphoma in Different Geographical Regions

With Dr. R. Dalla Favera we are correlating precise molecular abnormalities (break point within or outside the c-myc gene) with the geographical location and clinical features of Burkitt's lymphoma. Preliminary information suggests that there are distinctive features of the molecular changes observed in Burkitt's lymphoma in different geographical areas, and EBV associated versus EBV non-associated tumors. These findings will help to clarify the observed behavioral differences in these tumors, and may shed some light on the cell of origin of Burkitt's lymphoma in different parts of the world.

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 to 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, biological and immunological studies promise to increase our understanding of the mechanism whereby c-myc gene transcription is altered, and its consequences. Studies of the correlation between molecular rearrangements, EBV association, geographic location and clinical behavior of Burkitt's lymphoma will further our knowledge of pathogenesis and may ultimately lead to new approaches to intervention.

Proposed Course:

We shall continue to study immunoglobulin and oncogene expression in our cell lines when manipulated in various different ways including the utilization of agents which induce differentiation, and others which cause cell cycle blocks or influence proliferation in other ways. In this way, we hope to learn more about the function and regulation of c-myc, how its expression (or regulation of expression) differs in Burkitt's lymphoma and how it is that altered expression of this gene is important to the pathogenesis of Burkitt's lymphoma. We shall pursue our studies of the EBV association of Burkitt's lymphoma in different parts of the world including South America, North Africa and Asia, and also carry out more detailed characterization of lymphoid neoplasia occurring in developing countries in an attempt to generate clues to etiology. Correlation of molecular findings with clinical features may provide information of a quality hitherto unobtainable.

Publications:

1. Magrath, I., Cossman, J., Benjamin, D., Sieverts, H., and Triche, T.: Biological Features of Pediatric Non-Hodgkin's Lymphoma. Proceedings. New Perspectives in Human Lymphoma. Raven Press, New York, 1984, pp. 201-212.

2. Tsokos, G.C., Smith, P.L., Magrath, I.T., and Balow, J.E.: Characterization of the Prescursor Cells of the Epstein-Barr Virus Induced Suppressor Cells. J. Immunol Immunopharm., 1: 25-29, 1984.

3. Magrath, I.T.: Cell marker studies in leukemias and lymphomas. In Javadpour, N. (ed.): Biologic Markers in Cancer. Thieme Stratton Publishing Co., in press.

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D.V., Pearson G.R., and Kottaridis, S.D. (eds.): Proceedings of the First International Symposium on Epstein-Barr Virus and Associated Malignant Diseases. Human Press, in press.

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6. Sandlund, J.T., Kiwanuka, J., Marti, G.E., Goldschmidts, G., and Magrath, I.T.: Characterization of Burkitt's lymphoma cell lines with monoclonal antibodies using an ELISA technique. Proceedings of the 2nd International Congress of Human Leukocyte Antigens, in press.

7. Sieverts, H., Alabaster, O., Goldschmidts, W., and Magrath, I.: Expression of surface antigens during the cell cycle in different growth phases of American and African Burkitt's lymphoma cell lines. Cancer Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06811-03 PB

PERIOD COVERED

October 1, 1984, to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip Pizzo	Chief	PB, NCI
Others:	A. Miser	Visiting Fellow	PB, NCI
	S. Rosenberg	Chief	SB, NCI
	A. Baker	Senior Investigator	SB, NCI

COOPERATING UNITS *(if any)*

Pediatric Oncology Group, Gainesville, FL (M. Link); Dana Farber Cancer Institute, (A. Goorin); London Children's Solid Tumor Group, (J. Pritchard); Children's Hospital of Philadelphia, (J. Belasco).

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	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.)*

Treatment of localized osteosarcoma with amputation alone has historically resulted in long-term relapse-free survival of approximately 20%, although recently relapse-free survival of greater than 40% has been reported in surgically-treated patients. Although several chemotherapeutic agents have been found to cause tumor stabilization or regression in patients with overt tumor, their benefit in the adjuvant setting following surgical removal of all identifiable tumor is much debated. The objective of this multi-institutional study is to evaluate the efficacy of adjuvant chemotherapy using the currently available front-line drugs in children with localized extremity osteosarcoma. Following either amputation or limb salvage procedure, patients are randomized to receive either a 43-week course of chemotherapy using bleomycin/actinomycin D/cyclophosphamide, high-dose methotrexate, adriamycin and cis-platinum (regimen 1), or no immediate chemotherapy (regimen 2). Patients being observed on regimen 2 will receive chemotherapy only in the event of overt tumor recurrence, following attempt at surgical resection of all recurrent tumor.

Since May 1982, 36 eligible patients have been randomized on this study (16 from NCI), 18 to the immediate chemotherapy arm and 18 to the observation arm. An additional 77 patients refused randomization of whom 59 elected adjuvant chemotherapy and 18 elected observation alone. Analyzing the randomized group by assigned arm (4 patients refused the random arm assigned), there is a statistically significant difference in time to first relapse favoring the immediate chemotherapy group ($p=0.0013$, 2-sided log rank test), the 2 year actuarial relapse-free survival being 18% for the observation arm and 56% for the chemotherapy arm. Analysis of the non-randomized patients shows similar results. As yet there is no survival difference between the two arms. It is concluded that the natural history of localized, high-grade osteosarcoma of the extremity has not changed, since less than 20% of such patients treated with surgery alone will become long-term, relapse-free survivors. The administration of adjuvant chemotherapy has a significantly favorable impact on relapse-free survival.

Objectives:

Primary

1. To determine if intensive multi-agent chemotherapy given adjuvantly after surgical ablation of the primary tumor will significantly improve the disease-free survival (DFS) for patients with non-metastatic osteosarcoma when compared to a concurrent, non-adjuvantly treated control group.

2. To determine if freedom from second relapse and overall survival are different for patients treated with immediate post-operative adjuvant chemotherapy versus those treated with delayed adjuvant chemotherapy after relapse and metastatectomy.

Secondary

1. To determine if there are risk factors present at diagnosis in patients with osteosarcoma which can be utilized to predict the subsequent relapse hazard for these patients, and which can be utilized to predict high and low risk groups.

   The factors which will be analyzed prospectively include: histology of the primary tumor, site of primary, age of patient, sex, tumor size, duration of symptoms, alkaline phosphatase level, tissue alkaline phosphatase.

Methods Employed:

In this Pediatric Oncology Group (POG) multi-institutional study, 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 / cytoxan 600 mg/m^2/day x 2 / actinomycin D 0.6 mg/m^2/day x 2; adriamycin 30 mg/m^2/day 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 may either receive no further chemotherapy (where all metastatic disease has been resected) or a phase I or II agent (where active tumor remains).

Major Findings:

The study was opened in May 1982. To date, a total of 36 eligible patients have been randomized (including 16 from NCI), 18 to each arm. At 2 years, the actuarial relapse-free survival (RFS) for the control group is 18% compared to 56% for the group receiving immediate adjuvant chemotherapy (p=0.0013, 2-tailed log rank test). As yet, there is no difference in survival between the two arms, there being 4 deaths in the immediate chemotherapy group (including 1 from liver failure) and 2 in the observation alone group. Seventy-seven additional eligible patients from other co-operating institutions refused randomization but elected therapy according to the treatment arms of the protocol; 59 elected immediate adjuvant chemotherapy (2 year actuarial RFS 54%), and 18 elected observation alone (2 year actuarial RFS 11%). Toxicity has been tolerable, with one reported toxic death due to liver failure.

Thus, this study indicates that the long-term, relapse-free survival of patients with high-grade, extremity osteosarcoma treated with surgery alone is below 20%, consistent with most historical data. Adjuvant chemotherapy has a significant favorable impact on relapse-free survival in these patients.

During the conduct of this study, in addition to patients with localized disease, 17 patients with metastatic disease have non-randomly received this chemotherapy regimen, 9 following surgical resection of all tumor (of whom 4 continue disease-free at 20-37+ months) and 8 had overt disease. Of the 8 patients with measurable disease, no patient achieved a complete response with chemotherapy alone, but 4 were subsequently rendered NED by surgery. An additional 2/8 patients had stable disease for greater than 6 months. Two of the 8 patients had progressive disease.

Significance to Biomedical Research and the Program of the Institute

The role of adjuvant chemotherapy in osteosarcoma has been much debated in recent years, and the results of this crucial study will form the definitive basis for further chemotherapy trials in this disease at NCI and elswhere.

Proposed Course

The observation alone arm of this study is now closed to new patient accrual, and a new study is being formulated. All patients previously randomized on this study will continue on their assigned treatment arm and will be closely followed so that ultimately any survival difference between the 2 arms will become apparent.

Publications

Miser, A.W., Miser, J.S., and Pizzo, P.A.: Review: Therapy of Osteogenic Sarcoma: Local, Systemic or Both? Eur. J. Cancer Clin. Oncol. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06813-3 PB

PERIOD COVERED

October 1, 1984, to September 30, 1985

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

Molecular Biology of Pediatric Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark A. Israel	Head, Molecular Genetics Section	PB, NCI
Others:	L. Helman	Medical Staff Fellow	PB. NCI
	N. Rosen	Medical Staff Fellow	NMPB, NCI
	C. McKeon	Senior Staff Fellow	PB, NCI
	C. Thiele	Senior Staff Fellow	PB, NCI

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COOPERATING UNITS *(if any)*

Naval Medical Res. Inst., Bethesda (P. Reynolds); Univ. of PA, Philadelphia (B. Emanuel); Dept. of Pediatrics, UCLA, Los Angeles, (B. Seeger); Sloan Kettering, N.Y. (J. Biedler); Fordham Univ., Bronx, N.Y. (R. Ross).

LAB/BRANCH

Pediatric Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	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.)*

During the past year, our research program has pursued studies to elucidate the molecular events associated with the development of pediatric tumors, while terminating a previously major line of investigation directed at understanding the genetic basis of transformation and tumorigenesis induced by polyoma virus. Experiments focused on studying polyoma virus induced transformation included: 1) Characterization of a post-translational modification of pp60 c-src molecules physically associated with the polyoma virus transforming protein, middle T-antigen; 2) Characterization of the interaction between the middle T-antigen encoded by the transformation defective polyoma virus mutant, NG59, and pp60 c-src; and 3) Evaluation of the effect of SV40, adenovirus, and papillomavirus infection of rodent cells on pp60 c-src tyrosyl kinase activity. Experiments we have conducted to pursue our studies of pediatric tumors have included: 1) an examination of the cellular and genetic differences underlying two histiologically identical tumors: neuroblastoma and peripheral neuroepithelioma; 2) Characterization of the precise localization of the rcp(11;22) translocations found in peripheral neuroepithelioma and in a second pediatric tumor, Ewing's sarcoma; 3) Evaluation of the frequency of the rcp(11;22) translocation in pediatric solid tumors; 4) Evaluation of the importance of N-myc during the clinical progression of neuroblastoma; and 5) Evaluation of the importance of pp60 c-src activation in human cancer.

PHS 6040 (Rev. 1/84) GPO 914-918

Professional Personnel (Continued):

P. Cohen	Senior Staff Fellow	PB, NCI
J. Miser	Cancer Expert	PB, NCI
T. Triche	Head, Ultrastructural Pathology	LP, NCI
J. Whang-Peng	Head, Cytogenetic Oncology Section	MB, NCI

Objectives:

To characterize the molecular mechanisms which mediate the growth pattern and phenotype of pediatric tumors, we have focused our work on four specific goals:

1. Development of well-characterized biological reagents which can be utilized in molecular studies.

2. Characterization and evaluation of genetic alterations in pediatric solid tumors and tumor-derived cell lines.

3. Characterization of alterations in gene regulation which might be central to the development of pediatric tumors.

4. Identification and characterization of genes important for growth and tumor cell differentiation.

Methods Employed:

1. The development of well-characterized biological reagents which can be utilized in molecular studies

   Central to the genetic analysis of specific tumors is the availability of tumor tissues and corresponding normal tissues from patients with the disorder under study. The banking of tumor and normal tissue specimens, the development of tumor derived cell lines, and the immortalization of normal tissues which then can be grown in culture continue to be experiments of central importance to our laboratory research program. An important expansion of this effort has been recent attempts to clone multiple cell lines from tumor mass cultures in order to develop reagents which will allow us to study directly the clonal derivation and apparent genetic instability of the tumors we are currently evaluating. These questions are of particular importance in the study of pediatric tumors because of the possibility that they arise as disorders of normal differentiation, may be multi-centric, and are often-times rapidly progressive despite therapy which in some cases is very effective.

2. The characterization and evaluation of genetic alterations in pediatric solid tumors and tumor derived cell lines.

Neuroepithelioma and Ewing's sarcoma are characterized by a cytogenetically indistinguishable rcp(11;22) translocation. We have used a number of molecular probes which identify well-documented sites on 22q to demonstrate that the breakpoint in this translocation is distal to either the breakpoint of the Philadelphia chromosome which characterizes CML or the variant translocation involving chromosome 22 in some cases of Burkitt's lymphoma. Recently, we have identified a molecular probe which seems to recognize a rearranged chromosomal site in several different neuroepithelioma and Ewing's sarcoma tumor tissues. In a few cases for which normal tumor tissue is available from the same patient whose tumor we have examined, this rearrangement is not observed indicating that the alteration may be tumor specific and not simply a polymorphic chromosomal site. Also, we have identified in eight of eight consecutive cases of chest wall Askin's tumor a rcp(11;22) identical to that found in peripheral neuroepithelioma. This led us to study tissue and cell lines from this tumor for the expression of neural markers. We found that this tumor has an ultrastructural, biochemical, and cell surface marker profile indistinquishable from that of perpheral neuroepithelioma. These findings and the presence of the rcp (11;22) translocation demonstrate that this tumor is, in fact, peripheral neuroepithelioma. This is of particular importance in that it now provides a rational basis for the treatment of these patients.

3. Characterization of alterations in gene regulation which might be central to the development of pediatric tumors.

Studies examining the tumor specific regulation of genes has focused on two themes: 1) Altered regulation of genes likely to be important for the development of pediatric malignancies and 2) Identification of patterns of expressed genes which characterize specific tumors.

A major experimental thrust addressing the first of these themes has been to clone and determine the primary structure of N-myc, a gene invariably amplified in human neuroblastoma tumor cell lines. These experiments are well underway and it is likely that we will soon complete the molecular characterization of cDNA corresponding to the N-myc mRNA as well as the genomic DNA encoding N-myc. In other studies examining the expression of oncogenes in human neuroblastoma, we have found that in addition to N-myc, N-ras is invariably activated in human neuroblastoma tumor cell lines as determined by the NIH3T3 transfection assay. This finding is of importance because it documents for the first time the frequent activation of two "complementary" oncogenes in a particular human tumor type. Interestingly, the level of $pp60^{c\text{-}src}$ tyrosyl kinase activity in this tumor is also easily detectable by it phosphorylation of a variety of substrates raising the possibility that this oncogene activity is also abberantly regulated. Ongoing studies of this tumor may provide insight into the possibility that multiple oncogenes are involved in the specific metabolic pathways associated with tumorigenesis.

In other experiments, we have been able to develop batteries of cloned DNA molecules which identify mRNA species present in high copy number in specific tumors. We have used such cloned DNAs to develop molecular fingerprints which we believe will provide a new and uniquely rational basis on which to classify tumors. To date, our work has focused on the characterization of tumors whose tissue of origin is thought to be well-known allowing us to generate a series of fingerprints which define specific tumors and their tissue of origin. It should be possible to use such reagents to examine tumors whose tissue of origin is unknown in an effort to characterize them more exactly.

4. Identification and characterization of genes important for growth and tumor cell differentiation.

To identify and characterize genes important for the development of pediatric malignancies, we have devised a strategy to examine at the molecular level the possiblity that pediatric tumors arise from cells which are growing inappropriately because their ability to mature and begin senescence has been compromised. Cells arrested at a stage in differentiation during which they are specifically programmed to divide would be susceptible to the eventual acquisition of mutations which could then confer a malignant phenotype on the cells. We have now identified a large panel of genes which characterized either mature chromaffin tissue or malignant neuroblastoma and are currently studying the coordinated regulation of these genes in order to identify regulatory mechanisms which might be pathologically disturbed and interfere with the normal progression of embryonic adrenal tissue to form mature chromaffin tissue.

Significance to Biomedical Research and the Program of the Institute:

The identification of the molecular genetic alterations which characterize specific tumors will allow us develop therapeutic strategies which are directed at altering specific pathologic changes that lead to the formation of malignant tumors. Our recent identification of high levels of c-src activity as well as activated N-myc and N-ras in neuroblastoma raises the possibility that in some tumors the altered expression of multiple oncogenes is important for the development of this malignancy. Neuroblastoma tumor cells should provide an important model system in which the interaction of oncogene products might be examined and the possibility that their expression is coordinately regulated studied.

The identification of genetic differences between neuroepithelioma and neuroblastoma, two morphologically indistinguishable tumors of the autonomic nervous system raises the possibility that the molecular events underlying the development of these tumors is different and that each might have a distinctive pattern of gene expression characterizing its malignant behavior. The development of molecular fingerprints corresponding to a pattern of gene expression known to be associated with a particular tumor phenotype may provide a new, rational basis by which to classify tumors, as well as possibly providing insights into the genetic mechanisms underlying various aspects of malignant tumor growth.

This approach of establishing molecular fingerprints which identify specific patterns of gene expression should be generally applicable to many problems related to the classification of tumors such as identifying the cell of origin of specific tumors, estimating the likelihood of recurrance of a specific tumor, and estimating the metastatic potential of a particular tumor.

Proposed Course:

During the next year we will avidly continue our current efforts to develop tumor cell lines and immortalized normal tissues to use in the molecular analysis of the events underlying the development of tumors of the autonomic nervous system. In this regard, we will focus on the regulation of N-myc expression, c-myc expression, and the activation of the c-src gene product, $pp60^{c\text{-}src}$, in human tumors. An understanding of the molecular biology of these proto-oncogenes, each of which is active in the neuronal tumors we are studying, should provide important insights into the physiologic events which determine the behavior of these malignancies. Similarly, our efforts to elucidate the genetic alterations resulting from the reciprocal (11;22) translocation in peripheral neuroepithelioma should lead to a better understanding of the physiological alterations leading to the formation of this tumor.

To better characterize solid tumors, we will continue ongoing experiments to develop molecular fingerprints specific for well-characterized tumors in our effort to identify patterns of gene expression which correspond to particular tumor phenotypes. In this regard, we are studying tumors of the neural crest in an effort to determine if we can relate these tumors to one another on the basis of identifiable patterns of gene expression. Such fingerprints of various tumors of the autonomic nervous system may allow us to classify such tumors as ganglioneuroblastoma, ganglioneuroma, soft parts sarcoma, malignant pheochromocytoma, perpherial neuroepithelioma, and others in a biologically more accurate and clinically more useful manner than is presently available.

Publications:

1. Bolen, J.B., Thiele, C.J., Israel, M.A., Yonemoto, W., Lipsich, L.A., Brugge, J.S. Enhancement of cellular src gene product associated tyrosyl kinase activity following polyoma virus infection and transformation. Cell 38: 767-777, 1984.

2. Whang-Peng, J., Triche, T.J., Knutsen, T., Miser, J., Douglass, E.C., Israel, M.A.: Rcp (11;22)(q24;q12) in peripheral neuroepithelioma. N. Engl. J. Med. 311: 584-585, 1984.

3. Israel, M.A., Thiele, C., Whang-Peng, J., Kao-Shan, Chien-Song, Triche, T.J., Miser., J.: Peripheral Neuroepithelioma: Genetic Analysis of Tumor Derived Cell Lines. In Evans, A.E. (Ed.): Advances in Neuroblastoma Research. New York, Alan R. Liss, 1984, pp.161-170.

4. Donner, L., Triche, T.J., Israel, M.A., Seeger, R.C., and Reynolds, C.P.: A Panel of Monoclonal Antibodies which Discriminate Neuroblastoma from Ewing's Sarcoma, Rhabdomyosarcoma, Neuroepithelioma, and Hematopoietic Malignancies. In Evans, A.E. (Ed.): Advances in Neuroblastoma Research. New York, Alan R. Liss, 1984.

5. Bolen, J.B., Fisher, S.E., Chowdhury, K., Williams, J., Dawe, C.J. and Israel, M.A.: A determinant of polyoma virus virulence enhances virus growth in cells of renal origin. J. Virol. 53: 335-339, 1985.

6. Yonemoto, W., Jarvis-Morar, M., Brugge, J.S., Bolen, J.B., and Israel, M.A.: 1984. Novel tyrosine phosphorylation within the aminoterminal domain of pp60$^{c\text{-}src}$ molecules associate with polyoma virus middle tumor antigen. Proc. Natl. Acad. Sci. USA, in press.

7. Whang-Peng, J., Triche, T.J., Knutsen, T., Miser, J., Kao-Shan, S., Tsai, S., and Israel, M.A.: Cytogenetic characterization of selected small round cell tumors of childhood. Cancer Genet. Cytogenet., in press.

8. Bolen, J.B., Rosen, N., and Israel, M.A.: Elevated pp60$^{c\text{-}src}$ tyrosyl kinase activity in human neuroblastomas is associated with aminoterminal tyrosine phosphorylation of the src gene product. Proc. Natl. Acad. Sci. USA, in press.

9. Israel, M.A.: The evolution of clinical molecular genetics: neuroblastoma as a model tumor. Am. J. Ped. Hem. Onc., in press.

10. Emanuel, B.S., Nowell, P.C., Croce, C.M., McKeon, K., and Israel, M.A.: Translocation breakpoint mapping: Molecular and cytogenetic studies of chromosome 22. Cancer Genet. Cytogenet., in press.

11. Bolen, J.B., Israel, M.A.: Middle tumor antigen encoded by the polyoma virus transformation defective mutant NG59 is associated with pp60$^{c\text{-}src}$. J. Virol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06814-03 PB

PERIOD COVERED

October 1, 1984, to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James S. Miser	Expert	PB, NCI
Others:	P. Pizzo	Chief	PB, NCI
	E. Glatstein	Chief	ROB, NCI
	T. Kinsella	Senior Investigator	ROB, NCI
	J. Mulvihill	Chief	CEB, NCI
	M. Israel	Head, Molecular Genetics Sect.	PB, NCI
	T. Triche	Head, Ultrastructural Path. Sect.	LP, NCI
	D. Longo	Head, Experimental Immunol. Sect.	MB, NCI

COOPERATING UNITS *(if any)*

Rehabilitation Medicine, CC (L. Gerber)

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	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 study of pediatric sarcomas including Ewing's sarcoma, rhabdomyosarcoma, and undifferentiated sarcomas, as well as other sarcomas, 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; 5) in vitro radiation and chemosensitivity of cell lines derived from tumors of patients with these sarcomas; and 6) molecular biologic studies of these tumors. The cytogenetic characterization of Ewing's sarcoma, peripheral neuroepithelioma has confirmed the translocation t(11;22) in all cell lines studied with these disorders.

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 improving survival by utilizing intensive consolidation including high dose chemotherapy, total body radiotherapy, and autologous bone marrow reinfusion; 2) improvement in therapy for patients with moderate 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.

Since the 83-C-73 protocol was begun in early 1983 over 70 patients have been entered. For newly diagnosed patients the initial complete remission rate has been greater than 93% and greater than 70% remain in remission. These figures represent improvement over previous regimens utilized at this institution.

Objectives:

A. Therapeutic:

1. To devise an effective therapeutic regimen for patients with Ewing's Sarcoma of "high risk" (extensive rib, pelvis, sacral primaries, proximal extremity primaries and any primary with metastatic disease and to discuss similarities and differences between subgroups.

2. To devise an effective and relatively safe therapeutic regimen for patients with Ewing's Sarcoma of "moderate" risk (primaries from localized rib, and primaries other than pelvis, sacrum, proximal extremity, without metastatic disease).

3. To devise an effective therapeutic regimen for patients with poor prognosis Stage III and Stage IV rhabdomyosarcoma and undifferentiated sarcoma.

4. To devise an effective and relatively safe therapeutic regimen for patients with Stage II rhabdomyosarcoma and undifferentiated sarcoma.

5. To study the short and long term effects of therapy on cardiac, pulmonary, and other major organ systems including the immune system.

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 recovery of bone marrow after autologous bone marrow transplantation as measured by radionucleide imaging.

8. To study the effect of the rapidity of response to long-term outcome in patients with pediatric sarcomas.

9. To study the effect of Ifosfamide in refractory 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.

7. To study molecular biologic aspects of pediatric sarcomas.

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 including high-dose doxorubicin, high dose cyclophosphamide and weekly vincristine. 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 over 70 patients enrolled to date has shown that this intensive induction therapy is tolerated adequately, that the induction of complete remission occurs in more than 93% of patients, that the intensive consolidation therapy is tolerated adequately, and that over 70% of the original patient population remain disease free.

Patients with relapsed sarcomas have also been evaluated on this protocol. This therapy has been found not to be durably effective in patients who relapse on therapy; however, for patients who relapse off therapy, this form of retreatment has been effective at producing and maintaining a second complete remission in some patients.

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.
g. Immunologic function.

3. A Phase II Study of Ifosphamide in Refractory Pediatric Malignancies.

To date, 74 patients with sarcomas refractory to primary therapy have been treated with ifosphamide on a five day schedule (1800 mg/m^2 per day). The drug is clearly active even in patients previously treated intensively with cyclophosphamide. Twenty-three percent of patients have achieved partial response. Two patients treated with a combination of ifosphamide and followed by surgery are currently disease-free at 10 and 24 months. Toxicity has been relatively mild; some 25% of patients develop hemorrhagic cystitis (well controlled by mesna) while about 8% of patients have developed a confusional state. The latter appears to be particularly associated with the concomittant administration of a continuous IV infusion of morphine or other causes for central nervous system abnormalities.

Future plans are to develop a new combination including ifosphamide and VP16 for relapsed sarcoma patients, and to consider the introduction of this agent into Phase III studies in selected tumors e.g., osteogenic sarcoma, Ewing's sarcoma, rhabdomyosarcoma.

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.
e. To study the molecular biologic aspects of pediatric sarcomas.

2. Cytogenetics

Ten cell lines of patients with Ewing's sarcoma have been evaluated and found to have a (11;22) translocation. This finding has led to the further finding of the identical translocation in 3 patients with peripheral neuroepithelioma and 2 patients with the "Askin" Tumor.

Further evaluation of other pediatric sarcomas is ongoing.

Significance to Biomedical Research and the Program of the Institute:

Our studies in pediatric sarcomas are aimed at improving the survival of patients with these disorders as well as gaining a better understanding of these diseases and their treatments. The clinical studies will address important questions in the treatment of childhood soft tissue tumors by comparing and contrasting treatment results related to histology, by examining the benefit of short course intensive therapy to induce remission and by evaluating the efficacy and toxicity of intensification with total body irradiation and autologous bone marrow reconstitution to maintain remission.

Our current protocol appears to be of major importance. Results to date reveal that there has been a significant improvement in the complete remission rate for patients with extensive and metastatic disease. Further, the relapse rate, although follow-up is very short, is less than seen in our previous studies.

The proposed biologic studies have the potential to help gain important insights into biologic characteristics of these tumors. The finding of a common and consistent cytogenetic abnormality in two distinct pathologic entities is an example example of our early findings that may provide clues to the pathogenesis of these malignant processes.

Proposed Course:

Based on our initial studies we plan to further evaluate the therapy of pediatric sarcomas by:

1. Evaluating duration of therapy.
2. Evaluating the role of total body irradiation in patients with "high risk" localized disease.
3. Evaluating a new pilot therapy for the treatment of metastatic sarcomas.
4. Evaluating Ifosfamide in combination with VP16 in pediatric malignancies.

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. In particular, the molecular biologic evaluation of these tumors will be emphasized.

Publications:

1. Kinsella, T.J., Mitchell, J.B., McPherson, S., Triche, T.J., Miser, J.S., and Glatstein, E.: In vitro radiation studies on Ewing's sarcoma cell lines and human bone marrow CFU-c: Application to the clinical use of total body irradiation (TBI). Int. J. Radiat. Oncol. Biol. Phys. 10: 1005-1011, 1984.

2. Whang-Peng, J., Triche, T.J., Knutsen, T., Miser, J., Kao-Shan, C.S., Tsai, S., and Israel, M.A.: Cytogenetic of characterization of selected small cell tumors of childhood. Cancer Genet. Cytol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06815-03 PB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI.	Ian T. Magrath	Senior Investigator	PB NCI
Other.	Philip A. Pizzo	Chief	PB, NCI
	David G. Poplack	Head, Leukemia Biology Section	PB, NCI
	Mark A. Israel	Head, Molecular Genetics Section	PB, NCI
	James A. Miser	Cancer Expert	PB, NCI

COOPERATING UNITS *(if any)*

Division of Hematology/Oncology, The Hospital for Sick Children Toronto, Canada

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	8	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.)*

Eighty-eight 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 82% and 90% disease-free survival). Among the remaining patients the most important prognostic feature is bone marrow involvement. These findings have been utilized in the design of protocols in which treatment is 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 multiinstitutional study which showed no difference in outcome between these protocols). The results of 77-04-02 which includes IT therapy also appear to be improved compared to results prior to the introduction of CNS prophylactic IT therapy.

Two new protocols have been activated. One is for patients with extensive undifferentiated lymphomas and is based on protocol 77-04. The protocol is more intensive but specifies a much shorter total treatment duration (6 cycles). The second protocol is a new combination (ifosfamide, VP16 and high dose ara-C) which is being evaluated in relapsed patients prior to its possible incorporation into a treatment strategy for high risk patients.

Objectives:

1) To improve the outcome of treatment in patients with extensive non-lymphoblastic lymphomas.

2) To develop a new "non-cross-resistant" drug combination in patients with recurrent undifferentiated lymphomas which may be introduced into primary therapy.

3) To provide material for study of the biology of non-Hodgkin's lymphoma.

Methods and Major Findings:

A. Definition of Prognostic Groups

An analysis of the results of the first 75 patients entered into protocol 77-04 (all admitted more than 2 years ago) led to the following conclusions:

1. Overall, 91% of patients achieved a complete response and approximately 60% of patients achieved long term survival.

2. Of 10 patients with undifferentiated lymphomas involving the bone marrow, 3 achieved long-term survival and all others died (one from sepsis). All 5 patients with lymphoblastic lymphoma and bone marrow disease have relapsed.

3. Among patients without bone marrow involvement the best prognosis was enjoyed by patients in one of the following categories.

    a. lymphoblastic lymphoma (90% relapse-free survival in 11 patients)

    b. localized (stage A) or completely resected intraabdominal tumor (stage AR) in patients with undifferentiation lymphomas (82% relapse-free survival in 17 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 45-50%, this group of patients is worthy of the major focus in the next protocol.

B. Development of an Overall Strategy for the Improvement of the Results of Therapy in Patients with Extensive Undifferentiated Lymphomas

Two new pilot protocols have been activated. One is an improved version of 77-04, for use in high risk patients. The other is a drug combination consisting of high dose ara-C, VP16 and ifosfamide (VIPA) which will be explored in patients who achieve only partial response or relapse. The ultimate objective is to study the value of the addition of a new drug combination (first proven to be of value in relapsed patients) into the 77-04 type regimen. Ideally, this would take the form of a randomized study, in concert with other institutions. Alternatively, a single arm study incorporating the new regimen - if shown to be active in the pilot study - will be designed. By this means, we hope to improve the results of treatment in patient groups that currently have a relatively poor prognosis.

It is too early to present meaningful results of these protocols, although 3 of 3 patients with extensive undifferentiated lymphomas have achieved remission on the modified 77-04 protocol (one off treatment), while 3 patients have been entered into the VIPA protocol. One of the latter achieved CR but subsequently relapsed, the second died of an infection after completing 6 cycles of therapy and the third was too recently entered for assessment of remission status.

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 have been developed which will eventually lead to the addition of new agents into the regimen for patients with extensive disease.

Thus, progress has been made which should result in improvement in current approaches to management.

Proposed Course:

At the present time, patients are being accrued on the two pilot protocols After 10 patients have been accrued on each, the results will be assessed and a determination made as to whether to develop a new primary protocol, or to continue for a further limited period to accrue patients on the pilot protocols.

Publications:

1. Magrath, I.T., and Sariban, E.: Clinical Features of Burkitt's Lymphoma in the USA. Proceedings. Burkitt's Lymphoma - A Human Cancer Model. IARC Publications, in press.

2. Magrath, I.T., Sariban, E., and Edwards, B. Characteristics and results of treatment of non-Hodgkin's lymphoma in young people - experience of the Pediatric Branch, NCI, USA. Proceedings of the Conference on Malignant Lymphomas, in press.

3. Ziegler, J.L., Bragg, K., Abrams, D., Becksted, J., Cogan, M., Volberding, P., Baer, D., Wilkinson, L., Rosenbaum, E, Grant, K., Silverberg, I., Magrath, I.: High Grade Non-Hodgkin's Lymphoma in Patients with AIDS. Ann. N.Y. Acad. Sci. 437. 412-419, 1984.

4. DeChristoforo, R., and Magrath, I.T.. Oxazaphosphorine urotoxicity its mechanism and prevention. Antineoplastic Drugs 2: 4-5, 1984.

5. Magrath, I., Cossman, J., Benjamin, D., Sieverts, H., and Triche, T.: Biological Features of Pediatric Non-Hodgkin's Lymphoma. Ford, R.J., Fuller, L., and Hagemeister, F.B. (Eds.): Proceedings New Perspectives in Human Lymphoma. Raven Press, New York, 1984, pp. 201-212.

6. Magrath, I.T.. The management of Burkitt's lymphoma. In Gellis, S.S. and Kagan, B.M. (eds.): Current Pediatric Therapy. 12th Edition, W.B. Saunders Company, Philadelphia, in press.

7. Bates, S., McKeever, P., Masur, H., Levens, D., Macher, A., Armstrong, G., and Magrath, I.T.. Myelopathy following intrathecal chemotherapy in a patient with extensive Burkitt's lymphoma and altered immune status. Am J Med 78: 697-699, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06816-02 PB

PERIOD COVERED

October 1, 1984, to September 30, 1985

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

Studies of the Nature, Measurement and Management of Pain in Children with Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Miser	Visiting Fellow	PB, NCI
Others:	P. Pizzo	Chief; Head, Infect. Dis. Sec.	PB, NCI
	J. Miser	Expert	PB, NCI
	A. Chang	Senior Investigator	SB, NCI
	R. Wesley	Senior Staff	BR, NCI
	D. Poplack	Senior Investigator	PB, NCI

COOPERATING UNITS *(if any)*

Pharmacy Dept. CC (R. Greene, P.K. Narang); Neurol. & Anesth. Br. NIDR (R. Gracely); Developmental Human Genetics Br. NICHD (A. Mukherjee); Dept. of Rehab. CC (J. Hicks, M. Lampert, C. McGarvey); (continued next page)

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	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.)*

Research involving children with cancer experiencing pain is centered in three areas: clinical evaluation, descriptive studies, and therapy.

For clinical evaluation, 3 different modalities of pain measurement viz. a visual analogue scale, a verbal descriptor scale, and a picture face scale are being compared to evaluate their feasibility of administration and reliability in children of all ages experiencing acute or chronic pain. A cross-modality matching method for pain mearsurement is also being developed. Ongoing descriptive studies consist of (1) The prospective study of the predictive factors and nature of phantom limb pain and sensations in patients undergoing amputation and (2) The study of the prevalence and nature of pain in a childhood cancer population at initial presentation. A recently completed study of the etiology, prevalence and nature of pain in our entire patient population has demonstrated that approximately half of the in-patients and one-fourth of the outpatients are experencing pain at any given time, which was therapy-related rather than tumor-related in the majority of patients. Therapeutic studies in progress are: (1) Study of the efficacy and kinetics of fentanyl given by continuous intravenous infusion or transdermally in patients with malignancy who are experiencing pain and (2) Study of the use of nitrous oxide for children with malignancy undergoing painful procedures. A recently completely study has demonstrated the efficacy, kinetics and toxicity of morphine administered by a continuous intravenous or subcutaneous infusion to children and young adults with malignancy who are experiencing moderate to severe pain of various etiologies. Changes in plasma and red cell beta-endorphin occurring during intensive narcotic administration for severe pain are also being analyzed.

PHS 6040 (Rev. 1/84) GPO 914-918

Cooperating Units (continued):

Medicine Dept. CC (C. Natanson); Dept. of Anesth. CC (S. Jelenick); Sec. of Clinical Brain Research NIAAA (J. Johnson); Cancer Nursing Service CC (J. McCalla); Istituto Giannina Gaslini, Genoa, Italy (L. Massimo).

Objectives:

Clinical Evaluation

1. To develop and test pain measurement modalities in children.

Descriptive

1. To assess the predictive factors and nature of phantom limb pain and sensations in patients with malignancy undergoing amputation.

2. To assess the prevalence and nature of pain in a childhood cancer population at initial presentation.

3. To assess the overall prevalence, etiology and severity of pain in a pediatric cancer population.

4. To assess the value of art in the diagnosis and expression of pain in children with malignancy.

Therapeutic

1. To study the efficacy and kinetics of continuous narcotic infusions in children with cancer experiencing moderate or severe pain.

2. To study the use of nitrous oxide for children with cancer undergoing painful procedures.

3. To define dosage, efficacy and toxicity data for oral narcotics in pediatric cancer patients experiencing pain.

Methods and Major Findings:

Clinical Evaluation

Three different modalities of pain measurement viz. a visual analogue scale, a verbal descriptor scale, and a picture face scale are being completed by child, parent and physician on a serial basis to evaluate their sensitivity and reliability in pain measurement. Thus far, approximately 150 children have been studied. Other pain measurement modalities, e.g., cross modality matching methods, are being explored.

Descriptive Studies

1. The nature and duration of phantom pain and sensations are being studied prospectively in patients undergoing amputation; pre-operative measures of depression, anxiety, locus of control, pain and family environment, and intraoperative technique are being analyzed, and predictive factors sought. The study opened in February 1984, and data has been gathered on 14 patients thus far.

2. The epidemiologic features of pain in children with malignancy at initial presentation, in particular the presence, duration, severity and description of pain, is being evaluated by interview at presentation to NCI, and the duration of pain following the beginning of definitive cancer therapy is determined. Thus, far, approximately 95 patients have been evaluated on this study, and data is currently being analyzed. This study is also being performed by collaborators at Istituto Giannina Gaslini, Genoa, Italy.

3. During tha past year, the prevalence of pain in our in-patient and out-patient populations was assessed. The prevalence, nature, severity, etiology and functional result of pain in each patient seen in the Pediatric Branch on 10 separate days was assessed. A total of 101 in-patient days and 195 clinic visits in 139 patients were studied. Approximately half the in-patients and one-fourth of the out-patients were found to be experiencing pain; therapy-related pain predominated in both patient populations. Overall pain control was good with the median visual analogue scale score being 26mm or less on a 0-100mm scale.

4. Under the direction of Ms. Ellen Turok, Art Therapy Master's student, George Washington University, Pediatric Branch patients are asked to draw two pictures of their choice, and one or two pictures to express the etiology and nature of pain they are, or have been, experencing. The paintings will be analyzed for frequency of individual colors used to describe pain, frequency of the various sources of pain considered to be most severe by this population, and descriptive analysis. Forty-five of a projected 60 patients have thus far been studied since the Spring of 1984. An age and sex matched control cohort of normal children is being assembled for study. Analysis will be performed when the study is complete.

Therapeutic Studies:

1. A study of the efficacy and kinetics of continuous intravenous or subcutaneous infusions of morphine in children with severe pain inadequately relieved by frequent oral or parenteral narcotic boluses has recently been completed. In this study, the morphine dose was titrated to give each patient good to excellent pain control. Pain control was judged by the investigator's evaluation on a 0-100mm visual analogue scale to be excellent (0-20mm), good (21-35mm), fair (36-50mm), borderline (51-65mm), or poor (>65mm). Results are outlined in the table.

	Tumor Pain	Mucositis Pain
Patient No.	10	16
No. of Courses	13	17
Age Range	4-25 yrs.	11-25 yrs.
Median MS Dose	0.2mg/kg/hr	0.11mg/kg/hr
MS Dose Range*	0.04-31.3mg/kg/hr	0.03-0.29mg/kg/hr
Median Duration of Infusion	14 days	8 days
Length of Infusion (days)	2-154 days	0.75-26days
Control: excellent	3	4
good	7	6
good to fair/fair	3	4
fair to borderline	0	1
borderline	0	1
poor	0	1

*Morphine dose range does not include the initial 24-36 hours of dose escalation or the final 24-36 hours of dose tapering where appropriate.

In general, this was found to be a very effective means of pain control for this patient group. Dose-limiting respiratory depression to < 12/min occurred in 4 patients (including one respiratory arrest), all of whom had severe oral mucositis and had recently undergone intensive chemotherapy, total body irradiation, and autologous bone marrow re-infusion. This finding, and the observation that 2 of 3 patients receiving concomitant morphine and isophosphamide infusions appeared to demonstrate central nervous toxicity, might indicate a clinically important interaction between narcotics and other agents within the central nervous system.

In collaboration with NICHD (Dr. A. Mukherjee) red cell and plasma β-endorphin levels have been analyzed in patients undergoing autologous bone marrow transplantation, so that changes could be followed in patients experiencing pain progressing from the pain-free state, through mild to moderate or severe pain from oral mucositis requiring intensive narcotic management, to the pain-free again following healing of the oral membranes. Results are presently being analyzed, but it appears that a reversal of the normal red cell: plasma ratio (approx. 2:1) occurs at the onset of pain, even before the initiation of narcotic therapy, and that this ratio returns towards normal with healing of the painful lesions. This finding has not been previously reported; the significance is speculative at present.

Preliminary evaluation of the kinetic data (R. Greene, PDS, Pharmacy Dept., CC) shows that the plasma morphine concentration is directly correlated with the given dose of morphine per hour, indicating that the concept reported by other investigators of a definable "analgesic plasma morphine concentration" is incorrect. The median terminal half-life studies in 7 patients was 2.1 ± 1.4hrs. with a range of 0.25-4.3hrs. Five estimates of the CSF: plasma morphine ratio were made in 4 patients, showing a ratio ± S.D. of 0.73 ± 0.22. To consolidate this work, detailed kinetic data in the rhesus monkey model is now being accrued.

2. In July 1984, a study examining the efficacy, toxicity and kinetics of fentanyl given by continuous intravenous or transdermal infusion to patients experiencing moderate or severe pain was initiated. Fentanyl was chosen because its low dose requirement and physical properties are particularly suited to transdermal delivery, the major goal of this study. Thus far, analgesic efficacy and kinetics have been studied during 16 infusions in 10 patients, and a further 4 patients have been evaluable for kinetics alone during fentanyl infusions given for sedation during artificial vertilation. Two patients have been evaluable for transdermal delivery. While fentanyl appears to be an excellent analgesic when given by continuous infusion, further experience must be accrued before definitive conclusions as to its clinical role can be made.

3. The study of the use of nitrous oxide in children with malignancy undergoing painful procedures was originally designed as a double-blind cross-over study using 70% nitrous oxide. However, 3 of the 4 patients entered on the pilot nitrous oxide arm became restless and agitated during the procedure (Stage II anesthesia) although all 4 reported improved pain control. It was therefore decided to close this pilot study, and a patient-controlled 50% nitrous oxide 50% oxygen unit (Nitronox) is at present being evaluated, with a view to using this machine in a broader study of analgesia in children undergoing painful procedures.

   Thus far, 26 children and young adults undergoing painful diagnostic procedures have been studied. Sixteen of these had previously experienced the same painful procedure, of whom 13 indicated improved pain control with nitrous oxide, one indicated no difference, and 2 were considered inevaluable because of a change in clinical status between the two procedures. Toxicity has been minimal (nausea and vomiting in one patient each) and this method of analgesia appears worthy of further study. However, room air monitoring during two procedures documented extremely high nitrous oxide levels, and this is being further evaluated before this study is continued further.

4. Although the oral narcotics morphine and methadone are widely used in oncologic practice for pain control, dosage, efficacy and toxicity data and guidelines for use are extremely sparse for the pediatric age range. Whenever possible, therefore, Pediatric Branch patients requiring a stronger narcotic than codeine have been treated with either oral methadone or oral morphine and clinical data gathered.

Methadone: 22 courses of oral methadone were given to 19 children, of which 21 courses gave adequate pain control for periods of 5-267 days (median 24 days). In 16 courses, methadone was continued until death or until resolution of pain, whereas in 5 courses a change to parenteral narcotics was ultimately required. Toxicity was minimal, and it was concluded that a safe starting dose for oral methadone was 0.1mg/kg given every 4 hours, or the equivalent daily dose given less frequently, with escalation as required to achieve and maintain adequate pain control.

Morphine: data is being gathered, but is thus far too sparse for adequate analysis or comment.

Significance to Biomedical Research and the Program of the Institute:

Establishment of reliable pain measurement techniques in children will permit many valuable and more sophisticated pain studies in the future, and the current and future epidemiologic studies will aid in defining the major etiologic categories of pain for future study.

The therapeutic studies will yield efficacy data which will be generally applicable to pediatric oncology, and form the basis of future studies (see Proposed Course).

Proposed Course:

1. Clinical - All studies are continuing as outlined.

   Because of the high incidence of painful oral mucositis in our patient population and the morbidity it causes, the Pediatric Branch and Clinical Pain Section, NIDR will shortly open a double-blind randomized study to evaluate the efficacy of topical ibuprofen, a prostaglandin inhibitor, in providing pain control for these patients.

2. Laboratory

   One of the major issues facing those involved in pain management is the development of extreme tolerance to narcotic effect in a few patients who, therefore require massive doses of narcotics (up to 1.5 gms morphine per hour in our recent study) to control pain. Investigators at NIMH (Dr. E. Costa et al.) have recently isolated an inhibitory substance in rodents which appears to be secreted in large amounts from the central nervous system in response to narcotic therapy. The clinical implication of this finding for man is as yet unknown, although it is postulated that this may play a role in the development of narcotic tolerance. In the near future we plan to open a collaborative project with Dr. Costa to evaluate the significance of this finding in cancer patients receiving narcotics. This project will be especially relevant as the cholecystokinin - inhibitor proglumide has recently been advocated as worthy of clinical study in reversing and preventing tolerance to narcotics, proglumide also reversing the effect of the inhibitory substance isolated by Dr. Costa.

3. Publications

None

ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

OCTOBER 1, 1984 - SEPTEMBER 30, 1985

The Radiation Oncology Branch (ROB) of the National Cancer Institute now entered its third year in its new clinical facilities, which were occupied in the summer of 1982. Laboratory functions are still in transition, having been moved to the 1B space while B3, the original facility, is being renovated. This B3 renovation space will ultimately house the laboratory program.

The three major goals of the ROB continue unchanged: 1) major emphasis on clinical trials of a combined modality nature, predominantly collaborative with other clinical branches; 2) strong radiation-biology program with heavy emphasis on basic science, radiologic physics and questions of clinical relevance; 3) a training program in radiation therapy, equivalent in stature to the programs of training in medical, surgical and pediatric branches within the NCI.

All of these goals are being met. The clinical program appears strong within the confines of the diseases being studied by the various branches of the COP. This qualification is made because of the limited number of surgical subspecialties available within the NCI. The laboratory program has become internationally renowned very quickly; studies carried out within the ROB on radiation, experimental chemotherapy, radiation modifying agents, hyperthermia and phototherapy receive the attention of investigators all over the world. In addition, a growing program has emerged on radioimmunoglobulin, with interest aimed at treatment. Most of the present work has focused on human CFUC and human tumor cell lines, in collaboration with other branches.

Concerning the training program, approval has been obtained from the AMA Residency Review Committee for the Uniformed Services University of Health Sciences, working through the NCI, as well as Walter Reed Army Medical Center and the National Naval Medical Center in Bethesda. We have a three year program approved for the residency in Radiation Oncology, directed by Dr. Eli Glatstein. Half of the time is spent within the NCI and half within the military complex. This integrated program is required because of the complementary nature of the clinical material at the various hospitals, with GYN, head and neck and GU cancers in abundance at the military hospitals, whereas they are virtually completely lacking within the NCI. Each year we take four individuals for training, two of the positions being reserved for military personnel.

The clinical program within the ROB is centered on combined modality studies. Most of these are in collaboration with other branches. The most important of these are combined modality studies on small cell carcinoma of the lung, and on mycosis fungoides, both of which are in collaboration with the NCI-Navy Medical Oncology Branch. The findings

in small cell carcinoma of the lung are strongly suggestive of benefit of combined modality treatment for limited stage patients over chemotherapy alone. A number of interesting observations have been made which will be reported by the Navy Medical Oncology Branch. There are also important collaborative studies going on with the Surgery Branch in the soft tissue sarcomas, and also with the Pediatric Oncology Branch in areas of pediatric sarcomas. These latter pediatric studies appear to be extraordinarily promising, and if they continue to hold up they will represent a major step forward in the management of these patients. Again, the early findings will be reported by the POB rather than the ROB. There are also studies with the Medicine Branch in lymphomas and Hodgkin's disease.

Primary ROB studies center around intraoperative radiation therapy. A new treatment base/operating room has been opened, and the Microtron has been used for intraoperative therapy. Large single doses of electron beam treatment are applied intraoperatively to the tumor bed with critical normal viscera moved out of the way. There is also a dog program going on concurrently to determine tolerance levels. These programs represent major integration of surgery and radiation therapy in a cooperative way. Originally there were studies on pancreatic carcinoma, but a moratorium has been declared simply because those patients required enormous inpatient resources, and with the crunch on beds that has been in existence here, it was decided to eliminate that protocol for the time being. Gastric cancer, retroperitoneal sarcomas and other retroperitoneal problems are under investigation on randomized studies; in addition, a study has just begun on locally advanced lung cancer utilizing intraoperative radiotherapy. This latter protocol on the lung could not begin until the surgery and radiation could be delivered in the same room. At the present time, we can say that the treatment appears to be safe but its efficacy remains to be established.

Another major ROB study centers on Stage I and II breast cancer. In this randomized study, radical surgery is compared to definitive irradiation with preservation of the breasts following a lumpectomy. This study was originally organized by Dr. Allen Lichter, but following his departure to the University of Michigan, it is now headed by Dr. Peggy Findlay. There are now over 190 patients randomized, despite the very difficult randomization. In the first six years of this study, there is no obvious superiority of either arm, suggesting that the long-term results of treatment will be comparable. This study differs from the studies of Dr. Fisher of the NSABP in that the surgical excision makes no attempt to make the surgical margins negative but just simply remove the lump. Cosmesis is a major endpoint in addition to survival and freedom from relapse. In addition, the protocol is also open to patients who have masses up to 5 cm. This makes this approach of breast preservation applicable to the vast majority of patients who present with breast cancer initially. All patients with positive nodes receive adjuvant chemotherapy, regardless of the primary treatment.

Another area of intense clinical investigation has been that of radiosensitizers, with special attention directed to halogenated pyrimidines, specifically BUdR and IUdR. BUdR Phase I studies have been completed and unequivocal radiosensitization was demonstrated in human cells and

human patients. However, hematologic and especially cutaneous toxicity due to photosensitivity limited the applicability of intravenous BUdR. We were able to demonstrate here that IUdR was an equivalent to radiosensitizer but far less of a photosensitizer. Consequently, we have now completed our first Phase I study of intravenous IUdR with special attention in unresectable sarcomas and gliomas. The glioma information is difficult to interpret thus far, but we have seen some striking regressions of unresectable sarcomas and, indeed, have five unresectable masses that have gone away completely with IUdR and radiation in a Phase I study. We have additionally planned Phase I investigations to carry out with this compound, mostly seeing if it can be manipulated by having other agents which eliminate de novo thymidine synthesis, thereby driving IUdR further into the tumor cells. Ultimately, it is our aim to take patients with unresectable sarcomas and gliomas and randomize them to receive the radiosensitizer program or not, in conjunction with twice a day radiation fractionation. Under the direction of Dr. Jan van de Geijn our CT scanning has been fully incorporated into our radiotherapeutic treatment planning. Virtually all patients treated with curative intent are scanned in the treatment position and computerized treatment plans are routinely generated, superimposed on CT cross-sections. The program allows for adequate dose calculations, even accounting for tissue inhomogeneities and blocks within the radiation field.

Our long range plans still center highly upon intraoperative therapy as an investigational avenue. In addition to an opportunity to combine sensitizing drugs and hyperthermia in the treatment of abdominal neoplasms, it offers precise localization of tumor treatment and the ability to eliminate critical normal tissues, or at least protect them from high doses. Even though benefit has not clearly been established thus far, we plan to continue investigation in this area. We also hope to begin a study on bladder cancer in the near future, emphasizing radioactive implantation in an effort to save the bladder for invasive cancers, and also phototherapy for dealing with superficial cancers.

A final clinical program of interest is interstitial implantation for patients who have relatively small gliomas. In this study, selected patients with lesions that are appropriately located will undergo radioactive interstitial implantation therapy inserted stereotactically in conjunction with the Surgical Neurology Branch of the NINCDS.

In the laboratory, our major emphasis has been on mechanisms of sensitization and protection, resulting from radiation modifiers, and also investigation of the mechanism of the action of several different chemotherapeutic agents. Our interests have centered on sulfhydryl compounds, especially glutathione, its relationship to cell killing or protection, either by radiation or chemotherapy. Additional work has gone on in heat shock proteins and the characterization of human tumor cell lines (in conjunction with other branches). An embryonic program in molecular biology is also going on through Dr. Fornace, whose chief work is aimed at trying to clone heat shock protein genes, and also attempting to clone genes involved in radiosensitivity. Radioimmunoglobulin work has been going on through Dr. Gansow. His work is focused on pursuits of various chelates and being able to attach the chelate with radioactive heavy metals

to immunoglobulin. We are especially interested in alpha emitters as a potential means of delivering radiotherapy. There is also a large dog program run in conjunction with the Surgery Branch, whereby we are investigating the tolerance of various organs to intraoperative radiation therapy.

The major lab observations in the past year center on (1) the demonstration that cells which are pleiotropically resistant to drugs have, at least in most cases, no resistance to radiation, (2) the remarkable efficacy of phototherapy to cure mice with advanced ovarian cancer in the abdomen, and (3) lympholysis in animals treated with phototherapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 00650-30 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Zabell	Senior Investigator	ROB, NCI
Others:	T. Kinsella	Deputy Branch Chief	ROB, NCI
	P. Findlay	Senior Investigator	ROB, NCI
	B. Kelly	Chief, Rad. Therapy Tech.	ROB, NCI
	A. Zola	Rad. Therapy Tech.	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	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 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.

GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

J. Rowland	Nurse Specialist	CNS, CC
R. Smith	Nurse Specialist	CNS, CC
C. Gorrell	Nurse Specialist	CNS, CC

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 concomitant therapies.

Major Findings

There were 700 patients seen in formal consultation and an additional 400 (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., Mitchell, J.B., McPherson, S.J., Miser, J., Triche, T., and Glatstein, E.: In vitro radiation studies on Ewing's sarcoma cell lines and human bone marrow: Application to the clinical use of total body irradiation. Int. J. Radiat. Oncol. Biol. Phys. 10: 1005-1011, 1984.

2. Kinsella, T.J., Mitchell, M.B., Russo, A., Morstyn, G., and Glatstein, E.: The use of halogenated thymidine analogs as clinical radiosensitizers: Rationale, current status, and future prospects. Int. J. Radiat. Oncol. Biol. Phys. 10: 1399-1406, 1984.

3. Kinsella, T.J., Mitchell, J.B., Russo, A., Morstyn, G., Hsu, S-M., Rowland, J., and Glatstein, E.: Continuous intravenous infusions of bromodeoxyuridine (BUdR) as a clinical radiosensitizer. J. of Clin. Oncol. 2: 1144-1150, 1984.

4. Tester, W., Kinsella, T.J., Waller, B., Makuch, R., Kelley, P.A., Glatstein, E., and DeVita, V.T.: Second malignant neoplasms complicating Hodgkin's disease: The National Cancer Institute experience. J. Clin. Oncol. 2: 762-769, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 00684-30 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	F. Harrington	Biomed. Engineering Tech.	ROB, NCI
	R. W. Miller	Health Physicist	ROB, NCI
	J. Doolittle	Electronic Technician	ROB, NCI
	R. Creecy	Computer Specialist	ROB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	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.)*

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 are irradiated for radiobiology experiments. One current involvement is in I-125 dosimetry related to monoclonal antibody studies.

Considerable efforts have been made and are continuing in assisting both the Radiobiology Section and Radioimmuno Chemistry Section in regard to computerization and automation projects.

Project Description

Objectives: To provide radiation physics expertise and equipment to researchers involved with radiobiological projects.

Methods Employed

Assistance with dosimetric problems and radiation quality assurance continues to be given to radiobiologists in irradiating cells, tissue culture, mice, rats, and dogs using both linear accelerators and the Cobalt-60 and 250 kvp x-ray unit. Many devices have been fabricated to facilitate the irradiation of various biological specimen, including both in vitro and in vivo specimen. Extensive assistance is given in the area of automation and computerized data processing.

Major Findings

Cells, tissue cultures and animals are reliably irradiated using the Cobalt-60 unit and the Clinac 20. Basic methodology was developed as well as the linear accelerators.

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. High technology data processing greatly facilitates the evaluation of ongoing research.

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 06310-06 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. A. Findlay Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	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 study is to determine whether a breast-conserving treatment program of limited surgery and definitive radiation offers equivalent local control and survival to mastectomy in patients with early stage breast cancer. After work-up confirms localized disease, patients are randomly assigned to either primary surgery or primary irradiation. Patients treated with mastectomy are offered breast reconstruction. All patients undergo complete axillary node removal; those patients with pathologically positive lymph nodes receive chemotherapy.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

H. McDonald	Senior Surgeon	SB, NCI
D. Danforth	Senior Investigator	SB, NCI
M. Lippman	Head, Med. Brst. Cancer Sec.	MB, NCI
W. Schain	Clinical Care Consultant	Rehab. Med., CC
N. L. Gerber	Chief, Rehab. Medicine	Rehab. Med., CC
C. R. Gorrell	Cancer Nursing Specialist	CNS, CC
T. d'Angelo	Cancer Nursing Specialist	CNS, CC
C. Wood	Physical Therapist	Rehab. Med., CC
R. Makuch	Head, Biostat. and Data Mgmt. Sec.	BDMS, NCI

Objectives: If survival and recurrence data obtained with treatment that preserves a cosmetically acceptable breast are comparable to those obtained with radical surgical procedures, such treatment will probably be more acceptable to most women with localized breast cancer. Availability of an effective alternative to mastectomy may encourage woman to seek medical attention with earlier, hence more curable, cancers. The cosmetic and functional results of local treatment will be carefully evaluated. The psychological, sexual and sociological impact of mastectomy vs. lumpectomy and radiation will be noted. Ability to combine aggressive chemotherapy with either local treatment in node positive patients will also be assessed.

Methods Employed

Patients with stage T1-T2, N0-N1, M0 primary untreated breast cancer are candidates for the study. They will be randomized to receive either lumpectomy, axillary dissection and radiation therapy or total mastectomy with axillary node dissection. Patients receiving mastectomy will be offered breast reconstruction. Patients with pathologically positive lymph nodes wil receive chemotherapy.

Major Findings

This study has been active for 72 months. Currently 191 patients have been entered, of whom 95 have randomized to mastectomy, and 96 to radiation. Median follow-up is 32 months. No differences have been seen as yet between the two arms in terms of overall recurrence, local recurrence, or survival.

Significance to Biomedical Research and the Program of the Institute

The study is intended to determine whether breast conserving treatment (lumpectomy and radiation therapy) is equivalent to radical surgery as treatment for early stage breast cancer. If this is the case, this treatment option should

be much more acceptable to the majority of women. It is conceivable that the availability of such non-mutilizing treatment would encourage women to seek medical attention sooner, and therefore present with more curable disease.

Proposed Course

The study is ongoing.

Publications

1. Fraass, B.A., Roberson, P.L., and Lichter, A.S.: Dose to the contralateral breast due to primary breast irradiation. Int. J. Radiat. Oncol. Biol. Phys. 11:485-497, 1985.

2. Findlay, P.A., Lippman, M.E., Danforth, Jr., D., McDonald, H., d'Angelo, T., Gorrell, C.R., Gerber, N.L., Schain, W., and Lichter, A.S.: Mastectomy vs. radiotherapy as treatment for stage I-II breast cancer: A prospective randomized trial at the National Cancer Institute. World J. Surgery (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06320-06 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Response of Mammalian Cells to Chemotherapy Drugs

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	A. Russo	Clinical Associate	ROB, NCI
Others:	J. B. Mitchell	Radiobiologist	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if eny)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland, 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4	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.)*

Several chemotherapy agents with proven utility, e.g., Anthracylines, Bleomycins, and noble metal derivatives are being studied. The detoxification mechanisms, modification of cellular response by biochemical manipulation of intracellular redox status, and oxygen metabolism, in sensitive and resistant cells are of interest. Deleterious species produced by the antineoplastic drugs and cellular response to these species, as well as thiol compounds, and their metabolic interactions with the drugs, and labile species produced by the drugs are being examined. It has been demonstrated that depletion of cellular glutathione (GSH) by inhibitors of GSH synthesis sensitize cells to Adriamycin and Bleomycin while GSH elevation provides protection. Recently, we have shown that modulation of GSH has a profound effect on Neocarzinostatin biologic activity. Rescue of cells from chemotherapeutic treatment is being studied by utilizing compounds newly synthesized within the laboratory. Studies have begun on isolation and modulation of the genes responsible for GSH synthesis. Modulation of chemotherapeutic response will be studied either by substrate feed forward mechanisms or modulation of enzymic response by amplification of gene products by altering the promoter region.

Project Description

Objective: The objective of this project is to determine the importance of biochemical modulation of selected cellular redox compounds to drug cytotoxicity.

Methods Employed

In vitro cell culture will be exposed to the various agents mentioned above and assayed for cellular reproductive integrity using conventional tissue culture techniques. Both thymic and athymic mice are available to study the in vivo effects of modulation. Standard biochemical assays will be used to access biochemical modulations. Standard molecular biologic techniques are/will be employed. Standard synthetic organic techniques are used.

Major Findings

Dose response curves for Adriamycin, Bleomycin, Platinates, and Melphalan have been determined. Cell killing may be enhanced for Adriamycin, Platinum, and Bleomycin after removal of GSH from cells by either of two methods. Protection can be afforded if GSH is elevated by several means. Neocarcinostatin action can be blocked by removal of GSH.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of drug-induced resistance and cytotoxicity and are focused toward the potential of selective drug toxicity of tumor versus normal tissue via biochemical manipulation of the cellular redox cycle.

Proposed Course

Dose response curves are being generated for a variety of chemotherapy drugs and the role of intracellular chemoprotective compounds are being studied.

Publications

1. Russo, A., Mitchell, J.B., McPherson, S.J., and Friedman, N.: Alteration of bleomycin cytotoxicity by glutathione depletion or elevation. Int. J. Rad. Oncol. Biol. Phys. 10: 1675-1678, 1984.

2. DeGraff, W., Russo, A., and Mitchell, J.B.: Glutathione depletion greatly reduced neocarzinostatin cytotoxicity in V79 cells. J. Biol. Chem. (in press).

3. Russo, A., and Mitchell, J.B.: Potentiation and protection of adriamycin cytotoxicity by cellular glutathione modulation. Can. Treat. Rep. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06321-06 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. B. Mitchell	Radiobiologist	ROB, NCI
Others:	A. Russo	Clinical Associate	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	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.)*

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). The indirect effects of GSH removal will be assessed by high performance liquid chromatography and gel electrophoresis. In addition, Nitroimidazole radiosensitizers such as SR-2508 will be studied as hypoxic sensitizers as a function of intracellular Sulphydryl concentrations. These studies should provide a better understanding of the effects of radiation to aerated and hypoxic cells. Depletion of GSH by Buthionine Sulfoximine (BSO) enhanced 2508 sensitization while GSH elevation by Oxothiazobidine (OTZ) provided protection of 2508 hypoxic sensitization. Human tumor cell lines were found to be high in cellular GSH and thus less responsive to 2508 sensitization than hamster cell lines. These data may provide explanations for the failure of Nitroimidazoles in the clinic.

Project Description

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 radiation, either under aerated or hypoxic conditions. Oxygen enhancement radios (OER) will be determined. Cellular GSH will be measured by spectrophotometric methods and cellular levels altered by drugs that specifically modulate the GSH cycle.

Major Findings

GSH depletion to values of < 5% does not appreciably lower the OER. Diethyl Maleate treated cells, however, exhibited more hypoxic sensitization than did Buthionine Sulfoximine treated cells. Cellular GSH levels may be evaluated by Oxothiazolidine Carboxylate. GSH levels govern SR-2508 hypoxic sensitization. Human tumor cell lines are high in GSH and do not respond to 2508 at clinically achievable doses.

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 cell systems, explore effects of low and elevated levels of GSH on the OER alone in combination with SR-2508.

Publications

1. Morstyn, G., Russo, A., Carney, D.N., Karawya, E., Wilson, S.H., and Mitchell, J.B.: Heterogeneity in the radiation survival curves and biochemical properties of human lung cancer cell lines. J. N. C. I. 73: 801-807, 1984.

2. Russo, A. and Mitchell, J.B.: Radiation response of Chinese hamster cells after elevation of intracellular glutathione levels. Int. J. Rad. Oncol. Biol. Phys. 10: 1243-1247, 1984.

3. Russo, A., Mitchell, J.B., Kinsella, T.J., Morstyn, G., and Glatstein, E.: Determinants of radiosensitivity. Seminars in Oncology (in press).

4. Mitchell, J.B., Morstyn, G., Russo, A., and Carney, D.N.: The in vitro radiobiology of human lung cancer. Cancer Treatment Symposium (in press).

5. Phillips, T.L., Mitchell, J.B., DeGraff, W.G., Russo, A., Albright, N. and Rajpal, R.: Modification of SR-2508 sensitization in hypoxic V-79 cells by manipulation of glutathione levels. Radiation Research (in press).

6. Biaglow, J.E., Russo, A., Mitchell, J.B., and Varnes, M.: Factors influencing thiol oxidation and peroxide production in cell culture medium. Rad. Res. 100: 298-312, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06329-05 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Clinical Radiation Physics Service

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigetor.) (Name, title, laboretory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. W. Miller	Health Physicist	ROB, NCI
	A. Wolbarst	Radiation Physicist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI
	R. Creecy	Computer Specialist	ROB, NCI
	B. Arora Chin	Radiation Physicist	ROB, NCI
	K. Yeakel	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if eny)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	2.5	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 spece 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, continues to be improved for the three Varian accelerators (Clinacs 4, 18, and 20). A new quality assurance detector using five ionization chambers is being integrated into the QA program. This device will consolidate output, energy and symmetry checks and will be useful for electrons as well as photons.

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, Clinac 18 and Clinac 20 linear accelerators are now fully operational. Preparatory work for total skin and total body irradiation has been completed. Sufficient dosimetric work has been done to allow total body irradiation (TBI) and intraoperative radiotherapy to be performed

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on both the Clinac 20 and the Clinac 18. Work is progressing on the dosimetry necessary to conduct intraoperative therapy at the MM-22 Microtron.

4. Major innovative work has been completed on the Microtron electron accelerator, used mainly for intraoperative irradiation. A special adaptation of an existing operating table has been designed for use in the intraoperative program. Construction has been completed successfully.

5. The computer programs for clinical radiation treatment planning have been further extended in all three subfields: external beam, point-source, intracavity line-source, and interstitial radioactive seeds radiation fields.

6. Extension of the clinical usefulness of the VAX-750 Computer System is continuing. Considerable time and effort is being invested in improving and extending programs to the new system.

7. Supporting patient treatment and evaluation of clinical research.

Project Description

Objectives: To ensure high quality physics support for radiotherapy.

Methods Employed

A new highly efficient system is continually used for monitoring the performance of three linear accelerators, the Microtron, the simulator, and the CT scanner. Special mechanical supports and measuring devices are used 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. Computer-assisted treatment planning has been extended. Considerable efforts have been invested in the dosimetry of intraoperative, total-body, and total-skin radiotherapy. The clinical use of the Microtron continues to require the design and development of new accessories especially in regard to the intraoperative program. The CT scanner provides vastly improved quantitative data and superior image resolution, allowing thinner slices and more readily accommodates patients in the treatment position. This in turn allows much higher quality treatment planning.

The Section continues to provide non-routine in-vivo patient dosimetry by means of thermoluminescent dosimeters and diodes. Such ad hoc measurements are usually concerned with doses to sensitive organs and sometimes crucial to the continuation of a treatment technique.

Major Findings

The use of beam monitoring jigs enables daily monitoring of output, beam flatness, symmetry, and alignment of light field and x-ray fields for all three linear accelerators. The method allows simple documentation of performance. This

close surveillance of beam quality has proved to be a vital factor in treatment reliability and quality. Our system has proved to be impressive to visitors. The dosimetry of photon beam total-body irradiation as well as that of total-skin electron beam irradiation for mycosis fungoides requires further attention, especially improving the treatment of hands and feet.

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 continues 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.

The development of a mechanical back projection system for reconstruction of the positions of objects in the body has been highly successful.

Over the reporting period, the Section has been severely handicapped by understaffing. Over one half year the regular complement of four physicists was down to two.

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. The CT scanner is now the principal source of patient data for treatment planning.

Proposed Course

1. Continuation of adaptation of the computer programs to the new radiation machines and full usage of the VAX system.

2. Special attention to the quality assurance aspects of the Microtron, currently under installation.

3. Introduction and integration of NMR imaging into treatment planning purposes.

Publications

1. van de Geijn, J., and Harrington, F.S.: A simple system for manual image reconstruction from pairs of x-ray films. Int. J. Rad. Oncol. Biol. Phys. 10: 2375-2379, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06330-05 RO

PERIOD COVERED
October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. W. Miller	Health Physicist	ROB, NCI
	R. Creecy	Computer Specialist	ROB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK *(Use standerd 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 new radiation field 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 variations 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 investigations include the generalization for irregular fields modified by irregular blocks for photon beams and electron beams, and the influence of inhomogeneities. New developments in the description of the along-the-ray distribution as well as the field size dependence of the beam profile are being implemented. 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. 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, using the concept of Net Fractional Depth Dose (NFD). The NFD formalism is currently being extended to the description of the influence of inhomogeneities, such as lung tissues.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

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 60Co to 18 MV x-rays. These formulations are currently being extended to inhomogeneities.

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. A new concept (the collimator function) is being investigated, for the generalized description of beam profiles.

3. Special attention has been paid to verification of the model for the local radiation machines and to irregular fields modified by irregular blocks. This work is continuing and will be extended to the 6MV and 21MV x-ray beams of the Microtron.

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. Data are now available for 60Co, 4, 6, 10, 15 and 18 MV x-rays.

It has been established that the concept is applicable to irregular ("blocked") fields as well. Preliminary results for electron beams are most promising. The validity for neutron beams has been confirmed by investigators at Fermilab. All of these results have been incorporated in a clinical treatment planning system. The work is continuing with emphasis on the photon beams and electron beams of the Microtron. A more generalized treatment of cross beam profiles is showing some marked improvement.

Presently, development is towards incorporation of a new inhomogeneity correction method, in addition to existing ones for use in comparative clinical studies.

Significance to Biomedical Research and 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., and Fraass, B.A.: The net fractional depth dose: A basis for a unified analytical description of the FDD, TAR, TMR and TPR. Med. Phys. 11: 784-793, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06331-05 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. W. Miller	Health Physicist	ROB, NCI
	R. Creecy	Computer Specialist	ROB, NCI
	A. Wolbarst	Radiation Physicist	ROB, NCI
	B. Arora Chin	Radiation Physicist	ROB, NCI
	K. Yeakel	Dosimetrist	ROB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	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.)*

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 Clinac 4, Clinac 8 and Clinac 20 linear accelerators. More work is to be done on the implementation of the Microtron with its 2 photon energies and 9 electron energies and some unusual technical options. The current capabilities include interactive simulation of most irradiation techniques, including the effect of most beam modifying devices. Transverse patient contours are overlaid on corresponding CT scans so that dose distribution can be related to the anatomy. Three of the four new radiation machines have been implemented for routine treatment planning. Work is continuing on optimization of most of our computer programs on the VAX-11/750 system, from the old PDP 11/70 system. This work is, as before, complicated by the need for continuing reliable routine support for the clinical treatment.

Great attention is continuing to be paid to versatility, completeness and quality of documentation, as well as exchangeability. Generalization has progressed considerably by the introduction of patient representation by a density grid.

Project Description

Objectives: To develop and implement a generalized system for computer-assisted radiation treatment simulation.

Methods Employed

The dose field model, originally developed elsewhere by the present principal investigator, was further developed and experimentally tested for the local radiation facilities. The theoretical model covers irregularly shaped beams as well as irregularly shaped shielding blocks. Work continues on the improvement of the correction for inhomogeneities, such as lung tissues, and on electron beam dosimetry. Considerable improvement has been achieved by the implementation of the Net Fractional Depth Dose Concept.

Most of the associated computer programs for the local PDP 11/70 system have now been transferred to the VAX 11/750 and extended. The facility developed enabling the computation and display of dose distributions in planes perpendicular to the respective beam axes needs further work. 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. Much improved are the interactive operational characteristics, but the efforts to expand and facilitate the interactive capabilities continue. The hard copy documentation has been much improved by the phasing-in of a laser printer.

A major extension of the interstitial therapy computer programs has been started, and completed to a considerable extent, by Dr. U. Rosenow during his sabbatical stay from 4-1-84 through 1-2-85.

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 further developed 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.

The capabilities of the programs for interstitial treatment planning are of vital importance to the brain implant study.

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. Further development of the Beam's Eye View option, also 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; this has been delayed by budgeting problems.

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.

5. Development and implementation of digital x-ray imaging in conjunction with computerized treatment planning; this has also been delayed by both budgeting and personnel problems.

Publications

1. van de Geijn, J., Fraass, B.A., Miller, R.W., and Creecy, R.H.: The projective beam model: An update on model and characteristic data. Proceedings of the VIII International Conference on the Use of Computers in Radiation Therapy. July 9-12, 1984, pp. 94-98.

2. van de Geijn, J., and Fraass, B.A.: The net fractional depth dose: A basis for a unified analytical description of FDD, TAR, TMR and TPR. Med. Phys. 11: 784-793, 1984.

3. van de Geijn, J., and Harrington, F.S.: A simple system for manual image reconstruction from pairs of x ray films. Int. J. Rad. Oncol. Biol. and Phys. 10: 2375-2379, 1984.

4. Glatstein, E., Lichter, A.S., Fraass, B.A., Kelley, B.A., and van de Geijn, J.: The image revolution and radiation oncology: Use of CT, ultrasound and NMR for localization, treatment planning and treatment delivery. Int. J. Rad. Oncol. Biol. Phys. 11: 299-314, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06333-05 RO

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Dosimetry of Total Skin Electron Irradiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. W. Miller	Health Physicist	ROB, NCI
	B. Arora Chin	Radiation Physicist	ROB, NCI

COOPERATING UNITS *(if eny)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.2	.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 has now been updated and implemented on the new Clinac 20 linear accelerator.

A study has been started to selectively and locally modify the fluence rate to allow for the locally higher dose rates to the hands and feet, which now require cumbersome shielding over part of the treatment course.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on Project:

R. Morton	Radiation Physicist	RRP, NCI

Objective: Quantify and improve whole-skin treatments received by patients with mycosis fungoides.

Methods Employed

The dosimetry system of the Clinac 20 accelerator has been substantially modified, allowing electron treatment to the total skin at a much higher dose rate than normally available. This modification makes routine patient treatments short enough to be practical. A sieve method is being developed enabling local modification of beam intensity without changing the energy.

Major Findings

Careful modifications to the dosimetry circuits of the accelerator have improved the dose rate response of the system, allowing patient treatment with a dose rate of 1600 rad/min at the isocenter. It is desirable to modify the beam intensity without decreasing the penetrating power, at the positions of the hands and feet.

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. Modification of the beam intensity at the hands and feet of MF patients would greatly reduce their discomfort during treatment.

Proposed Course

Work toward improving the dose distribution is continuing. Also necessary is the improvement of methods to protect hands and feet against excessive dose.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06348-04 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. W. Miller	Health Physicist	ROB, NCI
Others:	R. Creecy	Computer Specialist	ROB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.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 tha space provided.)*

The current linear source brachytherapy program has been adapted to run on the VAX-750 with a Tektronix 4105 display terminal. The source inventory has been expanded to include Cs-137 needles as well as tubes in anticipation of a new protocol for treating bladder cancer with implants. Current work centers on the simplification of the current point dose algorithm into a look-up table as a function of distance and angle. This will allow the implementation of the algorithm on an array processor and will permit the integration of the line-source program and the point-source program into a single brachytherapy program.

Project Description

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 Sievert Integral, elsewhere developed by Dr. van de Geijn.

2. Coordination of I/O methodology with separate and external beam therapy.

3. 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

Work towards unification with point source brachytherapy program.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06349-04 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Clinical Associate	ROB, NCI
Others:	J. Mitchell	Radiobiologist	ROB, NCI
	B. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI
	N. Friedman	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland, 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	5	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.)*

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 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. Continued effort to inter-relate oxidative stress and the biochemical induction of genetic materials center around GSH metabolism. There is also interest in the role that GSH has on maintaining the integrity of the membrane. Moreover, the role membrane damage and how this damage modifies intracellular GSH with subsequent genetic expression of heat shock proteins is being utilized to study possible explanations for cellular heat and drug resistance.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objective: 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 Finding

There is a relationship in elevation glutathione and the induction of thermotolerance. Thermotolerance may be prevented by lowering cellular GSH or preventing its synthesis and an alteration in the extent of heat shock proteins synthesized.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of how heat kills cells, which might provide a clearer means for clinical utilization of hyperthermia as a treatment modality.

Proposed Course

Continue studying the relationship of glutathione (a cellular reducing compound) and thermotolerance.

Publications

1. Mitchell, J.B., Russo, A.: Thiols, thiol depletion, and thermosensitivity. Rad. Res. 95: 471-485, 1983.

2. Russo, A., Mitchell, J.B., McPherson, S.J.: The effects of glutathione depletion of thermotolerance and heat stress protein synthesis. Brit. J. Cancer 49: 753-758, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06351-03 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Response of Mammalian Cells to Halogenated Pyrimidines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Nama, title, laboratory, and instituta affiliation)*

PI:	J. B. Mitchell	Radiobiologist	ROB, NCI
Others:	A. Russo	Clinical Associate	ROB, NCI
	T. Kinsella	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	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.)*

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 lead 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. Further studies have questioned the role of low dose rate irradiation with halogenated pyrimidines. Additionally, pilot studies have been initiated to determine if halogenated purines are incorporated and provide x-ray sensitization.

Project Description

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. To determine if halogenated purines are also incorporated into DNA and radiosensitize.

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. Standard cell survival techniques have been used for other mammalian cell systems.

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. Positive identification of cells in tumor and normal tissue that had incorporated BUdR and IUdR has been made using the monoclonal staining technique. 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. Low dose rate radiation is potentiated by BUdR/IUdR incorporation into cellular DNA. This observation has major clinical implications.

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 and the possible efficacy of combining implant therapy to the use of halogenated pyrimidines.

Proposed Course

Evaluate bone marrow response for continuous infusion of BUdR/IUdR and continue work on cellular quantitations of BUdR/IUdR. Evaluate cell survival of other mammalian cells to halogenated purines.

Publications

1. Morstyn, G., Kinsella, T., Hsu, S.-M., Russo, A., Gratzner, H., and Mitchell, J.B.: Identification of bromodeoxyuridine in normal cells following therapy: Relationship to complications. Int. J. Rad. Oncol. Biol. Phys. 10: 1441-1445, 1984.

2. Kinsella, T., Mitchell, J.B., Russo, A., Morstyn, G., Hsu, S.-M., Rowland, J., and Glatstein, E.: Continuous intravenous infusions of bromodeoxyuridine (BUdR) as a clinical radiosensitizer. J. of Clin. Oncol. 2: 1144-1150, 1984.

3. Russo, A., Gianni, L., Kinsella, T., Klecker, R.W, Jenkins, J., Rowland, J., Glatstein, E., Mitchell, J.B., Collins, J., and Myers, C.E.: A pharmacological evaluation of intravenous delivery of 5-bromodeoxyuridine to patients with brain tumors. Cancer Research 44: 1702-1705, 1984.

4. Mitchell, J.B., Morstyn, G., Russo, A., Kinsella, T.J., Fornace, A., McPherson, S.J., and Glatstein, E. Differing sensitivity to fluorescent light in Chinese hamster cells containing equally incorporated quantities of BUdR versus IUdR. Int J. Rad. Oncol. Biol. Phys. 10: 1447-1451, 1984.

5. Morstyn, G., Miller, R., Russo, A., and Mitchell, J.B. 131-Iodine conjugated antibody cell kill enhanced by broodeoxyuridine. Int. J. Rad. Oncol. Biol. Phys. 10: 1437-1440, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06352-03 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: O. A. Gansow 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

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.)*

Nuclear Magnetic Resonance (NMR) 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.

A study of concentration dependence of T1 relaxation by various metal chelates and organic nitroxyl radicals has been prepared. Based on these studies, the metal chelates appear to be more efficient.

We have recently shown that such metal chelates are useful as an NMR contract agent in myelography and cisternography.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

R. Knop	Clinical Associate	DR, CC

Objectives: We proposed 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 have been measured by conventional inversion, recovery methods. New chelates for this purpose have been synthesized.

Major Findings

Preliminary studies have shown that paramagnetic chelates may be attached to antibodies or albumin without affecting the biological properties of the proteins. Results of T1 studies show that many paramagnets must be attached to one protein to have an effect *in vivo*.

Use of Gd(DTPA) as an NMR contract agent has demonstrated that myelography and cisternography may be practically useful in the clinic.

Proposed Course

The T1 measurements required to determine whether labeled paramagnetic proteins could be of use *in vivo* are in progress. Synthetic chemical procedures necessary for attachment of many chelates to protein are under development. Practical human use protcols have been submitted.

Publications

1. Di Chiro, G., Knop, R.H., Girton, M.E., Dwyer, A.J., Doppman, J.L., Patronas, N.J., Gansow, O.A., Brechbiel, M.W., and Brooks, R.A.: MR cisternography and myelography with gadolinium-DTPA in monkeys. Radiology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06353-03 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Metal Chelate Conjugated Monoclonal Antibodies for Tumor Diagnosis and Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	O. A. Gansow	Senior Investigator	ROB, NCI
Others:	R. W. Atcher	Expert	ROB, NCI
	M. Brechbiel	Chemist	ROB, NCI
	M. Magerstadt	Visiting Associate	ROB, NCI

COOPERATING UNITS *(if any)*

Johns Hopkins Medical School, Baltimore, MD (M. Strand); Argonne National Laboratory, Argonne, IL (A. Friedman).

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	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 spece provided.)*

Tumor-associated monoclonal antibodies are potential therapeutic agents as selective carriers of cytotoxic agents to malignant cells. We are testing this hypothesis in several animal model systems: one is a tumor virus induced leukemia of mice, another is human tumor xenographs in nude athymic mice.

The various cytocidal 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 Pb-212 or Bi-212. 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 or rational therapy of malignancies by selectively targeting cytocidal agents to tumors as well as metastases.

New chelates for use in this project have been synthesized and are in testing, and have thus far proven useful for Radiobiology studies of cell killing with alpha particle labeled antibody.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

D. Colcher	Senior Investigator	LCMB, NCI
T. Waldmann	Chief	MET, NCI

Objectives: The specific goal of these studies is to investigate in vitro and in animal tumor models the therapeutic efficacy of radionuclides attached to tumor associated monoclonal antibodies. These studies encompass the synthesis of new bifunctional chelates and cryptands designed for therapy employing a variety of radioisotopes and radiation types.

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., Tc-99m) and of less common, but potentially serviceable radionuclides (e.g., Ga-68, In-111, Bi-212), 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 project 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 purchase 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 antibody were dialyzed 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 isobutylcarboxy carbonic anhydride of DTPA. Since the yield of radiometal incorporation must depend on the amount of chelate attached to the antibody, C-14 or H-3 labeled chelates 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.

3. New chelates, p-Isothiocyanatobenzyl-EDTA and -DTPA have been prepared and are being evaluated for use in conjugating metals to antibody. In particular, In-111 labeled by using the above DTPA chelate has been employed for tumor imaging in nude mice. The tissue distribution and clearance properties of B72.3 attached to these chelates have been compared to similar results for the other available chelating groups. It is clear that the new chelate is superior. We plan to begin human use of the new chelate this summer.

4. Conventional radiation therapy has employed α-particles only sparingly, if at all, since they have very low penetration in tissue. We have proposed that it is precisely the lack of penetration that makes an α-particle emitting radionuclide the superior choice for use in therapy when linked to monoclonal antibody (MoAb). Here we present biological data to support this hypothesis together with the nuclear and inorganic chemistry necessary to form ^{212}Bi labeled antibody in a clinical environment.

A safe and easily used generator for the production of ^{212}Bi, ^{212}Pb is based on ^{224}Ra($t_{1/2}$=3.6d) and serves as a radiopharmacy source for the α-emitter ^{212}Bi.[1] After elution from the generator by .15M hydrogen iodide, ^{212}Bi is efficiently complexed by antibody chemically modified

with a DTPA chelate. Specific activities as large as 160 $\mu C_i/\mu gm$ are obtained. This delivers a dose of ≈4000 rad/μgm MoAb if delivered to 1 cc of tissue.

Extensive in vitro radiobiology studies support the observation that α-particle labeled antibody is exceedingly cytotoxic and cell selective. When the cell line HUT-102 with receptors specific for α-TAC-^{212}Bi labeled MoAb was treated in cell culture a cell survival curve with D_o=6.5 rad/ml (.23 μCi ^{212}Bi α-TAC/ml) was measured by a clonogenic assay, whereas a dose of 57.5 rad (2.6 μC_i ^{212}Bi-α-TAC) was seen for the receptor negative cell line MOLT-4. The interpretation of this data is that MoAb must be bound at the cell surface to be effective because of the short α-particle range (60-100μm).

To test this hypothesis, studies of cell survival with the positive receptor line were again performed, but with added unlabeled α-TAC to dilute ^{212}Bi concentration on the cell surface. The survival measured was nearly that seen for a receptor negative cell line. These studies have now been reproduced by using several other antibody-cell systems. Animal studies are in progress.

Significance to Biomedical Research and the Program of the Institute

The ability to attach metals to antibodies is significant for several reasons. It enables one to diagnose and detect cancer using radioactive metals in nuclear medicine tests, or using paramagnetic metals to enhance nuclear magnetic resonance images. The ability to attach particle emitters to antibodies opens up site specific therapy using a variety of radioactive metals which can be selected to maximize cell killing while sparing normal tissue. Finally, it appears that the bifunctional chelates currently being investigated have little effect on the viability and specificity of the antibodies, thus preserving their function.

Proposed Course

The effects of the number of chelates attached to the antibodies and the other conditions of preparation on the activity of the antibodies both in vitro and in vivo will be examined. Next, the conditions under which the metals are incorporated into the chelates will be examined for their effects on biological activity both in vitro and in vivo.

The radiobiology studies now underway will be expanded to test a number of human cell lines. Those cell lines adequately treatable will be considered for treatment in animal models and humans.

Publications

1. Gansow, O.A., and Kausar, A.R.: Diazo coupling of some lanthanide benzocryptates to proteins. Inorg. Chem. Acta. 91: 213-215, 1984.

2. Gansow, O.A., Strand, M., and Scheinberg, D.A.: Monoclonal antibody conjugates for tumor imaging and therapy. Cell Fusion: Gene Transfer and Transform. 14: 385-393, 1984.

3. Gansow, O.A., Strand, M., Scheinberg, D.A., and Friedman, A.M.: Monoclonal Antibody Conjugates for Diagnostic Imaging and Therapy. In Knapp, F.R. (Ed.): Monoclonal Antibodies and Cancer. New York, Academic Press, Inc., 1984, pp. 125-131.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06354-03 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: O. A. Gansow 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

We are developing 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, is being developed for that purpose.

Formerly, we reported results of an iron-57 NMR investigation of characteristic relaxation times and chemical shifts of some iron compounds. The data furnished information on the chemical shift range of iron coordinated to nitrogen, substituent effects on iron-57 chemical shifts, and relaxation mechanisms for iron-57, and thus provide the basic parameters needed for further development of iron-57 NMR. Most recently, the first observation of ^{57}Fe NMR in a protein, carbonmonoxymyoglobin, was achieved together with a determination of ^{57}Fe relaxation times and chemical shift anisotropy.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

E. Becker Section Head 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 effective.

Methods Employed

A specially constructed NMR probe for observing iron NMR was built and used as described in our recent publications. Enriched iron-57 proteins such as myoglobin have been synthesized. A new NMR probe for use with biological molecules is in construction.

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. A study of iron-57 relaxation times showed the advantage of the experiment being done at high magnetic fields.

Significance to Biomedical Research and the Program of the Institute

Our studies have shown that iron-57 NMR will be a new and valuable method for study of biological compounds. We have shown that ^{57}Fe NMR of proteins can be observed.

Publications

1. Baltzer, L., Becker, E.D., Tschudin, R., and Gansow, O.A.: ^{57}Fe NMR of heme proteins: Chemical shift anisotropy and relaxation parameters of Carbonmonoxymyoglobin. J. Chem. Soc. Chem. Commun. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06355-03 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 professionel personnel below the Principal Investigator.) (Name, title, laboratory, end institute affiliation)*

PI: P. A. Findlay Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.75	.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

Professional Personnel Engaged on the Project:

H. C. Lane	Senior Investigator	LIR, NIAID
H. Masur	Deputy Chief	CCM, Clinical Center
R. Steis	Clinical Associate	MB, NCI

Objectives: 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 are eligible to 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 have had individual skin lesions irradiated with graded test doses which were assessed for complete response one month later. This information has been 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

The dose seeking phase of the study indicated that the dose response of AIDS-associated KS is similar to non-AIDS KS.

Upper GI endoscopy and colonoscopy revealed previously unsuspected KS involvement of these sites in the majority of patients evaluated. Thus only an extremely small subset of patients with AIDS and KS could expect to benefit from a local treatment modality.

Significance to Biomedical Research and the Program of the Institute

The study has provided a better understanding of the course of KS and AIDS.

Proposed Course

The project has been terminated.

Publications

1. Patow, C.A., Stark, T.W., Findlay, P.A., Steis, R., Longo, D.L., Masur, H., and Macher, A.M.: Pharyngeal obstruction by Kaposi's sarcoma in a homosexual male with acquired immune deficiency syndrome. Otolaryngology - Head and Neck Surg 92(6): 713-716, 1984.

2. Patow, C.A., Steis, R., Longo, D.L., Reichert, C.M., Findlay, P.A., Potter, D., Masur, H., Lane, H.C., Fauci, A.S., and Macher, A.M.: Kaposi's sarcoma of the head and neck in the acquired immune deficiency syndrome. Otolaryngology - Head and Neck Surg 92(3): 255-260, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06356-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Treatment of Malignant Brain Tumors with Interstitial Radiotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. A. Findlay	Senior Investigator	ROB, NCI
Others:	R. Miller	Health Physicist	ROB, NCI
	P. Kelley	Nurse Specialist	ROB, NCI
	A. Wolbarst	Radiation Physicist	ROB, NCI
	K. Yeakel	Dosimetrist	ROB, NCI
	R. Creecy	Computer Specialist	ROB, NCI
	U. Rosenow	Visiting Scientist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	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.)*

Results of current therapy of most malignant adult brain tumors remain disappointing. Despite the most aggressive multimodality treatment, median survival in the most common tumor, glioblastoma multiforme, is 10 months and cure is anecdotal. These tumors extend beyond the limits of surgical resection and total dose of conventional external beam radiotherapy is limited by surrounding normal brain tolerance. By placing radioactive seeds of Iodine 125 directly into the tumor bed we hope to achieve: 1) a high radiation dose to the tumor; 2) a low radiation dose to surrounding normal brain; and 3) increased therapeutic ratio with radiation delivered at low dose rates.

Project Description

Professional Personnel Engaged on the Project:

D. C. Wright	Senior Investigator	SN, NINCDS

Objectives: To develop a technique of interstitial implantation of intracranial tumors; to determine the acute side effects and complications of such treatment; to explore the efficacy of such therapy; and to develop patient selection guidelines for future applications of this technique.

Methods Employed

Patients with primarily untreated high grade gliomas of less than 5 cm diameter receive 4000 rad of external beam radiation therapy prior to implantation. Patients with similar tumors recurrent after prior standard treatment receive implant only. Using a Brown Roberts Wells stereotactic frame and a customized template device, silastic catheters loaded with radioactive seeds of Iodine 125 are stereotactically positioned in the tumor. Catheters are anchored to the dura and the bone defect is closed.

Major Findings

The study has been active for 17 months. Ten patients have been enrolled. Two patients, both without previous treatment, have just begun external beam radiotherapy and are inevaluable. Four patients with recurrent tumors received implants. Of these, 3 have died at 3m, 5m, and 8m post-implant and one is alive 7 months post-implant. Four patients with no previous treatment were entered on study. Of these, one died of unrelated causes before undergoing implant. The other three are currently alive 3m, 8m, and 17m from beginning of treatment.

We have developed a technique for stereotactic placement of multiple catheters containing multiple radioactive sources, which can be used to implant tumor of all intracranial sites, exclusive of the posterior fossa which is technically inaccessible to stereotaxis. The technique is adaptable to a variety of radioactive isotopes and tumor configurations.

Significance to Biomedical Research and the Program of the Institute

This study should provide information on total radiation dose and dose rate effects on both tumor and normal brain. We hope that the treatment will prove more efficacious than current standard therapy.

Proposed Course

We are continuing patient accrual.

Publication

1. Findlay, P.A., Wright, D.C., Rosenow, U., Harrington, F.S., and Miller, R.W.: ^{125}I interstitial brachytherapy for primary malignant brain tumors: Technical aspects of treatment planning and implantation methods. 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 06357-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Clinical Studies on Intraoperative Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. J. Kinsella	Deputy Branch Chief	ROB, NCI
Others:	Z. Tochner	Visiting Associate	ROB, NCI
	E. Glatstein	Chief	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	10	

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 Oncology and Surgery Branches of the National Cancer Institute have been involved in prospective randomized trials evaluating the potential role of intraoperative radiotherapy in three major disease sites including resectable and unresectable carcinomas of the pancreas, resectable carcinomas of the stomach and resectable retroperitoneal sarcomas. We have also been involved in a single arm pilot trial involved in dose escalation of intraoperative therapy in selected patients whose locally advanced tumors are felt unlikely to be cured by standard therapy and at least there is a theoretical advantage for the use of intraoperative radiation therapy. In January 1985, we began a pilot trial of combining surgery and intraoperative radiotherapy for locally advanced lung carcinoma. To date, 96 patients have been treated with experimental intraoperative radiation therapy on these various protocols and there are an additional 59 other patients being followed as control patients on the various randomized prospective trials. We have clearly demonstrated that it is technically possible to combine intraoperative radiation therapy with a radical surgical procedure and that the acute morbidity from the combination is quite acceptable. To date, there does not appear to be any significant difference in the randomized prospective trials with respect to a local control, disease free survival, and overall survival. Obviously, these trials are ongoing and require more patients and further follow-up. Patients also need to be followed for any potential late effects of intraoperative radiation therapy.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

W. Sindelar	Senior Investigator	SB, NCI
H. Pass	Senior Investigator	SB, NCI
R. Smith	Cancer Nursing Specialist	CNS, CC
M. Maher	Cancer Nursing Specialist	CNS, CC

Objectives: These are phase I & II studies assessing the role of intraoperative therapy as an adjunct to surgical resection in primary tumors of the pancreas, stomach and retroperitoneum where local failure following surgery alone is unacceptably high. In the initial four years of the trial from 1979-1983, the surgical procedure necessitated gross tumor resection because our electron beam capabilities were limited to 11 MeV. Since September 1983, we have the electron capabilities up to 22 MeV. A dedicated intraoperative suite was opened in August 1984, in the Radiation Oncology Branch.

Methods Employed

The patients are considered for entry on these trials of combined surgical resection and intraoperative therapy who had specific malignant lesions within the abdominal or retroperitoneal space which were in advanced stage and which had no visceral metastatic spread. In general, these locally advanced cancers were felt to have little likelihood of cure by conventional surgical treatment alone. Patients are randomized prior to operation to receive either the conventional form of treatment which would combine surgery and post operative external beam radiation or the experimental form of treatment which includes surgery and intraoperative radiation therapy alone for carcinomas of the stomach and pancreas. Surgery, intraoperative and a reduced external beam dose is the experimental arm for resectable retroperitoneal sarcomas. All patients receiving intraoperative therapy are given a dose of Misonidazole, a known radiation sensitizer of hypoxic cells at grams per meter squared approximately 30 minutes prior to delivery in the 3.5 intraoperative radiation therapy. Patients are closely followed in the post treatment period to assess any toxicity as well as to address the issues of local control, disease free and overall survival.

Major Findings

To date, 95 patients have received intraoperative radiotherapy at the National Cancer Institute. The number of patients entered on the study are as follows:

1) Carcinoma of the Stomach - 26 patients have been randomized and received either intraoperative radiation therapy (10 patients) or conventional external beam radiation (16 patients).

2) Carcinoma of the Pancreas: Resectable Lesion - 26 patients have been randomized with 13 patients receiving intraoperative radiation and 13 patients receiving conventional post operative radiation.

3) Unresectable Carcinoma of the Pancreas: Unresectable - 26 patients have been randomized with 14 patients receiving intraoperative radiation combined with external beam radiation and 12 patients receiving external beam radiation alone.

4) Retroperitoneal Sarcomas - 34 patients have been randomized with 16 receiving intraoperative therapy with a reduced external beam dose while 18 patients have received high dose external beam radiation.

5) Pilot Trials of Intraoperative Radiation with Dose Escalation - 36 patients have been treated on this trial where the dose has been escalated from a dose of 2000 to as high as 3000 rad intraoperatively. Most patients also receive external beam radiation. The extent of each surgical resection has varied in this group of patients.

6) Sarcomas of the Bony Pelvis - 5 patients have been entered on this single arm study combining radical surgery usually hemipelvectomy with high intraoperative radiation alone or combined with external beam radiation if there is positive margins or tumor spill.

7) Pilot trial of intraoperative radiation with surgery in locally advanced lung cancer - 2 patients have been entered on this single arm study.

The preliminary results suggest that there does not appear to be any increased acute morbidity with the addition of intraoperative radiation to a major surgical procedure. The use of intraoperative radiation previously (until 8/84) required patient transport from the operating room to the radiation therapy area, which although increasing the anesthesia time does not appear to increase the operative mortality nor significant morbidity. While there appears to be an improvement in local control in the pilot trials of intraoperative therapy with dose escalation, the three randomized prospective trials to date show no difference in overall disease free survival between these relatively small groups of patients with variable follow-up.

Significance to Biomedical Research and the Program of the Institute

The Radiation Oncology Branch continues to be 1 of 4 major institutions in the United States involved in clinical research with intraoperative radiation therapy. While this technique of intraoperative radiation appears to be gaining considerable enthusiasm, these pilot and randomized prospective trials are very important in defining any significant future role for intraoperative radiation therapy. To date, the National Cancer Institute has the only group of controlled patients with which to make a meaningful comparison of intraoperative radiotherapy with the use of conventional external beam radiation.

Proposed Course

We plan to continue these pilot and the gastric carcinoma and retroperitoneal sarcoma randomized prospective trials in their present form. The studies with resectable and unresectable pancreatic carcinoma have been discontinued.

Publications

1. Kinsella, T.J., and Sindelar, W.F.: Intraoperative Radiotherapy. In DeVita, V.T., Hellman, S., Rosenberg, S.A. (Eds.): Principles and Practice of Oncology - ed. 2. Philadelphia, Lippincott, 1985, pp. 2293-2304.

2. Fraass, B.A., Miller, R.W., Kinsella, T.J., Sindelar, W.F., Harrington, F.S., van de Geijn, J. and Glatstein, E.: Intraoperative radiation therapy at the National Cancer Institute: Technical innovations and dosimetry. Int. J. Rad. Oncol. Biol. Phys. 11: 1299-1312, 1985.

3. Kinsella, T.J., Sindelar, W.F., DeLuca, A.M., Pezeshkpour, G., Smith, R., Kranda, K., Mixon, A., Yeakel, K., and Miller, R.: Tolerance of peripheral nerve to intraoperative radiotherapy (IORT): Clinical and experimental studies. Int. J. Rad. Oncol. Biol. Phys. (in press).

4. Sindelar, W.F., Kinsella, T.J.: Intraoperative Radiotherapy. In Karcher, H., (Ed.): Progress in Radio-Oncology. New York, Raven Press (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06358-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Effects of γ -Irradiation on Cells and Their Constituents

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	P. Riesz	Research Chemist	ROB, NCI
Others:	C. Murali Krishna Cherukuri	Visiting Fellow	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	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 spece provided.)*

The effects of ionizing, and ultraviolet radiation, of visible light, and of ultrasound on cells and their constituents are being studied. Phthalocyanines, a class of Azaporphyrins with maximum absorption in the 600-700 nm region, are efficient photosensitizers for killing of mammalian cells and may be candidates to replace hematoporphyrin derivatives in photodynamic therapy (PDT) of tumors. A comparison of the quantum yields for superoxide radical photogeneration at 615 nm from a variety of Phthalocyanines with their cytotoxicity to cultured mammalian cells suggests that superoxide is not the lethal agent. In a new photochemical reaction, the decarboxylation radicals formed from peptides and carboxylic acids by photolysis at 313 nm in the presence of various Metallo-phthalocyanines were identified by spin trapping and electron spin resonance. These reactions might be involved in the undesirable phototoxicity induced by sunlight following photodynamic therapy. The photochemical formation of super-oxide and of decarboxylated peptide radicals by visible light (405-615 nm) from the antitumor agents Carboquone, Mitomycin C and Streptonigrin were investiga-ted because of the possibility of combining chemo with phototherapy. Continuing our studies on the effect of continuous wave and pulsed 1 Megahertz (MHz) ultra-sound on aqueous solutions, the effect of varying the pulse repetition frequency on transient cavitation and on the formation of hydroxyl radicals was examined. The obstacles associated with spin-trapping oxygen-derived radicals inside cells were studied and the results indicate that the primary radicals react with the spin traps; however, the 5,5-Dimethyl-1-Pyrroline-N-Oxide spin adduct with hydroxyl radicals decays very rapidly in the interior of the cell.

Project Description

Objectives: The effects of ionizing and ultraviolet radiation on biological macromolecules and their constituents are being investigated. Radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals, or by the "indirect effect" where radical species are hydrated electrons, hydrogen atoms and hydroxyl radicals.

In the chain of events that leads to loss of biological activity, free radicals play an important role. Chemical compounds have been discovered which significantly modify radiation effects. These include: (a) electron affinity sensitizers which act on hypoxic tumor cells, (b) halogenated pyrimidines which are incorporated into DNA; and (c) cancer chemotherapy agents of the intercalating or alkylating type which sensitize tumor and normal cells. Studies of the mechanism of action of radio sensitizers and radioprotectors are necessary to design improved combinations of chemotherapy and radiation therapy.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity in macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

In the last few years it has become apparent that superoxide anion radicals and hydroxyl radicals are found in many biological systems in the absence of either ionizing radiation or UV-photolysis. Recent reports have indicated that radicals are produced in the presence of certain anti-cancer drugs such as Bleomycin and Adriamycin. The significance of radical reactions is therefore not confined to radiation biology. It has also been shown that damage to tissues following ischemia appears to occur during reperfusion with oxygenated blood. This damage is generally considered to be due to the excessive production of superoxide radicals and hydrogen peroxide. In support of this hypothesis it has been shown that in several model systems superoxide dismutase, catalase or allopurinol (a xanthine oxidase inhibitor) protect ischemic tissue from oxidative damage during reperfusion.

Methods Employed

Nucleic acids, proteins and their constituents were γ -irradiated either in the solid state or in aqueous solutions in a 800-curie Cobalt γ -source. Electron spin resonance studies were carried out with a Varian E-9 Spectrometer connected to a Nicolet Lab 80 computer. For photolysis studies at specific wavelengths, a 1000-watt high pressure Mercury-Xenon arc source and monochomator were employed. For ultrasound exposures aqueous solutions were insonated in a non-perturbing cylindrical cell with 1 mil mylar windows in an anechoic

ultrasound exposure apparatus at 30 ± 0.5°C. Specimens were exposed to either continuous wave or tone bursts of 1 MHz ultrasound to simulate both therapeutic and diagnostic exposure conditions. In the spin trapping method, the short-lived free radicals react with a diamagnetic scavenger (the spin trap) to produce longer-lived radicals (the spinadduct) which can be conveniently investigated by e.s.r. In our studies 2-Methyl-2-Nitrosopropane and 5,5-Dimethyl-1-Pyrroline-1-Oxide were employed as the spin traps.

Major Findings

I. Photochemical Generation of Superoxide Radical and the Cytotoxicity of Phthalocyanines. (with E. Ben-Hur, Department of Radiobiology, Nuclear Research Center-Negev, Beer-Sheva, Israel, I. Rosenthal, Department of Food Science, A.R.O. Volcani Center, Bet Dagan, Israel, and A.J. Carmichael).

Visible light (λ=615±10 nm) excitation of Phthalocyanines dissolved in Dimethylsulfoxide in the presence of oxygen generates superoxide radical anion. The efficiency of generating O_2^- was uncorrelated with the photodynamic activity of the same dyes, as judged by cytotoxicity to cultured Chinese hamster cells. It is concluded that O_2^- is involved very little, if at all, in the Phthalocyanine-induced photokilling of mammalian cells.

II. Phthalocyanine Photosensitized Reactions of Peptides and Carboxylic Acids. A Spin Trapping Study. (with C. Murali Krishna Cherukuri and I. Rosenthal.)

In a new photochemical reaction, the radicals formed from peptides and carboxylic acids by photolysis at 313 nm in Dimethylsulfoxide-water solutions in the presence of metallo- and metal-free Phthalocyanines were identified by trapping with 2-Methyl-2-Nitrosopropane. For peptides the predominant reaction was the decarboxylation of the carboxyl terminal residue. For the sodium salts of formic, acetic, malonic and linolenic acid, the decarboxylation reaction was observed. For linolenic acid containing allylic hydrogens, the H abstraction radical was also formed. The latter reaction suggests the possibility of Phthalocyanine photosensitized damage to biological membranes.

III. Photogeneration of Superoxide and Decarboxylated Peptide Radicals by Carboquone, Mitomycin C and Streptonigrin. (with A.J. Carmichael and A. Samuni).

Visible light (405-615 nm) excitation of Carboquone, Mitomycin C, and Streptonigrin dissolved in Dimethylsulfoxide in the presence of oxygen generates superoxide anion radicals (O_2). The quantum yields for these reactions were measured. The oxidation of the photoexcited drug molecules occurs via a direct electron transfer to dissolved oxygen in solution. Ultraviolet irradiation (λ = 313 ± 10 nm) of the aminoquinone drug solutions (80% H_2O, 20% Dimethylsulfoxide) in the presence of peptides results in the decarboxylation of the peptides. In this case the photo-

excited drugs are reduced, abstracting an electron from the peptides.

IV. On the Spin Trapping and ESR Detection of Oxygen-derived Radicals Generated Inside Cells. (with A. Samuni, A.J. Carmichael, J.B. Mitchell and A. Russo).

Recently several attempts to identify oxygen-derived radicals in whole cells by spin trapping have been reported using 5,5 Dimethyl-1-Pyrroline-N-Oxide. In the present study the feasibility of this method is examined. Chinese hamster V79 and human blood cells served as the test systems, while OH radicals were generated by γ radiolysis. The results indicate that the primary radicals react with spin traps, however these spin adducts decayed rapidly within the cells. The cellularly-induced decay of DMPO-OH presented the major difficulty in detecting the endogenous radicals, and potential experimental approaches to overcome this difficulty are discussed.

V. Free Radical Production in Aqueous Solutions Exposed to Simulated Ultrasonic Diagnostic Conditions (with A.J. Carmichael, M.M. Mossoba and C.L. Christman, Center for Devices and Radiological Health, Division of Physical Sciences, Electromagnetics Branch, FDA, Rockville).

Our results for pulsed exposures confirm Flynn's earlier theoretical predictions that microsecond pulses of 1 MHz ultrasound can produce transient cavitation and hydroxyl radicals in aqueous solutions.

VI. An Invited review article on "Free Radical Generation by Ultrasound in Aqueous and Non-Aqueous Solutions" was written for "Environmental Health Perspectives". (with C.L. Christman, FDA, Rockville, and D. Berdahl, Chemical and Engineering Laboratory, General Electric Corporation, Schenectady.)

Significance to Biomedical Research and the Program of the Institute

Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy; (2) carcinogenesis; (3) stability of the genetic pool; (4) the suppression of the immune mechanism; and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal cells and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained.

Proposed Course

To continue studies on the effects of ionizing radiation on mammalian cells and macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents and the interaction of radiation and cancer chemotherapy agents will be investigated. New areas of interest include photosensitized cell killing by porphyrins and Phthalocyanines in relation to photodynamic therapy and chemical and biological effects of ultrasound.

Publications

1. Mossoba, M.M., Rosenthal, I., Carmichael, A.J., and Riesz, P.: Photochemistry of porphyrins as studied by spin trapping and electron spin resonance. Photochem. Photobiol. 39: 731-734, 1984.

2. Faraggi, M., Carmichael, A.J., and Riesz, P.: OH radical formation by photolysis of aqueous porphyrin solutions. A spin trapping and ESR study. Int. J. Radiat. Biol. 46: 703-713, 1984.

3. Carmichael, A.J., Mossoba, M.M., Riesz, P., and Rosenthal, I.: Synthetic food color: Photosensitized decarboxylation of peptides. J. Agric. Food Chem. 32: 689-690, 1984.

4. Mossoba, M.M., Makino, K., Riesz, P., and Perkins, Jr., R.C.: Long-range proton hyperfine coupling in alicyclic nitroxide radicals by resolution-enhanced electron paramagnetic resonance. J. Phys. Chem. 88: 4717-4723, 1984.

5. Carmichael, A.J., Makino, K., and Riesz, P.: Quantitative aspects of ESR and spin trapping of hydroxyl radicals and hydrogen atoms in gamma-irradiated aqueous solutions. Radiat. Res. 100: 222-234, 1984.

6. Carmichael, A.J., Makino, K., and Riesz, P.: Photoinduced reactions of anthraquinone antitumor agents with peptides and nucleic acid bases: An electron spin resonance and spin trapping study. Arch. Biochem. Biophys. 237: 433-444, 1985.

7. Carmichael, A.J., Samuni, A., and Riesz, P.: Photogeneration of superoxide and decarboxylated peptide radicals by carboquone, mitomycin C and streptonigrin. An electron spin resonance and spin trapping study. Photochem. Photobiol. (in press).

8. Ben-Hur, E., Carmichael, A.J., Riesz, P., and Rosenthal, I.: Photochemical generation of superoxide radical and the cytotoxicity of phthalocyanines. Int. J. Radiat. Biol. (in press).

9. Riesz, P., Berdahl, D., and Christman, C.L.: Free radical generation by ultrasound in aqueous and non-aqueous solutions. Environ. Health Perspect. (in press).

10. Carmichael, A.J., Mossoba, M.M., Riesz, P., and Christman, C.L: Free radical production in aqueous solutions exposed to simulated ultrasonic diagnostic conditions. IEEE Trans. on Sonics and Ultrasonics. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06359-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

A Phase 1 Study of Iododeoxyuridine (NSC39661) Given as an Intravenous Infusion

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. J. Kinsella	Deputy Branch Chief	ROB, NCI
Others:	A. Russo	Clinical Associate	ROB, NCI
	J. B. Mitchell	Radiobiologist	ROB, NCI
	E. Glatstein	Chief	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	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.)*

Iododeoxyuridine (IUdR) is a known radiosensitizing drug which is being delivered as a constant intravenous infusion for 12 hours or 24 hours/QD for up to 14 days in patients with high grade primary brain tumors and other poorly radioresponsive tumors. The drug is being used as a clinical radiosensitizer and being combined with high-dose radiation therapy in an attempt to improve the response rate of these poorly radioresponsive tumors as well as to assess the toxicity both local and systemic of this radiosensitizer. Twenty-four patients have been entered onto the trial using a 12 hour infusion and 28 patients entered on the trial using 24 hour infusions. Patients are treated with twice daily fractions of radiation therapy given in two separate sessions and combined with two separate infusions of IUdR.

GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

R. Klecker	Technician	CPB, NCI
J. Collins	Senior Investigator	CPB, NCI
D. Wright	Senior Investigator	SNB, NINCDS

Objective: This is a Phase I/II study designed to assess the toxicity of IUdR given as a constant intravenous infusion for 12 hours or 24 hours/QD along with high dose hyperfraction irradiation. The local (within the radiation field) and systemic toxicity are being closely evaluated in this group of patients. The pharmacokinetics of the drug is under study in collaboration with Dr. Collins. Incorporation of the drug into tumors is also being studied on selected patients by tumor biopsy and the use of a monoclonal antibody to IUdR.

Methods Employed

Patients with histologically confirmed grade 4 gliomas and other patients with locally advanced non-CNS tumors are eligible for this Phase I/II trial. Eligibility criteria include normal peripheral blood counts, normal renal function and a life expectancy of at least two months. The IUdR is infused through a flexible silastic catheter into the superior vena cava via the subclavian vein. A portable automatic infusion pump maintains a constant infusion over the 12 or 24 hour duration and allows for outpatient treatment. Pharmacology studies in selected patients involve obtaining serial arterial blood levels drawn during and after the infusion using a radial artery catheter placed by the anesthesiology section of the clinical center. Patients are admitted for a period of approximately 24 hours for this pharmacology study. All other treatment is given as an outpatient. An HPLC assay for IUdR developed at the NCI in the Clinical Pharmacology Branch is used. Cellular incorporation of IUdR is being measured primarily in tumors by the use of a monoclonal antibody and immunohistic chemistry.

Major Findings

We have escalated groups of 3 patients on IUdR infusions starting at 250 milligrams per meter squared and escalating to a dose of 1200 milligrams per meter squared given as a 12 hour or 24 hour infusion daily x 14 days. We have recognized bone marrow toxicity primarily thrombocytopenia as a major systemic toxicity and it appears that the maximum tolerable dose will be in the range of 1200 milligrams per meter squared. At this dose, most patients tolerate an infusion of 10 to 14 days and will show bone marrow recovery within a period of 7 to 10 days from stopping the infusion. We have not noticed any skin toxicity as was found in our previous trials with Bromodeoxyuridine. There have been no other major systemic toxicities. The pharmacology studies have shown that the clearance of Iododeoxyuridine appears linear over the dose range used and at the dose of 1200 milligrams per meter squared per 12 hour infusion, a steady state arterial blood level is found in the range of 7 to 9 x 10^{-6} molar. This maximal tolerated dose results in higher pharmacology steady state levels than our previous experience with Bromodeoxyuridine.

We have also demonstrated that Iodouracil increases in a non-linear fashion during during the infusion period and this is under further study at the present time. We have performed tumor biopsies in two patients with unresectable sarcomas and the biopsies from both patients demonstrate incorporation of Iododeoxyuridine into tumor cells by the end of the infusion.

Significance to Biomedical Research and the Program of the Institute

The preliminary information on Iododeoxyuridine as a clinical radiosensitizer is very promising. There is a considerable amount of interest nationally in these trials and the information that we are able to achieve steady state arterial levels approaching 10^{-5} M is quite remarkable. As predicted from experimental studies comparing the light sensitivity of IUdR and BUdR, we have not noted any significant skin toxicity as a manifestation of systemic toxicity to IUdR.

Proposed Course

We have completed the evaluation of intravenous Iododeoxyuridine given by this constant infusion for 12 hours a day. Based on this information, we are proceeding with evaluation of a continuous 24 hour infusion assessing similar parameters as with the intermittent schedule. Pending the results of the 24 hour infusion study, it may appear reasonable to attempt to manipulate pyrimidine metabolism using metabolic inhibitors such as 5-FU or FUdR.

Publications

1. Kinsella, T.J., Russo, A., Mitchell, J.B., Collins, J.R., Rowland, J., Wright, D., and Glatstein, E.: Phase I study of intravenous iododeoxyuridine as a clinical radiosensitizer. Int J. Radiat. Oncol. Biol. Phys. (in press).

2. Klecker, R.W., Jenkins, J.F., Kinsella, T.J., Fine, R.L., Strong, J.M., Collins, J.M.: Clinical pharmacology of 5-iodo-2'-deoxyuridine and 5-iodouracil, and endogenous pyrimidine modulation. Clinical Pharmacology and Therapeutics (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06360-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Radionuclide Generators to Produce Alpha-Emitters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R. Atcher Expert ROB, NCI

COOPERATING UNITS *(if any)*

Chemistry Division, Argonne National Laboratory, Argonne, IL (A. Friedman).

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	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.)*

This work involves the design, testing and use of radionuclide generators to produce alpha-emitters to be attached to proteins for use in radiotherapy. A new radionuclide generator has replaced the previous design which used Th-228 as the parent. The long half-life, two years, made it unsuitable for use by personnel without training in the handling of long-lived activity.

We have undertaken a project with the Chemistry Division at Argonne National Laboratory to develop a new generator system based on the parent Ra-224. This radionuclide has a 3.5 day half-life reducing the potential problems associated with a longer lived radionuclide. We designed and tested a separation system to separate thorium and radium in a manipulator-equipped shielded cave. We have had this system in operation for one year.

Simultaneously, we developed a new generator which will use the radium parent. This system uses a disposable generator package to minimize shipping and handling. An organic cation exchanger is eluted with hydrochloric acid to yield either the bismuth daughter or lead daughter. Work with this system has shown that the generator is safe, has good operating characteristics and can be produced on a regular basis.

Similar results have been seen with the thorium-radium separation. The system can be used repeatedly with a minimum of trouble. We are currently building a new thorium separator to handle higher levels of radioactivity in a "senior" remote handling facility to minimize personnel exposure.

GPO 914-918

Project Description

Objectives: To design a radionuclide generator system which will produce Pb-212 and Bi-212 to be attached to proteins that contain bifunctional chelates. This system should be easy to use and yield radionuclidically and radiochemically pure products.

Methods Employed

Thorium-228 has been separated from Ra-224 in a shielded remote handling facility. This system has been in operation for one year. We have gained an understanding of breakthrough of the parent, yield of the daughter, radiation and chemical resistance of the generator, and radiochemical purity of the product. The new radionuclide generator has been used to produce alpha-emitters for labeling of proteins. It has been operating very well.

Major Findings

A generator has been developed which produces a radionuclidically and radiochemically pure product. The bismuth and lead daughters can be separated on the generator or can be separated after elution from the generator.

The separation system for thorium and radium works well in routine operation. The system is designed to be operated using external controls and master-slave manipulators in a shielded facility.

Significance to Biomedical Research and the Program of the Institute

The development of a new generator for the production of Pb-212 and its daughters would increase access of the biomedical community to alpha emitters which are well suited for use as radiotherapeutic agents. Further, the new generator could be used by personnel who are not extensively trained in radiochemistry.

Proposed course

The new remote separation system will be installed in a larger cave and larger quantities of radium will be separated on a regular basis. This radium will be used to make "clinical" scale generators. These generators will be used for continuing radiobiology experiments to test the use of bifunctional chelates attached to proteins for radiotherapy. Inorganic supports will be examined as the ion exchange medium for the generator to increase resistance to radiation degradation.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06363-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

DNA Damage by Alkylating Agents and Their Repair in Human Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

P.I.:	D. Yarosh	Senior Investigator	LMP, NCI
Others:	A. J. Fornace, Jr.	Cancer Expert	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1 (ROB)	.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.)*

R. S. Day, III and others have shown that approximately 20% of human tumor lines and viral transformed lines are hypersensitive to alkylating agents due to an apparent absence of Alkylguanine Alkyltransferase. We have not detected any consistent absence of a specific mRNA in these alkylation sensitive, mer$^-$, cell lines. We have been able to partially purify the enzyme from human liver. This enzyme removes alkylation damage at the 06 position of guanine but not at other sites in DNA. We plan to further characterize this enzyme and purify it to the point where amino acid sequence information can be obtained.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

R. S. Day, III Senior Investigator LC, NCI

Objective: To characterize the human Alkylguanine Alkyltransferase enzyme.

Methods

Standard molecular biology and protein biochemistry approaches.

Major Findings

A consistent cDNA clone absent in mer$^-$ cells was not detected. We are now approaching this subject by characterizing the enzyme.

Significance to Biomedical Research and the Program of the Institute

An understanding of this defect which occurs in approximately 20% of all human tumor lines would have obvious importance in both carcinogenesis and cancer treatment.

Proposed Course

See summary.

Publications

1. Yarosh, D., Fornace, Jr., A.J., and Day, R.: Human 06-alkylguanine-DNA alkyltransferase fails to repair 04-methylthymidine and methyl triesters in DNA as does the alkyltransferase from E. coli. Carcinogenesis (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06364-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Measurement DNA Base Damage Produced by X-rays in Human Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. J. Fornace	Cancer Expert	ROB, NCI
Others:	T. J. Kinsella	Deputy Branch Chief	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.7	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.)*

Measurement of DNA damage with endonucleases, which recognize lesions such as pyrimidine dimers or ionizing radiation base damage, has been used to study DNA repair in mammalian cells (Patterson, Adv. Radiat. Biol. 7: 1, 1978). The sensitivity of this approach has been increased 1 to 2 orders of magnitude by adapting it to the alkaline elution technique (Fornace, Mutation Res. 94: 263, 1982). We have adapted this technique to quantitatively measure very low levels of base damage produced by x-rays in various human cell strains. We have found that ataxia telangiectasia (AT) cells, which are hypersensitive to x-rays, remove this base damage normally after doses of 1 to 5 k rads. This is in contrast to Patterson's findings at higher x-ray doses where some AT cells, but not other AT cells, removed the base damage slower than normal. We conclude that the cause for the x-ray hypersensitivity in AT cells does not involve base damage or its repair.

We have also studied the repair of x-ray base damage in xeroderma pigmentosum (XP) cells. Cells from patients with this disorder are hypersensitive to UV-radiation and are deficient in the repair of DNA base damage, pyrimidine dimers, produced by UV; some XP cells are also moderately hypersensitive to x-rays. We found that most, >90%, of x-ray base damage is repaired in XP cells, but the remaining persists in all XP cell strains tested, complementation groups A, C, D, and G. This abnormality in DNA repair did not correlate with x-ray hypersensitivity. It is likely that the persistent base damage seen in XP cells are pyrimidine dimers since a very low level would be expected to be produced by Cerenkov radiation during x-irradiation.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objective: To determine if any abnormalities in repair exist in x-ray sensitive mutants at biologically relevant doses.

Methods Employed

Alkaline elution and standard cell culture conditions.

Major Findings

At more biologically relevant doses, AT cells repair x-ray base damage normally. A low level of x-ray base damage is persistent in XP cells. This base damage probably represents pyrimidine dimers; this is the first time such lesions have been detected in eukaryotic cells.

Significance to Biomedical Research and the Program of the Institute

Lesions important in x-ray lethality, mutagenesis, etc., are poorly understood. Our work in AT and XP cells indicates that x-ray base damage probably does not play a major role in x-ray sensitivity in these cells.

Proposed Course

We plan to continue our studies in AT cells with emphasis now on the repair of DNA double strand breaks and closely apposing DNA single strand lesions. As more x-ray sensitive mutants become available, we plan to study them with the same approaches.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06365-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

RNA Transcripts Induced by Hyperthermia in Rodent Cells

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Name, title, leboretory, and Institute affiliation)*

P.I.:	A. J. Fornace	Cancer Expert	ROB, NCI
Others:	J. B. Mitchell	Radiobiologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	.7	.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.)*

Prokaryotic and eukaryotic cells respond to environmental stress by the induction of a variety of stress-related proteins. In mammalian cells, the most well characterized group of stress proteins are induced by hyperthermia. Transcription of heat shock proteins increases markedly after hyperthermia and several of these genes have been cloned from HeLa cells in other laboratories. It is likely that transcription of other genes is also induced in mammalian cells since approximately 10-20 genes are induced in prokaryotes and lower eukaryotes. One approach to isolate such transcripts is to enrich for heat shock specific cDNA's by hybridization subtraction with mRNA from control cells. We have done this with rodent cells, V79, and we have also constructed a cDNA library from heat shock treated (HS) cells. Our results indicate that the most abundant transcript induced by HS in V79 and CHO cell lines is a small repetitive genetic element. The transcripts of this repetitive element are polyadenylated as are most mRNA species. Transcription of this repetitive element has previously been found to be increased in transformed rodent cells and rodent tumor cells. It is of interest that HS proteins are usually elevated in transformed cells. Sequence analysis of this element reveals that a region has very close similarities to the HS promoter consensus sequence of Drosophilia. The induction of this gene is not non-specific since equitoxic doses of x-rays or UV-radiation do not increase transcription.

GPO 914-918

Project Description

Objective: To identify genes induced by hyperthermia in V79 cells.

Methods Employed

Standard molecular biology approaches; hybridization subtraction procedures as used by Timberlake, Sargent, Davis, and others.

Major Findings

The major transcript induced by HS in V79 and CHO cells is from a repetitive genetic element. This sequence may function as an identifier sequence for HS.

Significance to Biomedical Research and the Program of the Institute

A more thorough understanding of the response of mammalian cells to hyperthermia would benefit both clinical hyperthermia research and also how mammalian cells respond to environmental stress.

Proposed Course

Further characterization of this repetitive element induced by HS is currently underway. The HS protein cDNA's will also be isolated from our HS cDNA library by similar approaches. If other types of cellular injury induce transcription of particular genes, they can be cloned with the same approach.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06366-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Nuclear Magnetic Resonance Studies on Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. B. Mitchell	Radiobiologist	ROB, NCI
Others:	A. Russo	Clinical Associate	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	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.)*

Cell systems have been developed to dynamically monitor ATP levels in cells by nuclear magnetic resonance (NMR). We have demonstrated that hyperthermia treatment and alteration of glutathione levels do not affect ATP metabolism. Further, the lack of ATP signals in cells was shown not to be an indicator of cell death as assayed by cloning forming ability. Studies are designed to study the effects of perturbations of the respiratory chain and redox cycle on ATP metabolism. Work is underway to synthesize non-toxic, specific NMR contrast reagents for resolving hypoxic centers within the tumor. This will allow for better treatment planning in the clinic.

Hyperthermia was found not to change cellular ATP levels and thus did not correlate to the synthesis of heat shock proteins.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

R. Knop	Senior Investigator	NMOB, NCI

Objective: Study ATP levels in mammalian cells by dynamic NMR and biochemical means of altering ATP levels.

Methods Employed

In vitro cell cultures immobilized in agarose strands and encased in an NMR perfusion tube will be used. Biochemical assays will be performed using standard UV-vis spectroscopy and NMR spectroscopy. Resolution of structures by NMR, mass spectroscopy by standard techniques is utilized.

Major Findings

ATP concentration can be monitored by NMR in living cells and ATP depletion does not result in cell death by colony forming ability. Glutathione depletion does not alter ATP levels. No correlation in ATP levels and synthesis of heat shock proteins were observed.

Significance to Biomedical Research and the Program of the Institute

These techniques will allow dynamic biochemical assessment of the effects of antitumor modalities such as hyperthermia.

Publications

1. Foxall, D.L., Cohen, J.S., and Mitchell, J.B.: Continuous perfusion of mammalian cells embedded in agarose gel threads. Exp. Cell Res. 154: 521-529, 1984.

2. Knop, R.H., Chen, C., Mitchell, J.B., Russo, A., McPherson, S., and Cohen, J.S.: Metabolic studies of mammalian cells by ^{31}P NMR using a continuous perfusion technique. Biochem. Biophys. Acta 804: 275-284, 1984.

3. Knop, R.H., Chen, C.W., Mitchell, J.B., Russo, A., McPherson, S., and Cohen, J.S.: Adaptive cellular response to hyperthermia: ^{31}P NMR studies. Biochimica Biophysica Acta (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06367-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Mechanisms of Radioprotection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Clinical Associate	ROB, NCI
Others:	J. B. Mitchell	Radiobiologist	ROB, NCI
	W. Degraff	Biologist	ROB, NCI
	N. Friedman	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

Thiols have long been studied as radioprotective compounds, yet the mechanism of protection is still poorly understood. We have developed means by which the major cellular thiol, glutathione, can be either depleted or elevated and then access radiosensitivity. We have shown that glutathione is not a major protector for x-ray. Plans are to synthesize compounds varying in chemical structure that may provide radioprotection. The importance of membrane in specialized tissue such as lymphocytes is being studied. The importance of separate thiol dependent detoxification enzymes versus general oxidative detoxification is being studied. The use of bacteria with great radiation sensitivity is being studied to determine the uniqueness of this model.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objective: To develop normal tissue radioprotective agents.

Methods Employed

V79 hamster cells and conventional cell cloning assays will be used. Biochemical assays for assessing the redox potential of cells have been developed.

Major Finding

Cellular glutathione does not play a major role in the radiation response. With lymphocyte population, the membrane is responsible for interphase death. Interphase death susceptibility can be correlated with oxidative detoxifying enzyme concentration.

Significance to Biomedical Research and the Program of the Institute

To provide a rational approach to radioprotective agents and to better understand the mechanism of radiation damage.

Proposed Course

Synthesize and evaluate new compounds. To continue exploring the mechanism of interphase death. To understand why certain bacteria are radioresistant.

Publications

1. Russo, A., and Mitchell, J.B.: Radiation response of Chinese hamster cells after elevation of intracellular glutathione levels. Int. J. Rad. Onc. Biol. Phys. 10: 1243-1247, 1984.

2. Russo, A., Mitchell, J.B., DeGraff, W., Spiro, I., and Gamson, J.: The effects of cellular glutathione elevation on the oxygen enhancement ratio. Rad. Res. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06369-02 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Radiation Characteristics of a 22 MeV Medical Microtron

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. W. Miller	Health Physicist	ROB, NCI
Others:	J. van de Geijn	Radiation Physicist	ROB, NCI

COOPERATING UNITS *(if any)*

Scanditronix, Essex, MA (T. Cook).

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.8	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.)*

The Physics Section is continuing studies of the radiation characteristics of the Scanditronix MM-22 medical Microtron. Current research is centered around our Intraoperative Radiotherapy Program (IORT) and concerns the dosimetry of special IORT applicators. Associated with this is a study of the effects of using asymmetric electron collimation on the dose distributions of these applicators. A new color camera television system for viewing the intra-operative portal is under development. This system will employ a permanent mirror of aluminized Mylar instead of the current retractable glass mirror. This should allow remote viewing of the radiation portal during treatment.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objectives: The optimization of the radiation and operational characteristics of the Mircotron.

Methods Employed

The high quality and versatile radiation measurement systems available to the Branch are used by staff personnel in cooperation with experts from the manufacturer to determine the basic performance of the various critical functions of the machine and its monitoring equipment. Several of these functions have been found less than optimal for the special purposes envisioned by the ROB and in several cases, dramatic improvements have been obtained already.

Major Findings

Several performance characteristics were unsatisfactory for our purposes: electron depth dose distribution and tranverse beam profiles, monitor characteristics. Interaction between representatives of our staff and experts from the firm have resulted already in performance characteristics much better than required by the specifications.

Significance to Biomedical Research and the Program of the Institute

The Microtron is to be utilized primarily for intraoperative radiotherapy. As such, its reliability and (especially) its beam characteristics are of critical importance to this program. Also, the machine offers some technical features such as independently adjustable collimator jaws, which are meaningful only with more than minimum performance characteristics.

Proposed Course

To be continued.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06370-01 RO

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Optimization of Treatment Planning for Brain Implants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	U. Rosenow*	Visiting Scientist	ROB, NCI
	A. Wolbarst	Radiation Physicist	ROB, NCI
	R. Creecy	Computer Specialist	ROB, NCI
	K. Yeakel	Dosimetrist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI
	P. Findlay	Radiotherapist	ROB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	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 purpose of this work is optimal physics, computer based and technical support for a brain implant protocol. In particular it aims at optimization of the chain of procedures comprising patient data acquisition, treatment planning, including optimal delineation of the target, determination of the number of radioactive sources, their strength and position, and determination of the surgical access route. The method includes the further development of special mechanical positioning devices and the further development and adaptation of locally developed computer programs.

*Dr. Rosenow left the project January 1, 1985.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

D. Wright	Neuro-Surgeon	SN, NINCDS

Objective: To develop a computer-assisted system for optimization of the physical and technical aspects of radioactive seed implants in brain tumors; criteria are:

1) accurate fitting of a critical dose rate surface around the chosen target volume;

2) a uniform dose distribution inside the target;

3) a minimum number of catheters entering the brain in the most economical way.

Methods Employed

1) Mathematical/Physical methods are employed to develop a generalized approach to seed placement and relative seed strength.

2) Computer-based image manipulation of diagnostic CT data to determine optimal access routes.

3) Development of mechanical positioning and directioning devices for use in data acquisition and in surgery.

Major Findings

Major results have been reached in the mathematical optimization, which is, partly for practical clinical reasons, in part computerized, in part manual-based, so that a semi-automatic interactive computer based system will shortly be available. Considerable work needs to be done to make the system universal.

Significance to Biomedical Research and the Program of the Institute

The system is essential to technically and physically safe and radiation-economic patient treatment. The development aims at general applicability. The theoretical and computer related procedures apply to implants in general.

Proposed Course

Continuation.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06371-01 RO

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Modification of the Cross Beam Fluence Rate Distribution in Electron Beams

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	B. Arora Chin	Radiation Physicist	ROB, NCI
	R. Morton	Radiation Physicist	RRP, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.9	0.1

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 treatment of mycosis fungoides involves the use of large beams of nominally 6 MeV electrons. The irregular shape of the human body and especially the complicated shapes of hands and feet make dosimetry according to the usual standards of uniformity difficult. Especially hands and feet require shielding over a large part of the course, by cumbersome and uncomfortable means.

An investigation has been started into the use of a "sieve" technique which would allow decreasing the intensity locally, without affecting the energy, that is the penetrating power, in a controlled manner. This would reduce the discomfort of the patients greatly.

Project Description

Professional Personnel Engaged on Project:

R. Morton	Radiation Physicist	RRP, NCI

Objective: Controlled modification of the electron beam fluence distribution without affecting the electron energy to a significant degree.

Methods Employed

The basic idea is to decrease the electron beam intensity by means of a "sieve": a (brass) plate with regularly spaced circular holes. The effect of the distance between sieve and surface, the hole diameter, hole spacing, and the thickness of the plate are investigated. The method aims at a uniform cross beam dose distribution of a controllable relative magnitude, with depth dose characteristics similar to those of the unimpeded beam.

Major Findings

Initial experiments are highly encouraging. Film dosimetry is the most convenient technique of measurement.

Significance to Biomedical Research and the Program of the Institute

The method may be of major importance in electron beam therapy in special categories of lesions such as mycosis fungoides. It is quite possible that abutment of electron fields, may be facilitated. It is also conceivable that electron beam "wedge fields" may be feasible.

The general understanding of broad beam electron dosimetry may also be improved.

Proposed Course

Systematic investigation of the significance of:

a) hole diameter
b) hole spacing
c) sieve-surface-distance
d) thickness of sieve
e) material of sieve
f) electron energy

in regard to - dose uniformity
- relative depth dose (beam energy)

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06372-01 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Extension of the Net Fractional Depth Dose for Inhomogeneity Correction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	B. Arora Chin	Radiation Physicist	ROB, NCI
	R. Creecy	Computer Specialist	ROB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	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.)*

The correction for the effect of inhomogeneity in body tissues is of considerable importance in the treatment optimization especially, of lesions in the thoracal region, such as the tumors of the breast, the esophagus, or the lung. The advent of CT has made this meaningful. The recently described NFD is being extended by the incorporation of the density scaling concept in the field size as well as the depth parameter, in the analytical formalism. Initial results are highly encouraging. The method appears to be accurate, versatile and easy to implement.

PHS 6040 (Rev. 1/84) GPO 914-916

Project Description

Objective: Accurate analytical description of the along-the-ray dose distribution in inhomogeneous media for the photon energies ranging from 4 - 20 MV, under clinically relevant conditions.

Methods Employed

The NFD formalism is extended by the introduction of the density scaling theorem: in principle, replacing the field parameter (the side of the equivalent square) by ρc, ρ being the relative electron density, and the depth by the radiologic depth $\sum \rho_i \Delta d_i$. A simple solution is introduced to deal with the "spill-over" across interfaces. Results are compared with experimental data from the literature as well as with local measurements.

Major Findings

No new parameters or new coefficients are required. Agreement with measured data is excellent, and often superior to existing methods. The method is easily implemented into the beam treatment program.

Significance to Biomedical Research and the Program of the Institute

1) The new method is more accurate than other existing "global" methods.

2) It improves the accuracy of computed dose distributions generated for tumors in the thoracal region.

3) It appears that the "effective depth" method overestimated the dose to lung for breast treatment, but is reasonably accurate in the unit density areas.

4) The method is easily implemented.

Proposed Course

Continued verification for various beam energies in clinically significant conditions.

Publications

1. van de Geijn, J., and Fraass, B.A.: The Net fractional depth dose: A basis for a unified description of FDD, TAR, TMR and TPR. Med. Phys. 11: 784-793, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06373-01 RO

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Radiation Therapy Treatment Planning Optimization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. Wolbarst Radiation Physicist ROB, NCI

COOPERATING UNITS *(if any)*
University of California
Lawrence Berkeley Lab, Donner Lab, Berkeley, CA (J. Lyman).

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	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.)*

To predict the likelihood of success of a radiotherapeutic strategy, one must be able to assess the effects of irradiation upon both diseased and healthy tissues.

This theoretical work explores a method for determining the probability that a healthy organ irradiated non-uniformly will escape complications. Starting with any treatment plan, an N-step dose vs. cumulative-volume histogram for the organ is generated. This is then reduced by means of an interpolation scheme to a slightly different histogram which corresponds to the same overall likelihood of complications but which contains only N-1 steps. The procedure is repeated until there remains a single-step histogram, for which the complication probability can readily be calculated.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objective: To find a means of quantifying the response of a diseased organism or patient to irradiation.

Methods Employed

This work extends the older Integral-Response and Critical-Voxel Radiological/Probabilistic model approaches to the problem of dose-response characteristics of healthy tissue or tumor irradiated non-uniformly.

Major Findings

A formalism was developed based upon an iterative histogram-reduction procedure; its predictions are consistent with those of earlier approaches, and it seems to agree qualitatively with clinical radiotherapy experience.

Significance to Biomedical Research and the Program of the Institute

As this type of model-building evolves, it may ultimately reach a form which will allow one to select an optimal treatment plan from among a variety of possible strategies; the optimal plan is that one which yields the greatest probability of affecting an uncomplicated cure.

Proposed Course

To be continued.

Publications

1. Wolbarst, A.B.: Optimization of radiation therapy, II: The critical-voxel model. Int. J. Radiat. Oncol. 10: 741-745, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06374-01 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Effect of Radioprotectors and Radiosensitizers on DNA Damage Produced by X-rays

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Neme, title, laboratory, and institute effiliation)*

P.I.:	A. J. Fornace	Cancer Expert	ROB, NCI
Others:	T. J. Kinsella	Deputy Branch Chief	ROB, NCI
	J. B. Mitchell	Radiobiologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

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.)*

Cellular radiosensitivity is affected by many factors which may be clinically important. For example, cellular O_2 concentration and the redox potential of the cell affect cellular radiosensitivity and probably are also important clinically. Several agents, BSO or 2508, have been shown to decrease the effective Sulfhydryl concentration in the cell and to act as hypoxic cell sensitizers. Addition of Cysteamine, on the other hand, protects cells x-irradiated under oxic conditions. With Alkaline and neutral elution, we have measured the effect of these agents on x-ray induced DNA damage, in particular, DNA double strand breaks (DSB), single strand breaks (SSB), and base damage (ESS). We have found that hypoxic irradiation markedly reduces the efficiency of DSB and SSB production by x-rays in V79 cells and reduces the yield of ESS to a lesser extent. When both BSO and 2508 were present during hypoxic irradiation, it markedly increased the yield of SSB and DSB, and had a lesser effect on ESS. The radioprotector Cysteamine produced a marked decrease in the yield of DSB with x-rays, had a lesser effect with SSB, and little or no effect on ESS. The yield of DSB was most affected by hypoxic irradiation, the addition of radiosensitizers, or the radioprotector Cysteamine. Although the lethal lesion produced by x-rays is unknown, indirect results of others indicate that DSB may be the critical lesion. Our work with radiosensitizers and radioprotectors support this hypothesis.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description

Objective: To study the effect of radioprotectors and radiosensitizers on particular types of DNA damage.

Methods Employed

Alkaline and neutral elution. Standard cell culture techniques.

Major Findings

The efficiency of production of particular types of DNA damage, especially double strand breaks, by x-irradiation of mammalian cells is affected by hypoxic irradiation, the hypoxic cell radiosensitizers 2508 and BSO, and the radioprotector Cysteamine.

Significance to Biomedical Research and the Program of the Institute

This work shows that agents which affect cellular radiosensitivity affect the production of different types of DNA damage. Such studies may aid in the development of effective radiosensitizers.

Proposed Course

Studies will be continued on the effect of various radiosensitizers and combination of radiosensitizers on x-ray induced DNA damage. The effect of such agents on the x-ray DNA damage *in vivo* will be considered.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06375-01 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

DNA Damage in X-irradiated Cells Treated with Halogenated Pyrimidines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	A. J. Fornace	Cancer Expert	ROB, NCI
Others:	T. J. Kinsella	Deputy Branch Chief	ROB, NCI
	J. B. Mitchell	Radiobiologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	.05	.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.)*

Treatment of patients with halogenated pyrimidines, in particular IUdR, has been found to increase tumor radiosensitivity. In mammalian cells, BUdR or IUdR pre-treatment increases their radiosensitivity in vitro. We have found that the initial level of DNA single strand and double strand breaks induced by x-rays in V79 rodent cells is increased in cells pre-treated with IUdR or BUdR. At clinically relevant doses, we have found that both DNA single strand breaks and DNA double strand breaks are increased approximately two-fold when 25% of the thymine bases are replaced with IUdR or BUdR. This level of substitution was obtained with a 10 uM dose of IUdR - a concentration which can be achieved in vivo.

Project Description

Objective: To determine the effect of DNA substitution with halogenated pyrimidines on DNA damage in x-irradiated cells.

Methods Employed

Alkaline and neutral elution. Standard cell culture techniques.

Major Findings

DNA single strand and double strand breaks were substantially increased in cells pre-treated with concentrations of halogenated pyrimidines which can be achieved in the blood clinically.

Significance to Biomedical Research and the Program of the Institute

This work demonstrates that increased levels of DNA damage can be detected in cells treated with clinically relevant doses of x-ray and with clinically achievable concentrations of halogenated pyrimidines. This work should provide a basis for *in vivo* studies on the effects of halogenated pyrimidines on radiosensitivity.

Proposed Course

We plan to further characterize the effect of varying concentrations of halogenated pyrimidines on cellular DNA damage produced by x-rays. This approach can be adapted for *in vivo* studies, and eventually we should be able to monitor DNA damage and repair in clinical specimens and determine the effect of IUdR.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06376-01 RO

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Repair Recombination in UV-sensitive CHO Cells.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: A. Fornace Cancer Expert ROB, NCI

COOPERATING UNITS *(if any)*

Eleanor Roosevelt Cancer Center, University of Colorado, Denver, CO (C. Waldren).

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	.05	0.05

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.)*

UVI cells were isolated by C. Waldren as a UV sensitive CHO mutant. The cells have an abnormality in post-replication repair - DNA synthesis on a damaged template. Since post-replication repair is dependent on recombinational repair in bacteria, we decided to determine if these cells have an abnormality in recombinational repair. We have recently shown in mammalian cells that a small fraction of pyrimidine dimers are exchanged into DNA synthesized after UV by a recombination process somewhat analogous to recombination repair in bacteria (Fornace, Nature 304: 552, 1983). The fraction of dimers exchanged in UVI cells was similar to that of the wild type cells.

Project Description

Objective: To determine if an abnormality in recombinational processes occurs in UVI cells after UV-radiation.

Methods Employed

Alkaline elution and standard cell culture conditions.

Major Findings

No difference in dimer exchange frequency between UVI cells and the normal wild type counterpart was seen with our approach.

Significance to Biomedical Research and the Program of the Institute

How DNA is synthesized on a damaged template is a central issue in DNA damage and repair, which is poorly understood in mammalian cells. A recombination process is probably involved, but the defect in UVI cells appears unrelated to such a process.

Proposed Course

Completed.

Publications

None.

ANNUAL REPORT SUMMARY

SURGERY BRANCH
NATIONAL CANCER INSTITUTE

October 1, 1984 to September 30, 1985

Laboratory efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients.

The most significant laboratory accomplishments of the Surgery Branch in the last year are as follows:

1. Therapy with lymphokine activated killer (LAK) cells and recombinant interleukin-2 has been shown to effectively treat established pulmonary and hepatic metastases in a variety of animal tumor models.

2. The mechanisms of the therapeutic effectiveness of LAK cells plus IL-2 has been shown to be due to the *in vivo* expression of transferred LAK cells.

3. Techniques have been established for the isolation of lymphoid cells with specific anti-tumor activity obtained from cells infiltrating growing murine and human tumors.

4. The exact phenotypes of the precursor and effector LAK cells in the mouse and human have been elucidated.

5. The toxicity of recombinant IL-2 administered to rodents has been defined.

6. Monoclonal antibodies have been produced that recognize antigens newly expressed by NIH 3T3 fibroblasts transfected with oncogenes from human tumors. These cell surface antigens result from the transformation of these lines and may be useful in defining the mechanism of oncogene-related transformation.

7. Several hamster animal models of ductal adenocarcinoma of the pancreas and several lines of human pancreatic cancer have been established as experimental models.

8. Several monoclonal antibodies with specificity for pancreatic and other gastrointestinal cancers have been developed and are currently being tested for reactivity in animal models and against panels of human tissues.

9. Monoclonal antibodies conjugated with alpha-emitting heavy metal radionuclides have been developed and successfully used to localize pancreatic cancers in tumor-bearing experimental animals and to destroy pancreatic cancer cell lines *in vitro*.

10. Studies in dogs have defined the tissue tolerance of various normal and surgically manipulated tissues to intraoperative radiation therapy and have enabled dose guidelines to be established for therapy in human patients.

11. Insulin treatment can reverse cancer cachexia in rats, improve host body composition, and survival following tumor resection.

12. Adriamycin impairs wound healing in rats. Chemoattroctants and growth factors, of which transforming growth factor-B is most important, reverse the healing impairment.

13. Hepatocytes from tumor-bearing rats with small tumors exhibit increased rates of gluconeogenesis. Lactic acid may be the cause of this abnormal energy-wasting metabolism.

14. The cytoplasm of human breast cancer cells has been found to contain several molecular weight species of the estrogen receptor. The distribution of molecular weight species from cell lines of differing hormone sensitivity are being studied.

15. The pineal gland hormone melatonin has been found to alter the growth of human breast cancer cells in vitro and in vivo. The plasma hormone melatonin has been found to alter the steroid binding and the nuclear binding of the estrogen receptor in human breast cancer cells.

16. A parathyroid hormone-like factor which causes bone resorption and is produced by human prostate carcinoma has been identified. This factor may provide a more basic understanding about the effect of prostate carcinoma on calcium metabolism in humans and may further our understanding of the pathophysiology of metastatic prostate carcinoma.

17. A prostate tumor derived growth factor which stimulates osteoblastic cells is being evaluated. This factor may have an autocrine effect and should further our understanding of the effect of prostate carcinoma on bone and other tissues.

18. The usefulness of a substance which inhibits 5-alpha reductase has been found to inhibit the growth of human prostate carcinoma in nude mice. This substance may provide a new direction for the treatment of metastatic prostate carcinoma in humans.

Clinical efforts in the Surgery Branch continue to emphasize combined modality treatments.

1. The successful regression of established cancer metastases in humans has been accomplished using therapy with lymphokine activated killer cells plus recombinant IL-2.

2. Clinical trials have been completed defining the immunologic effects of the administration of RIL-2 to cancer patients. Significant findings have been 1) a dramatic increase in IL-2 receptor bearing cells, 2) a 2-12 fold expansion of lymphoid cells in vivo and 3) early migration of IL-2 precursors out of the peripheral blood following IL-2 administration.

3. Clinical trials of recombinant IL-2 have been completed evaluating a range of doses administered intravenously or intraperitoneally, which define dose limiting toxicity. Unusual side effects including pronounced eosinophilia and weight gain have been reported.

4. Prospective randomized trials have demonstrated that adjuvant chemotherapy improves disease-free and overall survival in patients with high grade soft tissue sarcomas of the extremities. "Short" course chemotherapy is as effective as our previously used "long-course" treatment.

5. Prospective randomized trials have demonstrated that limb-sparing surgery is the equivalent of amputation in the treatment of patients with high grade soft tissue sarcomas of the extremities. Disease and overall survival rates in both groups are the same.

6. Analysis of eight years experience with the resection of pulmonary metastases in patients with osteogenic and soft tissue sarcomas has been completed. Improved survival resulting from resection of these metastases has been demonstrated and factors that determine prognosis in these patients has been defined.

7. A prospective randomized trial evaluating neoadjuvant chemotherapy for epidermoid carcinoma of the esophagus is in its third year. To date, 34 patients have been randomized.

8. A prospective randomized trial has demonstrated that local excision, axillary disection and primary radiation therapy is as effective as mastectomy in the treatment of stage I, II breast cancer in women. Further follow-up is necessary.

9. A prospective randomized trial has demonstrated that the psychosocial aspects of primary radiation therapy for breast cancer are well accepted and relieve many of the emotional problems associated with mastectomy.

10. A prospective trial has demonstrated the importance of a thorough disection of the axillary lymph nodes for staging and prognostic purposes in patients with breast cancer.

11. Eleven patients have been evaluated in a protocol designed to test the use of radionuclide coupled antibodies to melanoma injected subcutaneously for the evaluation of nodal disease. 3/3 true negatives and 1/8 true positives were found using this technique. Further work using whole antibodies are planned.

12. A prospective randomized trial comparing regional vs systemic FUdR continuous chemotherapy via the implantable pump for the treatment of colorectal hepatic metastases is continuing. To date, 36 patients have been accrued; no significant differences in overall survival have been seen.

13. Phase I-II studies of intraoperative radiotherapy in various advanced-stage malignancies have been proceeding for five years, providing information on efficacy of local tumor control, treatment toxicity, and tissue radiation tolerance. A dedicated intraoperative radiotherapy suite combining a fully-equipped operating room with a high-energy linear accelerator has been opened and is being used for patient treatment.

14. Various technical innovations for intraoperative radiotherapy have been developed, including a new treatment table, beam applicator system, monitoring devices, and procedure protocols.

15. The only prospective randomized protocols evaluating intraoperative radiation therapy that are being performed at any institution are entering their fifth year. Approximately 90 patients have been given intraoperative radiation therapy since the program was initiated. Randomized trials are proceeding in patients with pancreatic cancer, gastric cancer, and retroperitoneal sarcomas.

16. A prospective randomized trial has been completed demonstrating the efficacy of low molecular weight povidone-iodine solution in preventing intra-abdominal abscesses following surgery in the face of bacterial contamination causing peritonitis.

17. Patients with estrogen receptor or progesterone receptor positive breast cancer have been found to have lower plasma levels of melatonin than normal subjects or ER- or PR- patients. A significant inverse correlation is present between plasma melatonin and the receptor content of breast cancer.

18. An evaluation of tetracycline introduced into the axillary space at the time of axillary dissection has been carried out and demonstrated to be efficacious in decreasing amount and days of drainage through drainage catheters. Although efficacious, significant problems with decreased range of motion in the shoulder precludes its wide spread use. A prospective evaluation of its use in sarcoma patients is being considered.

TABLE I
Consultants
April 1, 1984 - March 31, 1985

Vascular:		
	Arterial exploration	1
	A-V fistula. revision/creation	6
	Carotid endarterectomy	1
	Embolectomy	1
	Placement IVC filter	2
	Repair arterial laceration	1
	Thoracic duct cannulation	2
Orthopedic:		
	Bone biopsy	18
	Arthroscopy	7
	Arthroplasty	1
	Arthrotomy and drainage	3
	Insertion femoral rods	1
	Garceau procedure	1
	Hoffman procedure	3
	Debridement nail bed	1
	Shoulder girdle resection	1
	Tikoff-Linberg procedure	1
	Phalangectomy/Ray resection	3
	Debridement infected amp. stump	1
	Laminectomy	1
Endoscopy:		
	Colonoscopy	2
ENT:		
	Antrostomy	1
	Laryngoscopy	5
	Myringotomy, bilateral	2
	Polypectomy	1
	Excisional biopsy	3
	Tracheostomy	2
	Mastoidectomy	1
	Ethmoidectomy	4
	Radical neck dissection, mandibulectomy	2
	Teflon injection vocal cords	1

TABLE II
Cases - General Surgery
April 1, 1984 - March 31, 1985

General:	
Abdominal-perineal resection	8
Anterior resection	2
Aortocaval lymph node dissection and biopsy for ovarian cancer staging	17
Appendectomy	2
Colon/bowel resection	55
Cholecystectomy	13
Colonoscopy	13
Denver shunt insertion/revision	5
Endoscopy, UGI	5
Exploratory laparotomy ± other procedures	92
Exploratory laparotomy ± Pelvic prosthesis insert/removal	3
Feeding gastroscopy	1
Gastrectomy	3
Gastrostomy	13
Hepatic resection	10
Hepatic resection w/insertion Infusaid(TM) pump, Cholecystectomy	6
Herniorrhaphy/hernia repair	10
Insertion systemic Infusaid (TM) pump/removal	20
Laparoscopy	3
Major soft tissue or muscle group excision	69
Pancreatectomy - partial/total	9
Peritoneoscopy	2
Polypectomy with sigmoidoscopy/sigmoid bx/EUA	2
Revision of colostomy	3
*Radiation therapy - intraoperative	19
Splenectomy	8
Staging laparotomy ± splenectomy for lymphoma	21
Total pelvic extenteration	1
Endocrine:	
Adrenalectomy	7
Excision Gastrinoma	4
Excision pheochromocytoma at Exploratory Laparotomy	5
Mediastinal exploration	6
Pancreatic exploration - insulinoma	5
Parathyroidectomy	22
Parathyroid autograft implant/removal	4
Thyroidectomy - complete/subtotal	21

TABLE II - Continued

Vascular:		
	Aorto-caval exlporation	1
	A-V shunt/shunt revision/shunt removal	2
	Embolectomy	2
	Filter placement, inferior vena cava	3
	Hickman atrial catheter placement	105
	Infusaport placement	8
	Subclavian line placement (Double-Lumen)	19
	Temporal artery biopsy	2
	Thoracic duct cannulation	9
	Vena caval graft	1
GYN:		
	Oophorectomy	1
	Total abd. Hysterectomy	2
ENT:		
	Caldwell-Luc Antrostomy	1
	Parotidectomy	1
	Radical neck dissection	3
	Tracheostomy	5
Surgery for Melanoma:		
	Excision solitary nodules/nevi	7
	Lymph node dissection: - Superficial groin	3
	- Axillary	3
Urology:		
	Aortocaval/retroperitoneal lymph node dissection	6
	Circumcision	2
	Cystoscopy + other procedures, biopsy	53
	Nephrostomy/nephrolitotomy/nephrectomy	7
	Orchiectomy/orchiopexy	6
	Prostatic biopsy	2
	Suprapubic prostatectomy	1
	Testicular biopsy	7
	Testicular implant	2
	Transurethral prostectomy	8
	Urethral dilation/Insertion ureteral stints	17
	Fulgeration bladder	9
	Insertion penile prosthesis	4
	Excision hydrocele	1
	Looposcopy	1
	Insertion suprapubic catheter	1

Breast:		
	Axillary node dissection with biopsy	25
	Breast biopsy	75
	Modified radical mastectomy with tissue expander insertion	20
	Modified radical mastectomy without reconstruction prep.	10
	Simple mastectomy	6
Orthopedics:		
	Above elbow amputation	1
	Below knee amputation	3
	Bone biopsy	8
	Finger amputation	1
	Hemipelvectomy	4
	Hip disarticulation	1
	Stump revision	2
	Sacrectomy	1
Plastic and Reconstructive:		
	Amnion implantation/biopsy amnion graft	19
	Debridement	6
	Dermabrasion	1
	Latissimus dorsi/myocutaneous flap reconstruction	10
	Revision/primary breast reconstruction	20
	Scar revision	3
	Skin grafting, STSG	13
Thoracic:		
	Unilateral thoracotomy	
	Biopsy nodules	47
	Chest wall resection	6
	Pneumonectomy	1
	Lobectomy	13
	Drainage empyema/ligation bleeding	4
	Bilateral thoracotomy/median sternotomy	44
	Pericardectomy/median sternotomy	2
	Mediastinoscopy	6
	Bronchoscopy	23
	Esophagogoscopy/dilatation ± other procedures	31
	Esophagogastrectomy	11
	Esophageal bypass/colon interposition	1
	Rib biopsy, open	6

TABLE II - Continued

Minor:

Biopsy: Node	97
Incision and drainage of abscess	11
Biopsy: Tissue mass, NOS	64
Insertion/removal abdominal catheter for chemotherapy	14
Wound repacking/debridement/resuturing	16
Excision nail	2
Application rigid dressing	1
Open liver biopsy	1

24 Surgical procedures performed in support of patients with AIDs (procedures listed elsewhere)

TABLE III
SURGICAL SERVICES DEPARTMENT
ANNUAL STATISTICS
APRIL 1984 - MARCH 1985

TOTAL PROCEDURES	HOURS	INSTITUTES/OTHERS	TOTAL PROCEDURES	
455.50	1742.25	Ward (NCI)	189	Emergencies
902.75	1865.75	Consult (NCI)	223	Add-Ons
130.00	243.00	Med. Br. (NCI)	432	Cancellations
1488.25	3851.00	TOTAL (NCI)	198	OPD's
			45	2WCSR
1488.25	3851.00	NCI	-	ICU-2J
320.25	1480.25	NHLBI	2	MICU-10D
129.50	597.50	NINCDS	13	Other Cath Lab
37.50	36.25	Med. Neuro		
99.00	202.00	NEI	2182	Total Cases
25.00	54.00	ENT	6388.25	Total Hours
21.50	26.25	ROB		
12.50	51.75	NIDR		
37.50	64.75	Orthopedics		
-	-	NICHD		
11.00	24.50	Other - Outside Consults		

MONTHLY SUMMARY

January	184	Total Procedures	July	202	Total Procedures
	547.50	Total Hours		591.00	Total Hours
February	174	Total Procedures	August	218	Total Procedures
	476.00	Total Hours		572.75	Total Hours
March	184	Total Procedures	September	151	Total Procedures
	526.25	Total Hours		468.00	Total Hours
April	186	Total Procedures	October	197	Total Procedures
	584.75	Total Hours		549.75	Total Hours
May	183	Total Procedures	November	166	Total Procedures
	584.00	Total Hours		488.25	Total Hours
June	185	Total Procedures	December	152	Total Procedures
	538.00	Total Hours		462.00	Total Hours

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM03800-15 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Surgical Consultants & Collaborative Research Involving Surgical Services at NIH

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.A. Rosenberg	Chief of Surgery, NCI	SURG, NCI
Others: Entire Staff	Surgery Branch	SURG, NCI

COOPERATING UNITS *(if any)*

GD Aurbach (NIAMDD), JL Doppman (CC), E Glatstein (NCI), J Robbins (NIAMDD), L Liotta (NCI), RC Young (NCI), P Pizzo (NCI), J Gardner (NIAMDD)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	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 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.

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 VII. Urology

Part VIII. Plastic

Part I. Endocrine Surgery

From July 1984 through June 1985, the Surgery Branch performed thirty-five operations for hyperparathyroidism (Thirty-one cervical operations, and four median sternotomies). All of these patients except one underwent successful procedures and were rendered normo- or hypocalcemic.

Nineteen patients have undergone surgical procedures for solitary thyroid nodules. Twelve underwent lobectomy for benign lesions, and seven underwent total thyroidectomy for malignant disease.

Fourteen patients underwent laparotomy for endocrine tumors of the pancreas. Four underwent surgery for insulinoma. Ten patients underwent laparotomy for gastrinomas.

Part II. Thoracic and Vascular Surgery

Consultative services for thoracic surgical problems are handled through the Surgery Branch. Seventh-eight thoracic surgical procedures were performed for patients referred by consultative services. The Thoracic Oncology Section has participated in major collaborative efforts with both the Pediatric Oncology Branch and the Naval Oncology Branch. A randomized trial of empiric antibiotic therapy for acute diffuse pulmonary infiltrates versus immediate open lung biopsy has been completed and demonstrates the effectiveness and safety of empiric therapy. Trials to evaluate the clonogenic assay for predicting therapeutic responses to chemotherapy for small lung cancer are in progress. Thoracic Surgery procures tissue for these trials.

Part III. Nutritional Support

The Surgery Branch continues to support Clinical Center patients nutritionally by intravenous feeding (TPN). The number of patients has continued to increase yearly.

We have instituted a prospective randomized study of the impact of pre- and postoperative TPN on surgical complications in patients with upper gastrointestinal malignancy. Since July 1983, we have entered 24 patients into the protocol. We have instituted a blinded prospective randomized study of standard TPN vs. branched chain enriched TPN in POB patients undergoing intensification therapy (total body radiation, chemotherapy and bone marrow rescue). Ten patients have been entered on this protocol.

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 Stage I or Stage II. All Stage Ia and Ib patients are being randomized to melphalan or observation for high risk patients following complete surgical tumor resections. Combination chemotherapy is being utilized to treat advanced-stage patients. Intraperitoneal chemotherapy is evaluated as an adjuvant 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. A protocol utilizing high dose cisplatin and cyclophosphamide as induction therapy has begun and demonstrated promising results in the first 24 patients (80% NED at second look surgery). Following 2-4 cycles of chemotherapy, selected patients with a partial response and patients found to be NED at peritoneoscopy will undergo second look laparotomy. 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 1984, the Surgery Branch performed over forty 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 are recently completed. Phase I trials with intraperitoneal misonidozole 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. Thirty-two catheters were inserted 4/1/84-3/31/85.

Part VI. Vascular Access

The Surgery Branch has provided vascular access for patients in the Clinical Center. Renal dialysis and leukaphoresis has been started by placing special double lumen subclavian catheters. Since July of 1984, over 100 Hickman and other catheters were placed to facilitate vascular access in Clinical Center patients. A protocol to study catheter related infections has been introduced with the POB.

Part VII. Urologic Surgery

Consultative services for patients with genitourinary problems are provided through the Surgery Branch. This includes evaluation for hematuria, cystitis, urinary outlet obstruction, nephrolithiasis, impotence, renal, testicular, adrenal or prostate masses, ureteral obstruction as well as evaluations for other benign or malignant abnormalities of the genitourinary tract. Patients with impotence and infertility are also evaluated as well as patients with congenital abnormalities. The Surgery Branch also collaborates with the Medicine Branch in studies of patients with metastatic testicular carcinoma and with a number of other Clinical Center scientists on evaluation and treatment of patients with adrenal neoplasms.

Part VIII. Plastic Surgery

The Surgery Branch provides plastic surgery consultation to the other services in the Clinical Center. This includes consultation in the care and closure of pressure sores, ulcers resulting from the infiltration of chemotherapeutic agents, and hypospadias. The Plastic Surgery Service has also participated in the closure of tissue deficits resulting from sternal wound infections on the cardiac service using musculocutaneous flaps. Breast reconstruction procedures

are offered to all women undergoing mastectomy for breast cancer. The Plastic Surgery Service also particpates in the treatment of head and neck tumors.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM03801-15 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (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 MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	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 spece 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 soft tissue sarcomas, osteogenic sarcomas, colorectal cancer, pancreatic cancer, and gastric cancer. The major emphasis in Surgery Branch cancer therapy is in adjuvant therapy with emphasis on the use of multiple treatment modalities in addition to surgery.

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 XIV.	Quality of Life in the Cancer Patient

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 lymph node dissection alone or lymph node 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. A protocol evaluating the role of radiolabeled anti-melanoma monoclonal antibodies as both diagnostic and potentially therapeutic reagents has begun. The Surgery Branch provides consultative services for these protocols and is directly involved in the Stage II protocol designed to evaluate subcutaneous injection of radioactively labeled anti-melanoma FAB fragments for diagnostic localization of metastases in the draining lymph nodes of melanoma patients with clinically positive regional lymph nodes. Patients then undergo local lymph node dissection (radical neck, axillary dissection or inguinal dissection).

Prospective randomized trials have established that adjuvant chemotherapy increases disease free and overall survival in patients with high grade soft tissue sarcomas of the extremities. Limb sparing surgery has also been shown to result in equivalent survival compared to amputation in these patients.

Prospective randomized trials of the value of adjuvant chemotherapy have also been conducted in patients with head, neck and trunk and retroperitoneal sarcomas. No improvement in disease free or overall survival was seen with chemotherapy in patients with retroperitoneal sarcomas. In patients with head, neck and trunk tumors, patients with high grade trunk wall sarcomas had a statistically significant increase in disease free survival when randomized to receive adjuvant chemotherapy. There is no difference in overall survival in this group.

In the past year, two new soft tissue sarcoma protocols have begun. Patients with Grade I soft tissue sarcomas are randomized to either receive or not receive adjuvant radiation therapy following surgical resection. Patients with high grade soft tissue sarcomas of the extremities undergo surgical excision and chemotherapy and are then randomized to either receive or not receive radiation therapy.

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 follow-up of 6 1/2 years and a maximum follow-up of 9 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. Recurrence free survival appears to plateau at about 38% compared to 18% for our own historical controls. Overall survival is 3-fold better for the study patients (63% at five years) compared to historical controls (20% at five years).

Twenty-six 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. With minimum follow-up of three years and maximum follow-up of six years analysis of the data still 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 is noted. 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 T_{10} regimen) with no immediate adjuvant treatment. During a 20 month accrual period, the cooperative group put 38 patients to study. Sixteen of these randomized patients have been registered by the NCI. Continued accrual to this study was closed because a highly significant advantage in disease-free survival (56% vs 16% recurrence free at 2 years, $p<0.0013$) favoring the group treated with immediate adjuvant chemotherapy has developed. This has not, as yet, translated itself into any difference in overall survival for the two study groups.

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. A second generation, randomized prospective study, has been written to address this question as well as assess the utility of "neoadjuvant" chemotherapy.

Part IV. Urology

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 masses, 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 alsosteronoma 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. 1) In patients with recurrent or extensive primary rectal cancer, surgery is used to remove the tumor mass. Following this a pelvic prosthesis is inserted and wide field high dose radiation therapy to the pelvis is used in order to achieve a local control. The pelvic prosthesis is to exclude radiation sensitive small bowel from the treatment field. 2) Patients with resectable hepatic metastases are worked up to exclude extra hepatic disease. If tumor masses can be resected then patients are randomized to receive or not receive intraperitoneal 5-FU. A protocol has just been concluded in which patients with Dukes B2 and C colorectal cancer were randomized to receive adjuvant intravenous 5-FU or intraperitoneal 5-FU. No difference in survival is yet seen between these groups.

Part VI. Breast Cancer

A prospective randomized trial (Protocol 79-c-111) is being conducted which compares modified radical mastectomy with lumpectomy, axillary dissection and post-operative radiation therapy in the treatment of clinical stage I or stage II breast cancer. The study was initiated in July, 1979, and to date 191 patients have been entered, 95 to the mastectomy arm and 86 to the radiation arm. Accrual appears to be increasing, with 47 patients entered in 1984, compared with 27 patients in 1983. Patients randomized to mastectomy are given the opportunity of reconstruction either immediately or at approximately 6 months post-operatively. Patients with pathologically positive lymph nodes receive Adriamycin/Cytotoxan chemotherapy for 11 monthly cycles post-operatively. Data collected include a comparison of the disease-free and overall survival for the two groups, site of biopsy procedure, lymph node counts and distribution in the axillary specimen, number of positive nodes, morbidity from mastectomy and radiation therapy, sites of recurrence, anatomical function, cosmesis, and psycho-social response to therapy. Median follow-up to date is 36 months, with 115 patients available for two year follow-up. There have been 23 recurrences,

11 in the mastectomy arm and 12 in the radiation arm. Seven mastectomy and four radiation patients have died of disease. Four mastectomy patients are alive with disease, and 8 radiation patients are alive with disease.

There are no statistically significant differences in overall or disease-free surviva between the two groups. Efforts are being made to further enhance accrual to the study. Ongoing interim analysis of the above parameters is being conducted.

Part VII. Endoscopy

The Surgery Branch continues to collect clinical data to help define the appropriate role of laparoscopy in cancer diagnosis and treatment. Laparoscopic tubal ligation, as a consultative service, is available for appropriate patients. Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The uility of this procedure from both the diagnostic and therapeutic point of view of colon lesions is well established.

Part VIII. Computer Applications.

Data for Surgery Branch continued to be collected, stored, and reported for three primary systems: 1) Cancer Patient Research Information System (CAPRI), 2) Protocol-Specific Studies, 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.

Custom programs as well as the soft ware packages available at the NIH Computer Center on the IBM-370 system and the DECsystem are utilized for data entry, program maintenance, remote job submission, interactive computing, and graphic presentations. The Surgery Branch Computing Services Office maintains a library of user manuals for these systems. One CRT editing display terminal (Megadata NIH8188), one high speed printing terminal (Atlanthus T-1222), and one graphics display terminal (Tektronix 4012) provide access to the central computers. An electrostatic copier attached to the Textronix 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 cisplatinum, bleomycin, and vindesine, or surgery alone.

The Surgery Branch, in cooperation with the Radiation Oncology Branch, has initiated investigations of the role of intraoperative radiotherapy (IORT) in combination with surgery for the local control of advanced malignancies. A series of pilot patients with a variety of poor-prognosis abdominal malignancies have been treated by surgical resection, by transportation under anesthesia between the operating room and the radiation therapy facility, by treatment with electron beam radiation directly to the tumor bed with operative displacement and shielding of normal abdominal viscera, and by returning the patient to the operating room for the completion of surgery. The initial experience with 20 patients showed the technique to be feasible and to result in local tumor control rates of approximately 70% for more than one year. A series of six prospective studies, including three randomized protocols, were then established in attempts to define the efficacy and toxicity of intraoperative radiation therapy. IORT studies include: (1) a phase I pilot trial of escalating doses of IORT to tumors in various anatomic sites, to establish efficacy in local tumor control, to determine toxicity, and to perfect technical manipulations; (2) a phase II trial of IORT in sarcomas of the pelvic girdle; (3) a phase II trial of IORT in carcinoma of the lung; (4) a phase III trial comparing IORT to conventional therapy in resectable gastric cancer; (5) a phase III trial company IORT to conventional therapy in resectable and non-resectable pancreatic cancer; and (6) a phase III trial comparing IORT with external beam radiotherapy to conventional radiation therapy in resectable sarcomas of the retroperitoneum. Protocol accrual as of 6/1/85 is summarized below. In 1985, a dedicated IORT facility combining an operating room with a radiotherapy unit was opened and has been utilized for patient treatment.

STUDY	NO. PATIENTS EVALUATED	NO. SUITABLE FOR STUDY	NO. TREATED ON STUDY	NO. GIVEN IORT
pilot	72	52	40	40
pelvis	8	5	5	5
lung	4	4	2	2
gastric	66	40	26	10
pancreatic	158	73	44	22
retroperitoneal	78	43	32	15
TOTAL	386	217	149	94

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 parenchym

as possible. Multiple procedures are often required. Within the past 12 months 54 operations have been performed for resection of pulmonary metastatic disease. A review of an eight year experience with resection of pulmonary metastases from osteogenic and soft tissue sarcomas have been completed. The major determinants of prognosis following resection have been determined. A study comparing computerized gomograms for detection of pulmonary metastases will be published. CT is more sensitive in detecting pulmonary metastases and criteria have been established to improve its specificity.

Part XII. Treatment of Liver Metastases

The Surgery Branch continues its aggressive approach to colorectal cancer metastatic to the liver. Patients who are referred to the NIH and have metastatic disease in the liver undergo resection if four, or fewer, suitably located nodules are identified. After resection, patients are randomized to receive or not receive adjuvant intraperitoneal 5-FU chemotherapy (Protocol 83-C-57). To date, 21 patients have been accrued to this protocol. In addition, protocol 82-C-183, which was initiated in August 1982, was designed to approach the problem of palliation for patients with greater than four metastatic lesions in the liver. Patients who are unresectable and randomized to receive either systemic or intrahepatic continuous infusion of FUdR. This protocol has accrued 36 patients, 18 on the hepatic artery arm and 18 on the systemic arm. Although the data is too preliminary to allow a statement about survival or even time to progression, we have sufficient experience to realize that the complication rate with the intrahepatic therapy will be substantial. This is primarily due to the toxicity of FUdR but is also due to the problem of duodenal ulcer disease which has been noticed by other investigators in the past. In addition, there is evidence that patients will develop metastatic disease outside the liver despite apparently good control of metastatic liver disease with regional chemotherapy. Thus the concept of regional therapy may be severely limited from the outset. The continuing accrual to this trial and continued follow-up will allow us to make statements about which treatment is most efficacious.

Part XIII. Reconstruction of the Breast

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 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. In July, 1984 we started to use tissue expanders placed at the time of mastectomy for immediate breast reconstruction.

Part XIV. Quality of Life in the Cancer Patient

The Surgery Branch is conducting various protocols to address different aspects related to the quality of life in the cancer patient. A protocol evaluating the efficacy of tazadolene succinate, a new non-narcotic analgesic, in postoperative pain has recently been approved. This protocol will spreahead the evaluation of new analgesics in the treatment of cancer pain in the Surgery Branch.

The Surgery Branch has had an interest in evaluating antiemetics in patients receiving chemotherapy. A randomized, prospective trial evaluating dexamethasone versus prochlorperazine in adriamycin and cytoxan induced emesis has been initiated. Chemotherapy induced emesis remains one of the major reasons for non-compliance in our sarcoma protocols.

A prospective protocol assessing the impact of adjuvant radiation therapy on the quality of life extremity sarcoma patients has been initiated. Assessments of economic status, sexual activity, functional living index and functional capacity of the treated limb are recorded prospectively.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03811-11 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.A. Rosenberg Chief of Surgery SURG, NCI

Others: L. Muul (Expert), S. Shu (Expert), J. Mule (Staff Fellow), S. Schwarz (Biologist), P. Spiess (Biologist), S. Ettinghausen (Medical Staff Fellow), M. Papa (Medical Staff Fellow), E. Shiloni (Medical Staff Fellow), E. Director (Microbiologist), C. Hyatt (Biologist), D. Slavin (Biologist), K. Burchenal (Biologist) SURG, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	6	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.)*

Attempts are being made to develop adoptive immunotherapeutic techniques utilizing the transfer of cells grown in long-term culture in interleukin-2. Techniques for the prolonged growth of cytotoxic and proliferative T cell lines and clones with anti-tumor reactivity have been developed. These cells have been shown to mediate the immunologic rejection of allografts and syngeneic tumors and attempts to use these cells in the adoptive immunotherapy of mouse and human tumors are in progress. A new class of cytotoxic cells has been described in both the mouse and the human. These lymphokine activated killer (LAK) cells develop selective cytotoxicity for cancer cells following incubation in the lymphokine, interleukin-2. The adoptive transfer of these cells into mice bearing established tumors can mediate the inhibition of pulmonary and hepatic metastases. The systemic administration of interleukin-2 has been shown to enhance immune responses in vivo.

In the past year, a new immunotherapeutic trial began studying the effects of adoptive transfer of lymphokine activated killer cells and recombinant IL-2 into patients with advanced cancer. Six objective responses have been seen in 12 evaluable patients including one complete response in a patient with malignant melanoma.

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2. Donohue, J.H. and Rosenberg, S.A.: In vivo administration of lymphokines: A new approach to immune regulation. Surgical Forum (in press).

3. 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 53: 896-905, 1984.

4. Rosenstein, M., Yron, I., Kaufmann, Y., and Rosenberg, S.A.: Lymphokine activated killer cells: Lysis of fresh syngeneic NK-resistant murine tumor cells by lymphocytes cultured in interleukin-2. Cancer Res. 44: 1946-1953, 1984.

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7. Mazumder, A., and Rosenberg, S.A.: Successful immunotherapy of NK-resistant established pulmonary melanoma metastases by the intravenous adoptive transfer of syngeneic lymphocytes activated in vitro by interleukin-2. J. Exp. Med. 159: 495-507, 1984.

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9. Donohue, J.H., Rosenstein, M., Chang, A.E., Lotze, M.T., Robb, R.J., and Rosenberg, S.A.: The systemic administration of purified Interleukin-2 enhances the ability of sensitized murine lymphocyte lines to cure a disseminated syngeneic lymphoma. J. Immunol. 132: 2123-2128, 1984.

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45. Kawamura, H., Rosenberg, S.A. and Berzofsky, J.A.: Immunization with antigen and interleukin-2 in vivo overcomes Ir genetic low responsiveness. J. Exp. Med. (in press).

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47. Lotze, M.T., Matory, Y.L., Rayner, A.A., Ettinghausen, S.E., Seipp, C.A., and Rosenberg, S.A.: Toxicity of interleukin-2 in patients with cancer. J. Clin. Oncol. (in press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06652-09 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Sugarbaker	Head, Colorectal Cancer Section	SURG NCI
Others:	H. Edington	Medical Staff Fellow	SURG NCI
	E.P. Steller	Visiting Fellow	SURG NCI
	R.T. Ottow	Visiting Fellow	SURG NCI
	W. Matthews, Jr.	Chemist	SURG NCI
	F.J. Gianola	Physician's Assistant	SURG NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgery Branch

SECTION

Colorectal Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland, 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	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 two major projects: 1) Assessment of alterations in the immune response brought about through the administration of passively administered alloantibody. The effects of alloantibody on retransplanted skin grafts is currently under investigation. 2) Studies on the use of activated cells to destroy tumor cells both in vivo and in vitro are underway. Biological response modifiers used to activate tumor cells include interleukin-2 and gamma interferon. An adjuvant immunotherapeutic attack on cancer cells remaining after surgery is planned using in vitro activated cells.

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2. McCullough, C.S., Sugarbaker, P.H., and Matthews, W.: Effects of passive enhancement on graft and host: Graft adaptation by alloantibody as the mechanism of prolonged skin allograft survival. Transplantation 37:91-96, 1984.

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5. McCullough, C.S., and Sugarbaker, P.H.: Accelerated murine skin allograft rejection accomplished by adoptively transferred in vitro sensitized Lyt-2 bearing lymphocytes (submitted for publication).

6. Havelaar, I.J., McCullough, C.S., and Sugarbaker, P.H.: Immunotherapeutic effects of F_1 lymphocytes sensitized in vitro by parental stimulator cells Oncology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06654-08 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.F. Sindelar	Senior Investigator	SURG NCI
Others:	S. Kurtzman	Medical Staff Fellow	SURG NCI
	G. Rong	Visiting Fellow	SURG NCI
	D. Ogrowsky	Visiting Fellow	SURG NCI
	H. Hoekstra	Guest Researcher	SURG NCI
	J. Scharff	Biologist	SURG NCI

COOPERATING UNITS *(if any)*

T. Kinsella, Senior Investigator, RAD ONCOL, NCI
A. Russo, Senior Investigator, RAD ONCOL, NCI

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	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.)*

Patients with 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 both hamster and human pancreatic cancers. Monoclonal antibodies have been demonstrated to be cytotoxic to pancreatic cancer cells both in vitro and in vivo. Conjugation of radionuclides to monoclonal antibodies has resulted in cell-kill both in vitro and in vivo as well as permitting imaging of tumor sites in tumor-bearing experimental animals. Tolerance of various normal and surgically-manipulated tissues to intraoperative radiotherapy is being investigated in dogs to determine both acute and long-term toxicity.

PUBLICATIONS

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7. Sindelar, W.F.: Isolation-perfusion of the liver with 5-fluorouracil. Ann. Surg. 201:337-343, 1985.

8. Kinsella, T.J., Sindelar, W.F., DeLuca, A.M., Pezehkpour, G., Smith, R., Maher, M., Terrill, R., Miller, R., Mixon, A., Harwell, J.F., Rosenberg, S.A., Glatstein, E.: Tolerance of peripheral nerve to intraoperative radiotherapy (IORT): Clinical and experimental studies. Int. J. Radiat. Oncol. Biol. Phys. (in press).

9. Rong, G.H., Alessandri, G., Sindelar, W.F.: Inhibition of tumor angiogenesis by hexuronyl hexosaminoglycan sulfate. Cancer (in press).

10. Rong, G.H., Sindelar, W.F. 1985. Experiments evaluating anti-tumor immunity induced by cholesterol hemissuccinate-treated syngeneic cell vaccines. J. Surg. Oncol. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06655-05 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jack A. Roth, M.D., Senior Investigator, SURG, NCI
Harvey I. Pass, M.D., Senior Staff Fellow, SURG, NCI

Others: Stanley P.L. Leong, M.D., Medical Staff Fellow, SURG, NCI
Louis Lanza, M.D., Medical Staff Fellow, SURG, NCI
Robert S. Ames, Biologist, SURG, NCI
Emile E. Trahan, Medical Technician, SURG, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgery Branch

SECTION

Thoracic Oncology Section

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4.0	2.0

CHECK APPROPRIATE BOX(ES)

xx ☒ (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 adversly influence responses to immunotherapy. The expression of tumor associated antigens by autologous human primary and metastatic sarcomas has been clearly defined using a panel of monoclonal antibodies. It has been demonstrated that primary tumors and their metastases express similar antigens but almost invariably, micro-heterogeneity of tumor antigen expression results from antigen absent populations of cells within each tumor. The mechanism of heterogeneity for tumor antigen expression of the B16 melanoma system has been defined using a tumor specific monoclonal antibody. Variation in antigen expression correlated with variations of tumor cell density in culture. Monoclonal antibodies have been produced that recognize antigens newly expressed by NIH 3T3 fibroblasts transfected with oncogenes from human tumors. These experiments define a new technique for the production of anti-tumor monoclonal antibodies and will also be useful in defining the mechanism of oncogene-related transformation. Two monoclonal antibodies have been produced and both are selective for human tumors. Conjugation to the A chain of ricin has produced a potent immunotoxin.

1. Davidson, D.D., Carney, D.N., Sugarbaker, P.H., and Roth, J.A.: Immunoregulatory factors derived from human tumors: Suppression of murine immunologic Functions by fresh and cultured human tumor extracts with identity of in vitro immunobiologic responses for murine and human lymphoid cells. J. Surg. Res. 38: 289-297, 1985.

2. Chang, A.E., Lotze, M.T., Ames, R.S., Roth, J.A. and Rosenberg, S.A.: A large scale method of separatiing multiple lymphokines secreted by the murine EL-4 thymoma. J. Immuno. Pharma. 7: 17-31, 1985.

3. Roth, J.A., Restrepo, C., Scuderi, P., Baldwin, R.W., Reichert, C.M., and Hosoi, S.: Analysis of antigenic expression by primary and autologous metastatic sarcomas using monoclonal antibodies. Cancer Res., 44: 5320-5325, 1984.

4. Roth, J.A., Scuderi, P., Westin, E., and Gallo, R.C.: A novel approach to production of anti-tumor monoclonal antibodies: antibody to a cell surface glycoprotein associated with transformation by a human oncogene. Surgery 96: 264-272, 1984.

5. Funkhouser, W.K., Neckers, L.M., Ames, R.S., and Roth, J.A.: Human tumor cell growth inhibition by a tumor-derived glycoprotein. Surg. Forum. 35: 394-396, 1984.

6. Grimm, E.A., Jacobs, S.K., Lanza, L.A., Melin, G., Roth, J.A., and Wilson, D.J.: Is there a role for IL-2 activated cytotoxic lymphocytes (LAK) in cancer therapy? Proc M.D. Anderson Cancer Symposium, Plenum Press (in press).

7. Lanza, L.A., Ames, R.S., Byers, V., Lee, H., Scannon, P., and Roth, J.A.: Immunotoxin-mediated cytotoxicity of human oncogene-transformed tumor cells. Surg. Forum (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM06657-03 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies in Cancer Cachexia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jeffrey A. Norton, M.D. SURG, NCI

Others: J. Moley, M.D., Medical Staff Fellow, SURG, NCI
T. Lawrence, M.D., Expert, SURG, NCI
R. Inculet, M.D., Visiting Associate, SURG, NCI
C.M. Gorschboth, Medical Technologist, SURG, NCI

COOPERATING UNITS *(if any)*

Seoras Morrison, Ph.D., Laboratory of Theoretical Biology, NCI, NIH

LAB/BRANCH

Surgery Branch

SECTION

Surgical Metabolism Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

An in vitro assay for hepatocyte glucose production (gluconeogenesis) has been developed. Hepatocytes from sarcoma-bearing rats have increased endogenous gluconeogenesis and increased gluconeogenesis from lactate.

Plasma amino acid deficiencies have been described in esophageal cancer patients.[1] Patients with upper gastrointestinal cancer and weight loss have lost more body cell mass than anorexia patients with weight loss.[2]

Exogenous insulin reverses cancer cachexia in rats[3], improves host composition[4], and survival following tumor resection. It has utility as an anti-cachexia agent.

Adriamycin impairs healing in rats.[5] Chemoattractants and growth factors like transforming growth factor-B reverse the Adriamycin-induced healing impairment.[6]

1. Norton, J.A., Gorschboth, C.M., Wesley, R.A., Burt, M.E., and Brennan, M.F.: Fasting plasma amino acid levels in cancer patients. Cancer, in press, 1984.

2. Moley, J.F., Morrison, S.D., and Norton, J.A.: Insulin reversal of cancer cachexia. Cancer Res, in press, 1985.

3. Moley, J.F., Peacock, J.L., Morrison, S.D., and Norton, J.A.: Insulin reversal of cancer induced protein loss. Surgical Forum, in press, 1985.

4. Lawrence, W.T., Grotendorst, G.R., and Norton, J.A.: Reversal of adriamycin induced healing impairment with growth factors. Surgical Forum, in press, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06658-03

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies of the Pineal Gland Hormone Melatonin and Estrogen Receptor Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.N. Danforth, Jr., M.D.	Senior Investigator	SURG NCI
Other:	B. Gabriel	Visiting Fellow	SURG NCI

COOPERATING UNITS *(if any)*

Medicine Branch

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	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.)*

The role of the pineal gland hormone melatonin in the regulation of estrogen receptor (ER) activity and the growth of human breast cancer cells is being studied in vitro and in vivo. We have shown that melatonin rapidly increases cytosolic and nuclear ER activity of human breast cancer cells, increases the binding of ER to DNA cellulose, but reduces binding to deoxyguanylic acid. The increased ER activity is salt extractible, and is not associated with a change in sedimentation rate on sucrose density gradients or molecular weight PAGE. Melatonin has been found to decrease ^{3}H-thymodine incorporation and alter cell growth of human breast cancer cells. We are currently determining the conditions which effect melatonin alteration of growth of human breast cancer cells in vitro and in vivo.

We have also studied the secretion of plasma melatonin in women with breast cancer, women at high risk for breast cancer, and normal subjects. We found that women with ER or PR positive tumors have a lower plasma level of melatonin than ER or PR negative patients of normal subjects. The pattern of secretion of women at high risk is similar to that of breast cancer patients and normal subjects, suggesting that the plasma levels of melatonin are important in determining the hormone dependency of human breast cancer.

We have also characterized the ER from variant human breast cancer cell lines and from patients using PAGE and a radiolabel, tamoxifen aziridine, which binds covalently to the ER. We have found that the molecular weight of the basic ER subunit structure is 62,000 dalton. Several molecular weight species have been identified in the cytoplasm of these cells, and the pattern of this distribution varies according to whether the cells are hormone responsive or nonresponsive.

We are presently determining the significance and the characteristics of these new molecular weight species.

GPO 914-918

1. Danforth, D.N. Jr., Tamarkin, L., Mulvihill, J.J., Bagley, C.S., and Lippman, M.E.: Plasma Melatonin and the hormone-dependency of human breast cancer. J. Clin. Oncol. (In press)

2. Markey, S.P., Higa, S., Shih, M., Danforth, D.N. Jr., and Tamarkin, L.: The correlation between human plasma melatonin levels and urinary 6-hydroxy-melatonin excretion. Clin. Acta. (In press).

3. Tamarkin L., OFX Almeida, and Danforth, D.N. Jr.: Melatonin and malignant disease. Ciba Foundation (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06659 03 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: W. Marston Linehan, M.D., Head, Urologic Oncology Section, SURG, NCI

Others:	Marie Kish	Microbiologist	SURG NCI
	Gerald Andriole	Cancer Expert	SURG NCI
	Tom McClain	Medical Staff Fellow	SURG NCI
	Chi Liang	Visiting Fellow	SURG NCI
	Richard Byrne	Guest Researcher	SURG NCI

COOPERATING UNITS *(if any)*

John Termine, NIDR

LAB/BRANCH

Surgery Branch

SECTION

Urologic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2.5	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 the Urologic Oncology Section of the Surgery Branch the effect of estrogen, LHRH and 5 alpha reductase inhibitors on prostate carcinoma are being evaluated. We also have described and characterized a model for humoral hypercalcemia in nude mice bearing a prostate carcinoma. This is the first description of a model for humoral hypercalcemia for prostate carcinoma. We have also found the hypercalcemic effect to be present in a number of other human prostate carcinomas in nude mice and nude rats. This indicates that the humoral hypercalcemia may be a more generalized effect of the tumor and not localized to the initial cell line in which the effect was observed. We have identified a parathyroid hormone-like factor which is produced by these prostate carcinoma cell lines and we are in the process of characterizing this factor. We have established the presence of bone resorptive activity in prostate carcinoma of tumor extracts and also in the conditioned media from prostate carcinoma cell lines. We are comparing this activity with that found in the hypercalcemia of malignancy seen with hypernephroma tumors. We have also identified an osteoblastic factor which is produced by the prostate carcinoma in tissue culture, i.e. the fact that the tumor-conditioned media stimulates thymidine and proline incorporation by the osteoblastic cell lines. We are in the process of further characterization of the substance, its effect on both osteosarcoma cell lines and human osteoblasts as well as its specificity for human bones and osteoblast precursors. We have found that a 5 alpha reductase inhibitor has a significant effect on the growth of both a human hormone responsive bladder and prostate carcinoma. We have also evaluated the effect of LHRH analogs on the growth of hormone-responsive human genitourinary tumors.

Publications

1. Linehan, W.M., Kish, M.L., McClain, T.D., Santora, A.C.: Identification of parathyroid hormone-like bioactivity and bone resorbing activity in human genitourinary tumors. Surg. Forum (in press).

2. Andriole, G.L., Rittmaster, R.S., Loriaux, D.L., Kish, M.L., Linehan, W.M.: The effect of 4MA, a potent inhibitor of 5 alpha-reductase, on the growth of PC-82, a human, androgen-dependent prostatic cancer. Submitted to J. Urol.

3. Linehan, W.M., Kish, M.L., Chen, S.L., Andriole, G.L., Santora, A.C.: Human prostate carcinoma causes hypercalcemia in athymic nude mice and produces a factor with parathyroid hormone-like bioactivity. Submitted to J. Urol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06660-02 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

The Study of Immune Adjuvants in Rodent Tumor Models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A.E. Chang, M.D. Senior Investigator SURG, NCI

Others: Hilda Wexler, Biologist, SURG, NCI
Donna Perry-Lally, Microbiologist, SURG, NCI
Yvedt L. Matory, M.D., Medical Staff Fellow, SURG, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

XX ☒ (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 laboratory is involved in defining the in vivo biologic effects of immune enhancing reagents in rodent models. In particular, the administration of interleukin-2 (IL-2), an immunoenhancing lymphokine, is being investigated in murine and rat models to establish rational approaches to the immunotherapy of cancer. The bioavailability, toxicity, immune effects and antitumor effects of IL-2 are being examined in these models. Mechanisms of tumor rejection in a specific adoptive immunotherapy model is being investigated. Requirements for successful adoptive immunotherapy are being defined in this model.

PHS 6040 (Rev. 1/84) GPO 904-917

1. Donohue, J., Rosenstein, M., Chang, A.E., Lotze, M.T., Robb, R.J., and Rosenberg, S.A.: The systemic administration of purified interleukin-2 enhances the ability of sensitized murine lymphocytes to cure a disseminated syngenic lymphoma. J. Immunol. 132:2123-2128, 1984.

2. Chang, A.E., Hyatt, C.L., and Rosenberg, S.A.: Systemic administration of recombinant human interleukin-2 in mice. J. Biol. Resp. Mod. 3:561-572.

3. Chang, A.E., Lotze, M.T., Ames, R.S., Roth, J.A., and Rosenberg, S.A.: A large scale method of separating multiple lymphokines secreted by the murine EL-4 thymoma. J. Immunopharm. 7:17-31, 1985.

4. Matory, Y.L., Chang, A.E., Lipford, N., Braziel, R., and Rosenberg, S.A.: The toxicity of recombinant human IL-2 in rats following IV infusion. J. Biol. Resp. Mod. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06661-02 SURG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Immunologic Studies in Patients with Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. - M.T. Lotze, M.D., Senior Investigator, SURG, NCI

Others - Mary H. Custer, Microbiologist, SURG, NCI
John M. Skibber, M.D., Medical Staff Fellow, SURG, NCI
Kevan Roberts, Ph.D., Visiting Fellow, SURG, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	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.)*

Our laboratory's major work is in the development and evaluation of immunologic reagents in patients with malignancy. Preparation of single cell suspension from human tumors and evaluation and derivation of cloned and bulk populations of autologous lymphocytes reacting to them remain a major goal. In the last year, over 60 tumor preparations have been evaluated and currently attempts to grow tumor reactive cells directly from tumors as well as lymph node samples is being evaluated. Results of our mixed lymphocyte tumor interaction projects as well as cloning projects have been published. As part of the overall laboratory effort occurring in the Surgery Branch to evaluate lymphokine activated killer cells as a promising immunotherapeutic approach conducted in Dr. Steven Rosenberg's laboratory, we have been evaluating the long-term growth of these cells and have defined the precursor relationship to mature T cells. We have evaluated numerous culture conditions and believe that a moderate 10-100 fold expansion can be carried out and hope to apply this to patient protocols. In addition, projects evaluating purification of the LAK precursor for use in binding studies as evaluated by flow microfluorimetry are being conducted 50-100 fold enrichment of LAK precursors has been obtained.

In addition, investigation of the cellular interaction with tumor as well as the LAK phenomenon, we've carried out an active program of investigation of the in vivo use of interleukin-2. Extensive studies with over 39 patients with both natural and recombinant IL-2 are being conducted as well as an extensive laboratory evaluation. Significant findings of these studies have defined a short half life of interleukin 2, profound immunologic effects with rapid regress of lymphokine activated killer precursor cells from the vascular compartment, development of Tac positive cells which appear to be leu 3+, 2-, indicating a possible expansion of helper cells as well as the demonstration of soluble IL-2 receptors

PHS 6040 (Rev. 1/84) GPO 904-917

in the serum of patients receiving IL-2. These findings have been reported in detail. Future efforts will be designed to evaluate the role of IL-2 alone in an adjuvant setting in stage 2 melanoma as well as a continued evaluation of its use in the treatment of primary peritoneal tumors.

1. Lotze, M.T.: Treatment of Immunologic Disrorders in Patients with AIDS. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Acquired Immune Deficiency Syndrome. Philadelphia, Lippincott, 1985.

2. Lotze, M.T., Rayner, A.A., and Grimm, E.A.: Problems with the isolation of lymphoid clones with reactivity to human tumors. In Behring Institute Research Communications, in press.

3. Lotze, M.T., Rosenberg, S.A.: Treatment of immunologic disorders in AIDS patients. In DeVita V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Acquired Immune Deficiency Syndrome. Philadelphia, Lippincott, 1985

4. Lotze, M.T., Matory, Y.L., Ettinghausen, S.E., Rayner, A.A., Sharrow, S.O., Seipp, C.A.Y., Custer, M.C., and Rosenberg, S.A.: In vivo administration of purified human interleukin-2. II. Half life, immunologic effects and expansion of peripheral lymphoid cells in vivo with recombinant IL-2. J. Immunol., in press.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE BIOLOGICAL RESPONSE MODIFIERS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1984, through September 30, 1985

INTRODUCTION

The Biological Response Modifiers Program (BRMP) was officially begun on April 27, 1981. It was established as a comprehensive program of the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), with both extramural and intramural components charged to investigate, develop and bring to clinical trials biological therapeutic agents that may alter host responses or have direct effects on cancer growth and metastasis. The first Associate Director of the BRMP was Dr. Robert K. Oldham who served from October 20, 1980, to February 1984. Dr. Ronald Herberman served as Acting Associate Director between February, 1984, and April, 1985, and Dr. Dan L. Longo became the Associate Director in April, 1985.

The BRMP has both extramural and intramural components. The extramural program is supervised by the Biological Resources Branch (BRB) through a balanced program of grants and contracts supporting preclinical and clinical research in biological response modifiers in the biomedical community. The branch monitors Phase I and Phase II clinical studies testing the biological effects of selected biological response modifiers in cancer patients and relates the changes to antitumor activity. The BRB supervises an intramural preclinical screening program for the assessment of BRMs. The activities of the extramural component of the BRMP are carried out in consultation with a Decision Network Committee composed of extramural advisors and representatives from a number of DCT and other NIH programs.

The intramural program consists of the Biological Therapeutics Branch (BTB) and the Laboratory of Molecular Immunoregulation (LMI). The BTB currently consists of four sections: the Clinical Investigations Section (CIS), the Monoclonal Antibody Section (MAB), the Natural Immunity Section (NIS), and the Immunopharmacology Section (IS). The BTB (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 BRMs that may be involved in the regulation of tumor growth; (3) developes new biologicals and BRMs and investigates the effects of selected BRMs on host and on tumor growth; and (4) developes clinical protocols for the optimal use of BRMs in the therapy of experimental animal tumors and in cancer patients.

The LMI consists of three sections: the Immunobiology Section (IS), the Lymphokines Section (LS), and the Biochemistry Section (BS). The LMI (1) investigates at a molecular level the intercellular and intracellar processes that regulate host defense mechanisms; (2) studies the lymphokine/cytokine modulation of cellular functions that participate in host defense;

(3) devises new diagnostic tests to better define the immune status and pursue more critical evaluation of BRMs; (4) evaluates the effects of BRMs on immunoregulator pathways and host defense mechanisms; and (5) generates new BRMs that may modify host defense mechanisms.

ACCOMPLISHMENTS - EXTRAMURAL PROGRAM

Grants Program

In fiscal year 1985, active grants numbered 105. About $14 million was awarded in fiscal year 1985. This includes 95 R01s, two R23s, five P01s, one R44, one R13, and one R43. An additional $85,000 was awarded to fund continuation costs for one grant activated in response to an RFA in fiscal year 1982.

Request for Applications

In fiscal year 1985, the BRMP issued one RFA: The Use of Oncogene Related Products for Cancer Therapy. Eighteen applications were received and five received fundable scores.

Program Announcements

In fiscal year 1985, the BRMP issued six Program Announcements: Use of Growth Factors, Maturation Factors, and Antigrowth Factors in Animal Tumor Models; Development of Genetically Engineered Cell Products; Use of Tumor Associated Antigens as Immunogens; Development of Cell Lines Producing Lymphokines and Cytokines; Determination of the Therapeutic Usefulness of Purified Cytokines and Anticytokine Monoclonal Antibodies in Cancer Models; and Determination of the Therapeutic Usefulness of Purified Cytotoxins. Grant applications are still being received for these Program Announcements. So far there have been 151 grant applications recefed and, of these, 27 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 fiscal year 1985, 21 contracts were active. During fiscal year 1985, the BRB awarded approximately $3.8 million for 18 extramural contracts, including $1.2 million for six new clinical task order contracts and two supplements and $2.6 million in ten nonclinical contract awards: two were new awards, six received incremental fundings, and two were recompeted. These contracts, in general, provided for (1) the collection, storage, testing and quality assurance of BRMs, (2) the development of monoclonal antibodies and cytokines, (3) technical support for the review and evaluation of BRMs, and (4) Phase I/II clinical trials. Contracts currently in the BRMP include those related to collection, storage, quality assurance, clinical development and distribution of BRMs, characterization of biochemical analysis and biochemical analysis of BRMs, production of hybridomas secreting monoclonal antibodies against lymphokines and cytokines, chemical coupling of cytotoxic agents to monoclonal antibodies, technical support for review and evaluation of BRMs, and procurement of BRMs for preclinical and clinical testing.

Phase I/II Clinical Trials

In fiscal year 1980, 27 institutions were awarded a Master Agreement permitting them to compete for the the Task Order Contracts to perform Phase I/II clinical trials with BRMs. In fiscal year 1984, the BRB recompeted and awarded the new Master Agreements to 17 institutions. Task orders for Phase I trials of the alpha interferons, thymosins and MVE-2 have been completed. Phase II trials with lymphoblastoid interferon (Wellferon) are continuing. Five clinical task orders have been awarded: the Illinois Cancer Council, Fred Hutchinson Research Center, and the University of California, San Diego will perform a Phase I/II clinical trial using monoclonal immunoconjugates. The Illinois Cancer Council and the University of Wisconsin will each perform a Phase I trial of recombinant interleukin 2. These studies are set to begin in the fall of 1985. Duke University has completed a Phase I trial with gamma interferon which accrued 16 patients. A dose of 60 x 10^6 u/m^2 was found to be the maximally tolerated dose with EEG abnormalities being consistently observed in patients at that dose level. Other toxicities included hypotension, gastrointestinal upset, fevers, chills, fatigue, and leukopenia. Phase I studies with gamma interferon (Biogen) and beta interferon (Cetus) were conducted at Ohio State University. Dose limiting toxicities for both interferons where fever, chills, fatigue, and anorexia, and minimal antitumor effects were seen. UCLA is completing a Phase I trial of recombinant beta interferon (B Ser 17). The maximum dose achieved was 500 x 10^6 u/m^2, and one patient with melanoma achieved a partial response lasting one month.

The Fox Chase Cancer Center has completed a Phase I trial using a monoclonal antibody called anti-Leu 2A (Becton-Dickinson) which has specificity for cytotoxic and suppressor T lymphocytes. These workers are about to begin a second study to determine the optimal biologic dose of this monoclonal antibody.

In addition to the contract trials noted, several unfunded collaborative trials have been conducted through the BRB Clinical Trials Section using BRMs made available through the BRB Distribution Desk. Trials are ongoing at the University of Hawaii, University of Cincinnati, and M.D. Anderson Hospitals which include studies of poly ICLC and gamma interferon. A study of intralesional administration of alpha and gamma interferons is being initiated at the University of Texas at Galveston in patients with advanced cutaneous malignancy.

Future plans include the competition and award of at least six additional clinical task orders in fiscal year 1985, including requests for clinical studies of monoclonal antibodies, lymphokines, and cytokines, polyribonucleotides, and interferons, alone and in combination with other therapeutic modalities.

The BRB Clinical Trails Section works closely with the Biologics Section of the CTEP. Agents receiving initial evaluation in Phase I and II trials sponsored by the BRMP will then be evaluated for Phase III trials administered by CTEP. Joint input of the BRMP and CTEP staff is provided by the establishment this year of a BRMP/CTEP working group.

Pharmaceutical Company Relationships

The BRMP has established relationships with several pharmaceutical companies including Burroughs-Wellcome, Hoffmann-LaRoche, Schering, Biogen, Cetus,

Genentech, Collaboative Research, and Becton Dickinson. Each of these companies is supplying agents for clinical trials sponsored by the BRMP.

The BRMP conducted a symposium on polyribonucleotides in an effort to focus the designs of clinical trials in this area.

The BRMP tested a variety of preparations of interleukin 2 supplied by industry. As a result of this comparative study, substantial individual variation in biological efficacy was detected, and a single reference standard for IL-2 was established. This reference reagent is intended for use in research and industrial programs to quantitate levels of activity of laboratory reference standard or other sources of IL-2. The BRMP expects to develop other lymphokines and cytokines for reference standards as materials become available in sufficient quantity and purity to warrant a reference reagent being established.

Preclinical Contracts

The BRMP has nine preclinical contracts designed to develop, test, standardize, store, distribute, acquire, and produce a variety of BRMs. In addition, the BRB plans to announce SBIR grant and contract proposals in 12 areas including monoclonal antibody conjugates, the detection of cell surface markers for drug resistance, the development of monoclonal antibodies to growth factors and oncogene-related products, and the detoxification of endotoxin among others. Depending upon the availability of funds, additional clinical task orders are planned in Phase I trials of monoclonal antibody immunoconjugates and liposomes containing biological response modifiers.

Preclinical Screening Program

The Preclinical Screening Laboratory under the direction of Dr. James Talmadge subjects putative BRMs to a systematic sequence of assays designed to evaluate their potential clinical efficacy. In addition to the compounds summarized and reported to the BRMP Decision Network Committee as of last year, the following compounds have been screened this fiscal year: Muramyl tripeptide-phosphatidyl-ethanolamine (MTP-PE), MTP-PE incorporated in multilamellar vesicles, six human natural IL-2 preparations, six recombinant human IL-2 preparations, and FK-565. Tumor necrosis factor, poly AU, tuftsin, levamisole with and without 5 FU, poly ICLC with or without 5 FU, Ampligen, and isoprinosine are currently under study.

The study of the human preparations of interleukin 2 have been very interesting. Their in vivo activities require the administration of high doses probably due to the short half-life of the compound. There is some differential effect upon the augmentation of NK cell activity after in vivo interleukin 2 such that hepatic and pulmonary NK cell activities are augmented greater than splenic and peripheral blood NK cell activity. Liposomes incorporating MTP-PE also have a compartmentalized augmentation of NK cell activity. It affects NK cells in the lungs and liver but does not augment splenic or peripheral blood NK cell activity.

It has previously been observed that chronic administration of agents designed to augment NK cell activity ultimately leads to a hyporesponsive state. There are two types of hyporesponsiveness: first, that associated with a generalized

depletion of NK responsiveness and, second, that associated with a hyporesponsiveness only in the spleen and peripheral blood. Studies are underway to evaluate whether these types of hyporesponsiveness are related to a depletion of NK cell precursors or reflect a selective trafficking of NK cells to particular organ stes not assayed.

Poly ICLC, MTP-PE incorporated into liposomes, and FK-565 are the agents with the best long-term macrophage activating capacity. Poly ICLC, OK-432, and FK-565 have been found to be excellent NK cell augmenting agents.

Treatment of mice with UV-induced skin tumors with liposomes incorporated with MTP-PE, poly ICLC, OK-432 have resulted in significant prolongation of survival. An interesting observation in the autochthonous tumor-bearing animals has been that the chronic administration of immunomodulators in the older tumor-bearing animals requires the administration of significantly lower doses of the agent than that required in either normal animals or younger animals bearing transplantable tumors.

In summary, correlations have been noted between the optimal immunomodulatory dose and the optimal therapeutic dose in tumor-bearing animals both of which were disparate from the maximum tolerated dose of a particular agent. It has been noted as a result of the preclinical screening program that many BRMs express a bell-shaped curve not only for immunomodulation but also for therapeutic activity such that high doses are not as effective as lower doses. Differences in effects have also been noted in normal animals versus tumor-bearing animals. Furthermore, the optimal therapeutic approach appears to vary with the tumor burden, the tumor site, and the age of the animals. We are eager to test the predictions of the preclinical screen in the design of clinical therapeutic protocols.

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 preclinical screen and to be studied clinically. Members include individuals with expertise in basic research, clinical medicine, pharmacology, toxicology, basic and clinical immunology, and Federal Government regulatory policies. Meetings are held on a quarterly basis.

BRMP Operating Committee (BRMPOC)

The BRMPOC acts as the steering committe for the BRMPDNC. It reviews and prioritizes agents as to their evaluation in the preclinical screen. The BRMPOC makes recommendations for evaluation by the BRMPDNC.

ACCOMPLISHMENTS - INTRAMURAL PROGRAM

The Biological Therapeutics Branch (BTB) and the Laboratory of Molecular Immunoregulation (LMI) concurrently comprise the intramural component of the BRMP. The BTB consists of four Sections: Clinical Investigations; Monoclonal Antibody; Natural Immunity; and Immunopharmacology. The LMI consists of three Sections: Biochemistry; Lymphokines; and Immunobiology.

1. Biological Therapeutics Branch

The Clinical Investigations Section has undergone a change of leadership with the departure of Dr. Kenneth Foon. The new head of the Clinical Investigations Section is Dr. Ronald G. Steis. This section consists of a four-unit inpatient facility at the Frederick Memorial Hospital and a ten-bed outpatient unit at the Medical Pavilion of the Frederick Memorial Hospital. With the addition of Dr. Ronald Steis, a clinical liaison with the Medicine Branch of the Clinical Oncology Program has been established. It is projected that there will be four inpatient beds in the Building 10 Clinical Center on the 12 West unit when that unit opens in September 1985. In addition, in collaboration with the Clinical Oncology Program, two new inpatient beds will be opened at the Frederick Memorial Hospital primarily to accommodate patients receiving lymphokine activated killer cells plus interleukin-2. The mission of the Clinical Investigations Section is to carry out Phase I and II trials of selected biological response modifiers and to perform innovative pilot studies integrating biologicals with other forms of therapies.

A variety of recombinant and nonrecombinant and interferons have been tested in Phase I and some Phase II trials. It has been found that similiar to the toxicities to interferons, interferons dose limiting toxicity consists of a serious flu-like syndrome and granulocytopenia. No antitumor responses have been seen to date. Studies have begun to discover the optimal immunomodulatory doses of recombinant leukocyte interferon and recombinant interferon.

In a previous Phase I trial of recombinant interferon, the maximum tolerated dose was determined to be 50 x $10^6/m^2$ i.m. 3 times weekly. A Phase II efficacy trial has been conducted with the maximal tolerated dose of recombinant leukocyte A interferon in patients with lymphoma. Eighty-four patients with a variety of lymphomas were treated. In 24 patients with a favorable histology lymphoma, a response rate of 56% was seen. In patients with cutaneous T-cell lymphoma, a response rate of 45% has been seen. Only two of 19 patients with chronic lymphocytic leukemia and three of 16 patients with unfavorable histology lymphoma have responded. Currently, patients with favorable histology lymphoma have been randomized to received low-dose daily recombinant interferon (3 x 10^6 units) versus 50 x 10^6 units twice per week to see if the well-tolerated low dose regimen is as efficacious as the high dose schedule. We have also treated 30 patients with hairy cell leukemia with recombinant leukocyte A interferon in low doses (3 x 10^6 units). Complete or partial responses have been seen in 30 of 32 patients. Both of the nonresponders were patients with particularly massive splenomegaly. Eighteen patients with metastatic breast cancer were treated with recombinant A interferon with no patients responding to therapy.

The biological response modifying effects of the interferon inducer poly ICLC has been evaluated. This agent is known to have potent in vitro effects to augment natural killer cell, macrophage, and cytotoxic T cell activity. We infused poly ICLC intravenous twice weekly to patients with biopsy proven malignancy who had previously received standard therapies. The two doses of poly ICLC were 1 mg/m^2 or 4 mg/m^2. To date twenty patients with a variety of different kinds of tumors have entered on this study. Although no antitumor effects have been seen, patients have developed toxicity which has included fevers, fatigue, nausea and hypotension. The agent was demonstrated to induce interferon levels at both doses. However, patients receiving 4 mg/m^2 had consistently higher levels of interferon. Immunologic monitoring has demonstrated

consistent enhancement of monocyte mediated cytotoxicity. However, natural killer cell activity and mitogen-stimulated T cell proliferative responses have in general decreased in response to the therapy.

Two Phase I trials of antitumor monoclonal antibodies have been performed using T-101 in chronic lymphocytic leukemia (CLL) and cutaneous T cell lymphoma (CTCL) patients and using 9.2.27, an antimelanoma monoclonal antibody in patients with disseminated malignant melanoma. Thirteen patients with CLL were treated with T-101 and 12 patients with CTLC received T-101. In general, the patients with CLL demonstrated transient short-lived reductions in circulating leukemia cells with attendant modulation of the target antigen from the cell surface. However, there were no reductions in the size of enlarged organs or lymph nodes in any of the patients. Five of the 12 patients with cutaneous T-cell lymphoma had minimal improvement in skin lesions. Toxicity from the administration of T101 has included mild fever and some shortness of breath upon rapid administration of the antibody preparation. Twenty patients with metastatic melanoma were treated with the 9.2.27 monoclonal antibody that detects a 250,000 molecular weight protein on the surface of melanoma cells. There have been no reductions in the size of any metastatic lesions in these 20 patients. Both monoclonal antibodies T101 and 9.2.27 have been labeled with ^{111}Indium and used to image patients with tumors that react with the antibody. Both antibodies label viseral tumor with a high degree of sensitivity and specificity. An interesting observation from the use of T101 to label CLL cells has been the demonstration that the labeled cells freely percolate into involved lymph nodes. This suggests that there is a substantial exchange of cells from the peripheral blood and the lymph node tissue involved with CLL.

An adoptive immunotherapy protocol has been initiated to test the efficacy of elutriator purified autologous monocytes activated in vitro with interferon in the treatment of colorectal cancer metastasized to the peritoneum. Patients have received weekly administration of their own elutriator purified monocytes activated with interferon administered into the peritoneal cavity via Tenckhoff catheter. The therapy has been administered on an outpatient basis, and two patients have shown clear-cut partial responses that has allowed them to undergo surgical debulking after therapy. Neither patient was felt to be a surgical candidate before the administration of this treatment. Studies of this therapy will be expanded to ovarian cancer in the next year.

The capacity to isolate human monocytes in a resting state and activate them in vitro has allowed some insight into the genetic regulation of the states of activation of monocytes. Dr. Henry Stevenson has demonstrated that low molecular weight messenger RNA for interferon is produced by human monocytes activated to secrete interferon by poly ICLC. However, agents which activate monocytes but do not result in the secretion of interferon such as muramyldipeptide result in the production of a messenger RNA of higher molecular weight that hybridizes with the same interferon probe. It is likely that this model system will allow the genetic dissection of genes activated by monocytes undergoing a variety of stimuli.

Monoclonal Antibody Section

The Monoclonal Antibody Section has been using monoclonal antibodies alone or conjugated with drugs, radionuclides or toxins in a variety of animal tumor models of immunotherapy. Using a xenograft of the human melanoma line FeMX,

workers in this section have demonstrated that the administration of 50 μg grams of antibody 9.2.27 conjugated to gelonin or pokeweed antiviral protein can delay the emergence of tumor in nude mice bearing prepalpable FeMX tumors. This small effect did not result in any difference in the median survival time. Localization studies demonstrated that 1 to 2 percent of a labeled 9.2.27 localizes in the FeMX tumors while 2 to 5 percent of the labeled antibody was found in the liver, kidney, and spleen. Less than 0.05 percent of a radiolabeled gelonin or PAP conjugate of 9.2.27 reached the site of FeMX tumors in tumor bearing animals. Two to 5 percent of the conjugates were taken up by the liver, kidney, and spleen. Thus it appears that the conjugation of antibodies to toxins may alter their biological distribution. The use of a nonspecific monoclonal antibody to saturate nonspecific uptake sites failed to enhance the specific uptake of the labeled monoclonal antibody in the tumor site or to decrease the uptake of the specific monoclonal antibodies by the reticuloendothelial system.

Efforts are being made to conjugate actinomycin-D to the monoclonal antibody 9.2.27. Preliminary experiments in vitro suggest that such conjugates can result in specific kill of melanoma cells bearing the antigen for which 9.2.27 is specific, and such conjugates have no effect on melanoma cells not bearing this antigen. Work is continuing to define the optimum conjugation ratios and to extend these studies to in vivo models.

Natural Immunity Section

The Natural Immunity Section, under the direction of Dr. John Ortaldo is concerned with the study of natural cell mediated immunity to tumors in man and animals, characterizing the effector cells and studying the effects of a variety of modulators on the development, activation, and distribution of these cells. This section is also studying the role of natural immunity in resistance against tumor growth.

The cell surface phenotype of mouse NK cells has been extensively studied. The data support the concept that spleen or bone marrow large granular lymphocytes (LGLs) exhibiting NK activity are more related to the T lymphocyte lineage than to the myelomonocytic subsets of hematopoietic cells. They are Thy-1^+ but do not bear the T lymphocyte subset markers LYT2 and L3T4. Liver and spleen LGLs have been studied with a panel of 25 monoclonal antibodies against cell surface markers. There are major quantitative differences in the surface expression of a number of these antigens between liver-derived LGL and splenic LGL identifying subsets of natural killer cells.

Studies have been performed to evaluate the mechanism of cytotoxicity by natural cytotoxic (NC) cells as opposed to NK cells. It was found that the NC susceptible target cells, in general, are highly susceptible to tumor necrosis factor; and antibodies to tumor necrosis factor inhibit NC activity. In contrast, NK susceptible target cells are resistant to lysis by tumor necrosis factor, and the killing activity of NK cells was uneffected by anti-TNF antibodies.

The rat LGL leukemias have been extensively studied. Biochemical analysis of the cytoplasmic granules has demonstrated the protein called cytolysin which is a molecule of approximately 60 kD molecular weight that is responsible for the cell lysis by NK cells. Antibodies against these granules purified from

the LGL leukemia inhibit rat and human NK cell activity, antibody dependent cellular cytotoxicity and inhibit the killing of the fungus cryptococcus neoformans by these cells. Molecular biologic studies have demonstrated that the LGL cells have not rearranged the β-chain of their T cell receptor genes suggesting that they are not of mature T cell lineage.

The mechanism of lysis of cells by LGLs has been examined. Comparisons have been made between the cytolysin of NK cells and recombinant lymphotoxin and tumor necrosis factor. The results demonstrate that cytolysin is distinct from both of the other cytotoxic cloned factors. LGLs have been cultured and have been demonstrated to respond to interleukin 2. IL-2 stimulation of LGLs results in the cytotoxicity of a large variety of target cells. In addition, the LGLs have been shown to produce α and β interferons, interleukin 1 and 2, and B cell growth factor.

Natural, recombinant, and hybrid recombinant α, β, and γ interferon molecules have been demonstrated to augment human NK activity, but they varied widely in their potency relative to antiviral activity. A recombinant J species of α interferon has recently been shown to be incapable of augmenting NK activity; however, it was capable of augmenting other leukocyte activities and demonstrated antiproliferative and antiviral activities similar to other cloned α interferons. This has led to studies regarding the structure/function relationships of interferon α and NK boosting activity. As previously mentioned, IL-2 has been demonstrated to augment NK cell activity. Although this augmentation appears to parallel the production of γ interferon by LGLs, the abrogation of antiviral activity with antibody directed against gamma interferon did not abolish NK boosting suggesting that these are distinct pathways.

The adoptive transfer of LGLs into rats with depressed NK activity has been demonstrated to restore in vitro tumor cell cytotoxicity, in vivo clearance of tumor cells from the lungs and to inhibit the development of artifical pulmonary metastases. These results provide the first direct evidence for an important in vivo antitumor role for LGL and suggest that the adoptive transfer of highly enriched LGL populations may be of potential immunotherapeutic value in cancer patients.

An interesting animal tumor model using a murine renal carcinoma (Renca) has been used to evaluate the ability of recombinant IL-2-stimulated cytotoxic lymphocytes plus IL-2 to enhance the antitumor effects of Adriamycin. Chemoimmunotherapy of stage I Renca cured 67 percent of the mice while adoptive immunotherapy or chemotherapy alone cured less than 20 percent of the tumor-bearing animals. The effectiveness of the chemoimmunotherapy alone was improved in stage II or stage III Renca by a bicompartimental approach in which administration of therapy both iv and ip cured over 75 percent of the tumor-bearing mice. In contrast, either iv or ip treatment alone produced no cures. These results demonstrate that adoptive immunotherapy can enhance the efficacy of chemotherapeutic drugs against tumors.

Anticoagulants such as PGI_2, heparin, and warfarin were found to prevent the coating of tumor cells with hemostatic factors. This inhibition was found to augment the capacity of NK cells to eliminate tumor cells and inhibit metastasis formation. The antimetastatic effects of these drugs could be abrogated by

eliminating NK cell activity. This suggests that the combined use of an anti-coagulant which prevents the protection of tumor cells from NK activity along with an agent to boost NK cell activity may have immunotherapeutic potential.

The differentiation capacity of thymocyte subsets has been examined in vivo and in vitro. The most immature intrathymic subset of adult thymocytes appears to be a population of $dLyl^+$, $Ly2^-$, and $L3T4^-$ cells. This subset is capable of repopulating the thymus of irradiated animals but not repopulating other hematopoietic compartments. These $dLyl^+$ positive cells are capable of generating both Lyt2 and $L3T4^+$ T cells upon differentiation in the thymus. Some of the dLyl cells can also be shown to differentiate into double ($Lyt2^+$ and $L3T4^+$) positive cells in short-term culture in vitro. These studies are hoped to lead us to a more detailed understanding of thymocyte differentiation.

Immunopharmacology Section

Dr. Michael Chirigos is the Head of the Immunopharmacology Section which conducts studies to evaluate specific agents for their capacity to boost humoral or cellular immune response when used alone or together with other cytoreductive therapies.

It appears that the majority of biological response modifiers augment both NK cell and macrophage tumoricidal activity. However, Imuthiol appears to selectively augment NK cells and Picolinic acid appears to selectively activate macrophages. Several BRMs have been examined in vitro and in vivo for their capacity to induce the production and secretion of regulatory factors such as colony stimulating factor, interferon, and Prostaglandin E species. It appears that serum levels of colony stimulating factor remained significantly elevated for seven days after the injection of poly ICLC or MVE-2. This is substantially longer than the approximately 7 minute half-life of exogenously injected colony stimulating factor. The effects of poly ICLC and MVE-2 to raise the endogenous CSF level was found to be accompanied by an increase in bone marrow cellularity, and appropriate timing of MVE-2 treatment after cytoreductive chemotherapy can result in earlier recovery of both NK activity and bone marrow function.

The use of both chemotherapy with cyclophosphamide and either MVE-2 or poly ICLC leads to longer survival and increased number of long-term survivors of animals injected with the MBL-2 lymphoma. The therapeutic effects of adding MVE-2 or poly ICLC appears to be related to their capacity to enhance NK and macrophage tumoricidal activity and to reconstitute bone marrow cellularity.

2. Laboratory of Molecular Immunoregulation

Biochemistry Section

The Biochemistry Section has been depleted by the attrition of certain investigators. One of our highest priorities is the recruitment of a suitable leader for this section.

Research in this section has centered around the receptors for interferon α and γ. It has been found that the level of interferon receptor expression varies with the activation state of normal T lymphocytes. Normal proliferating lymphocytes express threefold to fivefold more receptors for interferon γ than resting lymphocytes. However, they decrease their expression of γ interferon receptors

by tenfold after activation. In addition, studies of the receptors for interferon α on hairy cell leukemia cells has been studied. There does not seem to be a correlation between the number of interferon receptors and the likelihood of responsiveness to leukocyte A interferon. The average number of interferon receptors on hairy cell leukemia cells is about 500. Hairy cell leukemia lines have been established, and it has been demonstrated that it takes around 1,000 units/ml of α interferon to interfere with the proliferation of these cell lines. This is a much higher level of interferon than is normally achieved clinically with the injection of 3 million units of interferon. This implies that the interferon may be having an indirect effect to control the proliferation of hairy cells in vivo.

Lymphokine Section

The Lymphokine Section under the direction of Dr. Francis W. Ruscetti investigates the mechanism of action of lymphokines and other lymphocyte-derived growth and differentiation factors. Interesting results have been obtained from the study of the intracellular events of hematopoetic cells upon interaction with stimulatory lymphokines. For example, it has been demonstrated that both IL-2 and IL-3 have nearly identical effects upon targets of their action. In each case there is initially the rapid mobilization of calcium, the stiumulation of phosphatidylinositol turnover, subcellular redistribution of protein kinase C from the cytosol to the cell membrane, and the rapid phosphorylation of a number of proteins which are substrates for protein kinase C. Both IL-2 and IL-3 stimulated the phorphorylation of a 68,000 molecular weight protein within the cytosol of their target cells. Among the membrane associated proteins phosphorylated by protein kinase C is the IL-2 receptor. The relationship between phosphorylated IL-2 receptor and the low and high affinity IL-2 receptors is still under active investigation. Protein kinase C activation is also associated with the IL-2 stimulaton of γ interferon and IL-2 receptor messenger RNA synthesis. Using radio-labeled IL-2 binding and cytofluorometric assays for the expression of the IL-2 receptor, it has been demonstrated that IL-2 stimulates an increase in IL-2 receptor expression on T cells within 24 hours. There is an increased level of IL-2 receptor messenger RNA present in the cells exposed to IL-2, however after IL-2 stimulation there are major differences between the number of IL-2 binding sites (6 - 9,000 per cell) and the number of apparent T cell growth factor receptors as measured by reactivity with anti-Tac (35 - 45,000 per cell). It appears, as a result of these studies, that IL-2 stimulation of T cell growth results in an increased number of Tac antigen binding sites that have lower affinity for IL-2 on the cell surface.

In collaboration with Kendall Smith, dexamethasone sensitive and resistent clones of a given lymphoma cell line that constituitively produces IL-2 have been studied. Dexamethasone blocked cell proliferation of the dexamethasone-sensitive clones and the addition of IL-2 overcame the dexamethasone-mediated growth inhibition. Incubation of the clones with an antibody against the IL-2 receptor inhibited cell growth. This is the first direct evidence that some lymphoid tumors can grow by an autocrine-stimulated growth mechanism.

The above noted observation that MVE-2 can induce CSF secretion in vivo has been exploited in a tumor model of mice bearing the Wehi-3B myelomonocytic tumor which differentiates in response to CSF. Treatment of Wehi-bearing animals with cytoxan followed three days later with MVE-2 increased survival time significantly and rendered nearly half of the animals disease-free.

No effects were seen with tumors that were unresponsive to CSF. These results suggest that the induction of CSF by biological response modifiers may reconstitute hematopoiesis and macrophage function and may block leukemogenesis by inducing terminal differentiation of certain susceptible target cells.

Two murine tumors, a Moloney transformed T lymphoma and a spontaneous lung carcinoma called M109, have been found to secrete factors that stimulate their own growth in a clonogenic assay and in suspension culture. Efforts are underway to explore the effects of antibodies to growth factors or growth factor receptors in these models.

Immunobiology Section

The Immunobiology Section is headed by Dr. Luigi Varesio. It conducts studies on the role of various cytokines on cell growth and differentiation, investigates the intracellular biochemical events associated with cell activation, and the control mechanisms of gene expression in hematopoietic cells.

It has been demonstrated that different biochemical pathways are involved in the activation of macrophages by interferon γ on the one hand and interferon α and β on the other. However, despite these distinct pathways of activation, all three types of interferon induce a major alteration in RNA synthesis in macrophages that are differentiated to express cytotoxic cell activity. Activated macrophages show a specific alteration in ribosonal RNA metabolism causing a selected disappearance of the 28S ribosomal RNA precursor. Ribosomal RNA is synthesized as one long transcript, and it appears as though there are loops of double stranded RNA between the 18S, 5S, and 28S fragments. It appears as though interferon induces enzymes specific for double stranded RNA molecules and that the selective alteration of ribosomal RNA processing is what signals the cell not to proliferate but to mature into a cytotoxic cell.

Recombinant retroviruses carrying activated V-myc and V-raf oncogenes have been demonstrated to immortalize murine macrophages from bone marrow cells. Initially after infection, cells proliferate and subsequently slow down their proliferation. The slowing down of their proliferation has been shown to correlate with their differentiation process. If one exposes the cells to any surface, for example sepharose beads, the macrophages proliferate again instead of differentiating. Removal of the beads then allows the cells to differentiate. This model should provide large numbers of cells for the analysis of the genetic events involved in proliferation and differentiation of macrophages.

Cloned human interferon γ genomic DNA has been successfully inserted into mouse fibroblasts and T cells. The gene is not expressed in fibroblasts under any known conditions of gene induction; however, the human γ interferon gene can be expressed in the murine T cells if the T cells are stimulated with IL-2. The fact that the human gene and its product is under the control of a mouse lymphokine suggests that the gene is under physiologic regulation. This should allow dissection of the DNA surrounding the gamma interferon coding sequences to determine which sequences are involved in the control of the expression of γ interferon.

Using a cDNA expression library and antiserum to the purified 60,000 Dalton molecular weight cytolytic component of LGL granules termed cytolysin, it appears as though a cDNA clone has been isolated which strongly reacts with

the antiserum to cytolysin. This cDNA clone also reacts weakly with whole granule antiserum, and preliminary results suggest that the immunoreactive cDNA clone is about 700 nucleotides in length and represents a multicopy gene.

The production of IL-1 by a human monocyte tumor cell line called THP1 has been shown to be increased by 50 percent by treatment of the cells with 5-azacytidine. These effects last for only six to eight weeks. However, they suggest that demethylation can promote the gene expression for cytokine production in some tumor cell lines. The effects of IL-1 on an IL-1 responsive mouse thymocyte cell line has been studied. It has been demonstrated that the exposure of this cell line to IL-1 results in the increased expression of the c-myc oncogene as well as the expression of messenger RNA for both IL-2 and the IL-2 receptor.

The mechanism of production of IL-1 is unknown. Macrophages release high levels of IL-1 in response to a wide variety of immune and inflammatory stimuli. However, in the presence of anti-Ia antibodies, although IL-1 is still synthesized by murine macrophages, IL-1 does not appear to be released from the cell. Biochemical studies suggest that Ia may participate in the processing of the IL-1 precursor that accompanies the release process. It is also apparent that unlike macrophages which produce IL-1 and secrete it into the culture medium, B cells require contact with T cells in order to produce IL-1. The murine B cell IL-1 appears to remain cell associated and is not released into the surrounding milieu. The reasons for these differences are being investigated. It is clear that IL-1 is produced by normal B cells, NK cells, EBV transformed B cells, and dendritic cells that have accessory-like function. Normal B cell derived IL-1 appears to be biochemically and antigenically similar to monocyte or macrophage derived IL-1. In contrast, IL-1 produced by EBV transformed B cell lines shows some unique biochemical properties and is not inhibited by rat or antihuman monocyte derived IL-1. This transformed cell IL-1-like activity may be a product of a unique or altered gene.

Studies on the rate of production of IL-1 by monocytes reveals it to be present intracellularly within 30 minutes after stimulation and to be secreted at 60 minutes. The molecular weight of the intracellular IL-1 is 23,000 with a minor peak at 30,000. The intracellular IL-1 is largely associated with cytosol and only one to two percent is present in the membrane and particulate fractions of the cells. The secreted form of IL-1 is 17,000 molecular weight. Thus, some processing from a biologically inactive 30,000 or 23,000 molecular weight precursor is involved in the production of the final 17,000 molecular weight soluble IL-1. A partial amino acid sequence has been obtained from the 17,000 molecular weight form of human IL-1. The sequence agrees with the reported cDNA sequences for human IL-1, and radiolabeled purified 17,000 molecular weight IL-1 has been to demonstrate specific IL-1 receptors on some human B cell lines.

IL-1 has been shown to have an antitumor effect on certain tumor cell lines. Clones of IL-1 sensitive tumor cell lines can demonstrate complete sensitivity or complete insensitivity to IL-1. The study of the mechanism of these in vitro effects is in progress.

SUMMARY REPORT

BIOLOGICAL RESOURCES BRANCH

October 1, 1984 Through September 30, 1985

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 (BRMs) research in the biomedical community. The Branch monitors Phase I and early Phase II clinical studies which assess biological effects of BRMs 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 BRMs. A resource distribution system encompassing both information acquisition and assessment, as well as agent acquisition, and testing has been established.

BRANCH PERSONNEL

The offices of the Branch are in Building 426 at the Frederick Cancer Research Facility, Frederick, Maryland.

OFFICE OF THE CHIEF

Chief, BRB ----------	Carl M. Pinsky, M.D.
Program Support Specialist	James D. Doyle
Secretary ----------	Virginia Axline

PRE-CLINICAL SECTION

Head, PFPTS ----------	Cedric W. Long, Ph.D.
Health Scientist Admin.--	Andrew J. Vargosko, Ph.D.
Clerk-Steno (PPT) -------	TBN

CLINICAL SECTION

Head, CTS ----------	Freddie Ann Hoffman, M.D.

BIOLOGICAL RESPONSE MODIFIERS PROGRAM, PROCUREMENT, FORMULATION AND PRECLINICAL TRIALS SECTION (PFPTS)

Cedric W. Long, Ph.D. is the Section Head. This Section is responsible for the identification of BRMs 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 BRMs 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 BRMs and the coordination, planning and monitoring of detailed evaluations of BRMs in relevant systems. The Section Head serves as Project Officer on all BRB contracts in the preclinical area and administratively supervises grant management within the BRB. Dr. Andrew Vargosko serves as the Program Director for Cellular Immunology, Molecular Immunology and Cell Surface Immunology grants in the BRB.

BIOLOGICAL RESPONSE MODIFIERS CLINICAL TRIALS SECTION

Freddie Ann Hoffman, M.D. is the Section Head. The BRM Clinical Trials Section initiates and monitors Phase I and early Phase II clinical trials through the contract Task Order mechanism involving the use of BRMs and administers clinical grants. An important aspect of this function is the close liaison with the Cancer Therapy Evaluation Program (CTEP) of DCT in assessing correlations between changes in immunological reactivity and clinical efficacy and toxicity in these studies.

Monitoring of Phase II clinical trials with CTEP personnel is an ongoing arrangement in which the BRMP and CTEP work conjointly.

The Section Head is also responsible for coordinating efforts with CTEP in the planning and review of Phase III clinical trials which include BRMs.

SUMMARY OF FY 85 ACTIVITIES

During this year the BRB has been involved in the following:

- Issued 6 Program Announcements and received 151 grant applications as a result. Twenty-seven awards were made as a result of the Announcements.
- Maintained 13 contracts for testing BRMs in Phase I clinical trials.
- Initiated 4 Request for Proposals (RFPs) and will award 4 new contracts in FY 1985.
- Awarded 6 new clinical Task Order Contracts under the Master Agreement held by 17 institutions.
- Evaluated 15 compounds in the BRMP preclinical Screen.
- Continued distribution of interleukin-2 (IL-2) Reference Standard to scientific community worldwide.

° Issued 12 SBIR Announcements for contracts and received 46 applications.

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 the leading coordinator of preclinical and clinical research on BRMs. 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.

Liasons 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 areas. A regular working relationship is maintained with the Developmental Therapeutics Program to coordinate drug and biologic development. A cooperative clinical evaluation system with the CTEP has been established to coordinate Phase I, Phase II, and Phase III BRM Clinical Trials. This includes membership of several staff members of CTEP on the BRM Decision Network Committee and the establishment of a BRMP/CTEP working group.

GRANTS PROGRAM

In FY 1985, active grants numbered 105. Some $14.0 million was awarded in FY 1985. This includes 95 ROls, two R23, five POls, one R44, one R13 and one R43. An additional $85 thousand was awarded to fund continuation costs for one grant activated in response to an RFA in FY 1982.

The grants can be categorized in specific areas as follows:

Cell Surface Immunology

Antibodies; Antigens

Molecular Immunology

Interferons; Thymic Factors; Lymphokines

Cellular Immunology

Adjuvants; 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 BRMs in Cancer Therapy. (Includes studies relevant to the safety of BRMs when used therapeutically.)

Table 1 lists each grant title by referral area category.

REQUEST FOR APPLICATIONS

In FY 1985 the BRMP issued one RFA: Use of Oncogene Related Products for Cancer Therapy. Eighteen applications were received, and five received fundable scores.

PROGRAM ANNOUNCEMENTS

In FY 1985 the BRMP issued six Program Announcements: Use of Growth Factors, Maturation Factors and Antigrowth Factors in Animal Tumor Models; Development of Genetically Engineered Cell Products; Use of Tumor Associated Antigens as Immunogens; Development of Cell Lines Producing Lymphokines and Cytokines; Determination of the Therapeutic Usefulness of Purified Cytokines and Anticytokine Monoclonal Antibodies (MoAb) in Cancer Models; and Determination of the Therapeutic Usefulness of Purified Cytotoxins. Grant applications are still being received for these Program Announcements. There have been 151 grant applications received and, of these, 27 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 1985, 21 contracts were active. During FY 1985, the BRB awarded approximately $3.8 million for 18 extramural contracts, including $1.2 million for six new clinical task order contracts and two supplements and $2.6 million in ten non-clinical contract awards: two were new awards, six received incremental funding, and two were recompeted. The contracts active and projected for funding in FY 1985 are shown in Table II. These contracts in general, provided for (1) the collection, storage, testing and quality assurance of BRMs, (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. Contracts currently in the BRMP include those related to collection, storage, quality assurance, clinical development and distribution of BRMs, characterization and biochemical analysis of BRMs, production of hybridomas secreting MoAb against lymphokines and cytokines, chemical coupling of cytotoxic agents to MoAb, technical support for review and evaluation of BRMs and procurement of BRMs for preclinical and clinical testing.

PHASE I/II CLINICAL TRIALS

In FY 1980, 27 institutions were awarded a Master Agreement permitting them to compete for the Task Order Contracts to perform Phase I/II clinical trials with BRMs. In FY 1984, the BRB recompeted and awarded the new Master Agreement to 17 institutions, listed in Table III. In previous fiscal years, fourteen task orders for testing interferons, thymosins and MVE-2 were awarded under the original 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. Two Task Order RFPs were issued in FY 1984 which have resulted in the award of five Task Orders: three institutions (Illinois Cancer Council; Fred Hutchinson Research Center; University of California, San Diego (UCSD)) will perform a Phase I/II

clinical trial using monoclonal immunoconjugates; two institutions (Illinois Cancer Council; University of Wisconsin) will each perform a Phase I trial using IL-2. The Illinois Cancer Council will perform a trial using the anti-T-cell MoAb T101-I^{131} conjugate, which is to be provided by Hybritech. The Fred Hutchinson Cancer Research Center is planning to conduct a trial in melanoma patients with an anti-melanoma antibody conjugate. UCSD is planning on performing a clinical trial with a T101 drug conjugate and is currently performing a preliminary preclinical evaluation to pick the most efficacious conjugate. In these trials, pharmacokinetics, clinical toxicity, and relevant biologic responses will be monitored. The toxicity and biologic activity of IL-2 will be examined in the second Task Order. The Illinois Cancer Council is examining the differences between recombinant IL-2 (Hoffmann - LaRoche) and natural IL-2 (Collaborative Research), while the University of Wisconsin will compare the effects of recombinant IL-2 (Hoffmann - LaRoche) given by single rapid daily versus daily twenty-four hour infusions over a one week period. The above five clinical trials are to be initiated in the spring of 1985. During the previous fiscal year, several Phase I trials of gamma IFN (Duke, Ohio State, UCLA) were initiated or ongoing. Duke University has completed a Phase I trial with gamma IFN accruing 16 patients. A dose of 60 x 10^6 u/m^2 i.v. infusion was given over 20 minutes, four hours and 24 hours. Although no antitumor effects were observed, consistent increases in 2,5' oligo-A-synthetase were observed. Natural killer (NK) and monocyte-mediated tumor killing were inconsistent: control levels increased to very high levels, as do ACTH and growth hormone levels. At the above dose level and schedule, EEG abnormalities were consistently observed and common toxicities included hypotension, gastrointestinal symptomatology, fever and rigors, fatigue and leukopenia. Phase I studies with gamma and beta IFNs conducted at Ohio have just been completed. Eight patients received gamma IFN (Biogen) (dose ranges: 0.01 x 10^6 u/m^2 - 450 x 10^6 u/m^2) and eleven patients received beta IFN (Cetus) (dose ranges: 0.01 x 10^6 u/m^2 - 600 x 10^6 u/m^2). Dose limiting toxicities for both the IFNs were fever, chills, fatigue and anorexia, which was seen in all patients. Although no antitumor responses were observed in the gamma trial, two PRs were seen in patients with renal cell carcinoma. One patient treated with beta IFN had transient proteinuria with no increase in BUN or creatinine. UCLA is completing a Phase I recombinant beta IFN (B Ser 17) trial utilizing two dose-escalation schedules in fifteen patients, followed by a maintenance dose of 30 x 10^6 u/m^2 i.v. given biweekly. Maximum dose achieved was 500 x 10^6 u/m^2 which was well tolerated in 14/15 patients. One patient with melanoma achieved a PR one month after starting maintenance therapy.

Through a subcontract from the Fox Chase Cancer Center, Jefferson Medical Center has completed the initial phase of their trial of anti-T suppressor antibody, anti-Leu 2A (Becton-Dickinson), which evaluated the pharmacokinetics, clinical toxicity, and immunologic responses of anti-Leu 2A in patients with advanced malignancy, at several dose levels. Following a careful evaluation of the data, a second study will be performed to determine the efficacy and toxicity of anti-Leu 2A at the optimal biologic dose, i.e. the dose producing the most consistent reduction of T-suppressor cell population.

In addition to the above contract trials, several unfunded collaborative trials have been conducted through the BRB Clinical Trials Section, using BRMs made available through the BRB Distribution Desk. Trials are ongoing at the University of Hawaii, University of Cincinnati, Portsmouth (Virginia), and M.D.

Anderson which include studies of poly ICLC and gamma IFN. A study of intralesional administration of alpha and gamma interferons will be initiated at the University of Texas at Galveston in patients with advanced malignancy.

Future plans include the competition and award of at least six additional clinical Task Orders in the spring of FY 1985. These will include requests for clinical studies of MoAb, lymphokines and cytokines, polyribonucleotides, and the IFNs, alone and in combination with other therapeutic modalities.

The BRB Clinical Trials Section works closely with the Biologics Section of the CTEP. Agents receiving initial evaluation in Phase Ia, Ib and early Phase II trials by the BRMP will then be evaluated for their introduction into last Phase II and Phase III trials administered by CTEP. Members of both programs serve on the CTEP Protocol Review Committee and on the BRMP Decision Network and Operating Committees. Additional joint input of BRMP and CTEP staff is provided by the establishment this year of a BRMP/CTEP working group. In addition to the BRB Project Officer, clinical trial monitoring is also conducted by an NCI Support Contractor, Theradex, Inc., which is sponsored by the Quality Assurance Section of CTEP.

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 IFN Wellferon through Phase I 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 - LaRoche, Schering, Biogen, Cetus-Shell, Genentech, Collaborative Research and Becton Dickenson. Each of these companies is currently supplying agents for clinical trials or has supplied biologics for past clinical trials.

Additional Task Orders were initiated and awards made in FY 1983, for studies evaluating a MoAb supplied by Becton Dickenson, which recognizes antigens on suppressor T cells. This study is designed to determine both the tolerability and feasibility of administering this MoAb and to study its potential effects on decreasing suppressor cell activity and thereby increasing immunocompetence of patients with malignancy. Additional trials evaluating recombinant gamma IFN (Biogen) and/or recombinant beta IFN (Cetus) were also funded and initiated during FY 1983. These trials will be completed in FY 1985, and will establish the tolerability, toxicity and the biological modifying capabilities of these new recombinant IFNs . New trials were initiated in FY 1984, to study natural human IL-2 (Collaborative Research) and recombinant human IL-2 (Hoffmann - LaRoche).

The BRMP conducted a symposium on polyribonucleotides to determine the current "state of the science" in this area by bringing together many of the investigators actively exploring immunomodulatory effects of Poly IC:LC, Poly A-Poly U and ampligen. Several presentations were made by investigators representing industry, universities and government. Studies focused on both preclinical animal model studies and clinical trials. Based upon results presented and discussions following the meeting, the BRB will sponsor a Task Order for clinical trials in this area.

REFERENCE REAGENTS

At a Lymphokine Standardization Workshop (Nov. 17-18, 1983, Washington, DC), the desirability and feasibility of establishing an IL-2 standard was documented. Further efforts to establish a World Health Organization approved IL-2 standard are underway but may require several years to complete. In the interim, it was felt it would be extremely useful in comparing results and resolving discrepancies, if researchers using IL-2 would quantitate the amount of IL-2 being used in relation to a reference reagent of defined unitage. The BRMP has established a large lot of JURKAT-derived IL-2 of defined unitage for use by investigators as a "Reference Reagent." This reference reagent is intended for use in research programs to quantitate levels of activity of laboratory reference standards or other sources of IL-2. The BRMP expects to develop other lymphokines and cytokines for reference reagents as the materials become available in sufficient quantity and purity to warrant a reference reagent being established.

PRECLINICAL CONTRACTS

The following preclinical contracts have been functioning in FY 1985.

1. Characterization and Analysis of Proteinaceous Materials.

   This contract provides capabilities to chemically characterize peptides, proteins, and glycoproteins that may be used experimentally and/or clinically to modify tumor growth. Assay methods are developed to analyze the substance in bulk dosage form and in common pharmaceutical vehicles. Studies include determination of purity under native and denaturing conditions, amino acid composition, molecular weight, isoelectric point, terminal sequence and development of suitable immunological measurement (radioimmunoassays, etc.) and suitable biological assays for qualitative and quantitative evaluations. In the past year this contractor has analyzed and characterized several lots of naturally occurring alpha, beta and gamma IFNs for activity and purity; examined structural characteristics of the polysaccharide Lentinan solubilized by different procedures; purified the tetrapeptide tuftsin for preclinical screening by HPLC; examined a murine tumor necrosis factor preparation for purity and provided near homogeneous material for MoAb production; and evaluated the biochemical characteristics of several natural and recombinant human IL-2 preparations, natural B-cell growth factor preparations, human IL-1 and CSF and recombinant and natural human beta interferon preparations. This work is currently being performed under contract to the University of Iowa.

2. Technical Support for Review and Evaluation of Biological Response Modifiers.

   This contract provides technical support for the collection, review and compilation of available information on BRMs possible development through preclinical and clinical trials. The contractor has responsibility for obtaining pertinent information from sources in industry, institutes, universities as well as through literature review. Information and references obtained are submitted to the program in the form of five page review articles and one page synopses. Information gathered is stored in a computer data bank. Each year the program specifies about 100 compounds for the contractors to research. The contractor has currently developed reviews and

synopses on MoAb, lymphokines, cytokines, and a variety of immunomodulatory agents. The work is currently being performed under contract to Koba Associates.

3. Chemical Coupling Cytotoxic Agents to Monoclonal Antibody.

This contract performs coupling of chemotherapeutic drugs, toxins and radioisotopes to MoAb directed against specific antigens found on human tumor cells. Appropriate tests are carried out on conjugates to demonstrate that the cytotoxic agent-antibody conjugates retain antigen antibody specificity comparable to the unmodified antibody and cytotoxicity in excess of the nonderivatized cytotoxin. The contractor is required to scale up the appropriate conjugation procedure to provide sufficient quantities of a human use product for preclinical and preliminary clincial trials. Experiments have been ongoing to couple adriamycin, mitoxantrone vindescine, methotrexate, ricin A chain, Yttrium-90, Iodine-131 and Indium-111 to three MoAb: T101, an antibody directed against a human T-cell differentiation antigen; 9.2.27, an antibody directed against a human melanoma cell antigen; and D-3, an antibody directed against a tumor specific guinea pig antigen. The contractor has been supplying T101 and 9.2.27 conjugated to indium for biodistribution and imaging studies and is providing T101, Yttrium-90 and methotrexate for preclinical evaluation prior to clinical trials. This contract is currently held by Hybritech, Inc.

4. Collection, Storage, Quality Assurance and Distribution of BRM

The purpose of this contract is to provide effective inventory, distribution and quality assurance confirmation for BRM. The contractor is responsible for receipt, dispensing, storage, distribution and inventory control of biological agents. Quality assurance evaluation involves specific assays to confirm sterility and assays to determine pyrogenicity and endotoxin levels. The contractor performs general safety tests for biological agents in compliance with Government regulations intended for clinical use and helps in the development of master files and INDs for biologics. Currently, the contractor provides for storage and distribution of more than 50 different biologics. In the past year, the contractor has performed general safety, pyrogenicity, purity and other relevant testing on several lots of MoAb preparations for use in clinical trials from within the program as well as preparations submitted from other NCI and NIH scientists. The contract also provides for ascites production and purification of MoAb and has produced monoclonals specific for melanoma, colon, breast and B-cell cancers, as well as monoclonals directed against TAC receptor, human gamma IFN and human IL-2. Meloy Laboratories, Inc., is the current contractor.

5. Lymphokine Testing

The objective of this contract is to confirm the stated biological properties of lymphokine and cytokine preparations and to quantitate those properties and evaluate purity by testing for other activities. Studies are carried out to evaluate and verify the potential of each BRM to produce biological effects specific to that particular lymphokine. Bioassays routinely performed in the Lymphokine Testing Laboratory (LTL) include assessment of IL-1 (lymphocyte activating factor, LAF), IL-2 (T cell growth factor), BCGF (B cell growth factor), CSF (colony stimulating factor), PDGF

(platelet derived growth factor), IFNs, macrophage activation factor, and B cell differentiation factors. The LTL has also been active in the development and assessment of new bioassays for routine use, such as the fluorometric assay for peroxidase to measure macrophage activation, and the monitoring of ionic fluxes and transmembrane potentials as possible early markers of activity. The LTL has been involved in administration of the BRMP interim IL-2 standard. Data from laboratories around the world receiving the standard have been received, and are being examined statistically to provide information to the scientific community regarding the stability and reliability of the standard.

6. Feasibility Study for the Acquisition and Distribution of BRMs.

The purpose of this contract is to establish the need and requirements of the extramural community for an acquisiton, quality assurance and distribution program for BRM. The contractor has conducted a survey to define potential suppliers and general availability of biologics as well as the amount, time intervals, levels of quality assurance, and standardization, toxicity testing, formulations and relevant in vitro and in vivo biological testing required by potential scientific investigators involved in testing and evaluating biologics. The survey has been conducted by phone, mail questionnaires, and personal contact. Individuals are being contacted in private industry, academic institutions, and government. More than 1,000 guestionnaires were mailed out and about 270 responses were received. The final document will be a written report summarizing all survey results and providing conclusions and interpretation. It is expected that this contract with Technassociates will be completed by July 1985.

7. Production of Hybridomas Secreting Antibodies Reactive Specifically with Human Cytokines.

This contract is for the production of murine MoAb directed against human lymphokines and cytokines. The contractor develops appropriate immunizing protocols to confirm the immunogenicity of the human cytokine in mice, and produces and isolates individual hybridoma clones secreting MoAb. Appropriate radioimmune and biologic assays are developed for screening individual hybridoma clones for antibody reactivity and ability of MoAb to specifically bind to and inhibit each cytokine. The contractor provides anti-cytokine secreting hybridomas and semipurified immunoglobulin derived from the various hybridomas. The contract has developed hybridomas secreting MoAb against human IL-2, and human gamma IFN and has undertaken development of MoAb against human and murine tumor necrosis factor, human B-cell growth factor, human alpha and beta IFNs and IL-1. This contract will be recompeted in late FY 1985.

8. Preclinical Assessment of Monoclonal Antibodies.

This contract will be awarded late in FY 1985. The ability to produce murine monoclonal antibodies against tumor antigens now exists in many laboratories. There is at present a tremendous amount of research and development in progress in industry, academics and government to determine the potential usefulness of monoclonal antibodies and immunoconjugates in tumor classification, tumor diagnosis, tumor detection and cancer treatment. Before a monoclonal antibody can be used in a clinical setting one must

thoroughly establish the specificity of the antibody. This includes molecular characterization of the reactive antigen and determination of its cellular and tissue distribution. Screening of antibody against a variety of tissues to identify antigen positive tissues can greatly facilitate design of the best diagnostic and therapeutic modality. It would also be valuable to have information comparing new monoclonal antibodies with monoclonal antibodies in existence to define new specificities as well as cross reactive relationships among monoclonal antibodies for antigen recognition. The purpose of this contract is to develop a centralized, coordinated program for uniform preclinical testing and evaluation of monoclonal antibodies and their immunoconjugates prior to entry into clinical trials. The contractor will test and evaluate MoAb and immunoconjugates in several test systems: 1) immunoreactivity against a panel of known tumor cells to define relationships with other MoAb and establish epitope reactivities by molecular or serologic means; 2) in vitro cytoxicity assays, soft agar cloning assays; 3) virus testing for LCM, retrovirus and the MAP test; 4) immunohistologic screening to define antigen positive tissues and specificity; 5) antitumor effects in the nude mouse model and subrenal capsule assay; 6) animal toxicology evaluation in rodents and perhaps primates. MoAb will be evaluated at each level of testing and must pass the requirements of specificity and sterility before proceeding to the next level of evaluation and consideration for clinical evaluation.

9. Development of Screening Procedures for Testing the Potential Antitumor Efficacy of Human Lymphokines on Human Cells.

This is a new contract that will be awarded late in FY 1985, and will deal with the development of a screening mechanism for testing purified lymphokines for their potential therapeutic usefulness. Certain lymphokines have already been shown to have antitumor effects in vitro (i.e. lymphotoxin) and in vivo (i.e. tumor necrosis factor). Others (i.e. IL-2) have been shown to be able to expand cell populations with tumor cell killing capabilities. Such systems can be further developed to screen other lymphokines for beneficial anti-tumor cell effects. New and novel systems also need to be developed to screen purified lymphokines either alone or in combination with other lymphokines or with other treatment modalities. This will assist the NCI in determining which of these factors have the greatest therapeutic potential and which factors should be further developed as BRMs. The objectives of this contract are: 1) to develop and standardize methods of effectively screening human lymphokines for direct and indirect anticancer effectiveness in vitro utilizing human cells as effector cells and several different human tumor cells as targets, 2) for human lymphokines that are not species specific, to develop methods of testing the effectiveness of in vivo administration of human lymphokines in animal models to: (a) modify the ability of cancer bearing host to react to its tumor and (b) effect restoration of depressed immune reactivity caused by chemotherapeutic or radiotherapeutic treatment procedures or by the tumor itself, and 3) to evaluate human lymphokines supplied by the BRMP in the above mentioned screening procedures.

NEW INITIATIVES

° The BRB plans to reissue announcements to solicit SBIR grant and contract proposals in the following areas:

1. Monoclonal Antibody (MoAb) Conjugates for Therapy and Diagnosis
2. Development of MoAb to Cell Surface Markers for Drug Resistance
3. Development and Characterization of MoAb to Tumor Growth Factors and Oncogene Related Products
4. Large Scale Production of Human Retroviruses and Their Individual Structural Components and Production of High Titered Polyclonal and Monoclonal Antibodies
5. Development of Novel Methods for Preparation of Monoclonal Antibody (MoAb) Conjugates with Toxins, Drugs and Radioisotopes for In Vitro and In Vivo Therapy
6. Production of Liposomes with Biological Response Modifiers and Medicinals for Therapy
7. Production of Monoclonal Antibodies Directed Against Cytokines
8. Production of Monoclonal Antibodies (MoAb) Directed Against Tumor Associated Antigens and Cell Receptors
9. Production of Tumor Cell Lines Sensitive to Specific Cytokines
10. Production of Detoxified Endotoxin
11. Production of Genetically Engineered Cells Making Cytokines
12. Production of Immunoaugmentive Agents

° A new RFP for the Acquisition, Quality Assurance and Distribution of BRMs is being prepared for release in FY 1986.

° Additional clinical Task Orders are planned for FY 1986, pending the availability of funds: Phase I Trials of MoAb Immunoconjugates and Phase I Trials of Liposomes Containing BRM.

° In FY 1986, the BRB will recompete the projects for Characterization and Analysis of Proteinaceous Materials and Technical Support for the Review and Evaluation of BRMs. These activities are currently being performed under contracts to the University of Iowa and Koba Associates respectively.

PRECLINICAL SCREENING PROGRAM

The Preclinical Screening Laboratory encompasses a broad-based screening program which uses a system of sequential and progressively more demanding studies, designed to determine the therapeutic potential of a BRM as well as to obtain some understanding of mechanism of activity. The "common track screen" provides a systematic preclinical screening of defined chemical and biological agents. This step-by-step approach to the screening of potential BRMs is designed to define their effects on T cells, B cells, NK cells, and macrophage functions. The sequence and progression of assays is as follows:

1. In vitro activation: in vitro testing
2. In vivo activation: in vitro testing
3. In vivo activation: in vivo testing (Therapy and Prophylaxis of transplanted tumors)
4. Therapy of autochthonous tumors

This sequence of studies allows parameters of dose and toxicity, immunomodulation, adjuvancy, and therapeutic potential to be determined in an orderly fashion for all BRMs. Additional information is also obtained for each BRM on optimal therapeutic parameters including dosages, scheduling, duration, and functions to be monitored.

Initial assays are performed with cell populations obtained from normal donors (in vitro:in vitro) or normal donors treated with the BRM (in vivo:in vitro) to define the baseline criteria for positive responses and to exclude the influence of tumor-induced or tumor-associated immunosuppression. Next, BRMs are evaluated for their ability to treat preexistent experimental and spontaneous metastases from syngeneic animals whose primary tumors have been surgically excised. Within the therapeutic models, poly ICLC or liposomes containing MTP-PE have demonstrated outstanding therapeutic potential. In addition, thymosin, OK-432, and Bestatin have also demonstrated excellent therapeutic efficacy.

Finally, agents found effective in these hosts are evaluated for their therapeutic efficacy in rodents bearing autochthonous tumors. These latter therapy models include UV-induced skin tumors (fibrosarcomas, hemangiosarcomas, rhabdomyosarcomas, and squamous cell carcinomas) in mice or NMU-induced mammary tumors in rats. Both of these autochthonous tumor models spontaneously metastasize, which allows the analysis not only of therapeutic effects against primary tumors but also therapeutic potential against systemic disease.

The agents which have been or are to be entered into the Preclinical Screening Laboratory are listed in Table V. Testing has been completed and summary reports or interim reports presented to the BRMP Decision Network Committee (DNC) on MVE-2, Nor-MDP, Azimexone, thymosin alpha-1, thymosin fraction five, rHu IFN-alpha A, nHu IFN-alpha, rHu IFN-alpha A/D, FK-565, Muramyl tripeptide-phosphytidyl -ethanolamine (MTP-PE), MTP-PE incorporated in multilamellar vesicles (MLV), six nHu IL-2, and six rHu IL-2, N-137, poly ICLC, Lentinan, OK-432, Bestatin, and ALP. The agents presently under study in the screen include poly AU, tuftsin, rM IFN-gamma, rHu TNF, rM IL-1, nM TNF, levamisole, levamisole combined with 5 FU, pICLC combined with 5 FU, Ampligen, and rHu IL-2 (Biogen).

To date, thymosin alpha-1, thymosin fraction five, and the recombinant IL-2s, have been found to be the best in vitro T cell immunomodulators. Equidoses of thymosin alpha-1 and thymosin fraction five are required to provide equal T cell stimulation both in vitro and in vivo for adjuvant activity. Although the rHu IL-2s are extremely active in vitro for T cell and NK cell augmentation, their in vivo activities require the administration of high doses perhaps due to renal filtration. There also appears to be some organ sequestering of IL-2 activities such that following i.v. injection, there is a preferential augmentation of hepatic and pulmonary NK cell activities compared to splenic and peripheral blood NK cell augmentation. In addition, there is a marked bell-shaped curve for IL-2 adjuvant activity for cytotoxic T lymphocytes. Liposomes incorporating MTP-PE also have a compartmentalized augmentation of NK cell activity. This particulate immunomodulator selectively augments NK cell activity in the lungs and liver but is incapable of augmenting splenic and peripheral blood NK cell activity. The NK cell augmentation occurs in the absence of any demonstrable systemic or in-situ IFN production.

Animal models for the induction of the NK cell hyporesponsive state, similar to that observed clinically with the chronic administration of IFN, were developed. It has been found that a hyporesponsive state for splenic NK cell activity could be induced not only with rHu IFN-alpha A/D and rM IFN-gamma but also MVE-2, C. parvum, OK-432, and rHu IL-2. This hyporesponsive state could be subdivided

into two different classes. First, that associated with IFN and IL-2 (lymphokines) which resulted in a systemic NK cell hyporesponsive state, including not only splenic NK cells but also peripheral blood, pulmonary, and hepatic NK cells. In contrast, the splenic NK cell hyporesponsive state associated with MVE-2, OK-432, and C. parvum was limited to the spleen and blood, with greatly increased NK activity in the lungs and liver suggesting that the splenic hyporesponsiveness was due to cellular sequestering to the lung and liver. Additional studies revealed that the lymphokine induced hyporesponsive state was reversible by a single injection of a non-lymphokine, NK cell augmenting agent whereas the splenic NK cell hyporesponsive state associated with MVE-2, C. parvum, or OK-432 could be reversed by an injection of either the lymphokines or any agent other than the ones capable of inducing the hyporesponsive state.

Poly ICLC, MTP-PE incorporated into liposomes, and FK-565 have been found to be the best long-term macrophage activating agents studied to date, while both poly ICLC, OK-432, and FK-565 have been found to be excellent NK cell augmenting agents.

At present the best therapeutic activity has been found with poly ICLC, FK-565, liposomes incorporating MTP-PE, rHu IFN-gamma A/D, OK-432, Bestatin, thymosin alpha-1, and rHu IL-2. Less or no activity has been observed with the other immunomodulatory agents. In recent studies using poly ICLC, a determination was made of the maximum tolerated dose, the optimal immunomodulatory dose (in normal mice) and the optimal therapeutic protocol. Using these doses and schedules, an attempt was made to correlate therapeutic activity with immunomodulatory properties for tumor associated macrophages and host spleen cells in animals bearing MBL-2 tumor ascites. In these studies, an inverse correlation (linear regression analysis) was found between the percent of macrophages in the tumor ascites ($p = 0.04$), the cytotoxic activity of the tumor associated macrophages ($p = 0.01$), the CTL activity of the splenic lymphocytes (0.05) and a near significant correlation with splenic NK cell activity (0.08). This study as well as similar studies have revealed that the optimal immunomodulatory protocol in tumor bearing animals is the same as the optimal therapeutic protocol for animals bearing the MBL-2 tumor ascites. Similar studies have revealed that the optimal therapeutic protocol and dose are disparate from the maximum tolerated dose for all BRMs studied to date. Similar approaches have revealed that the optimal immunomodulatory dose or protocol differs in normal animals compared to tumor bearing animals. Furthermore, there appears to be a bell-shaped curve not only for immunomodulation but also therapeutic activity with many immunomodulators. This latter observation is consistant with poly ICLC, FK-565, free MTP-PE, and rHu IL-2. In addition, it has been found that the optimal therapeutic protocol for tumor ascites and systemic (pulmonary) metastases is disparate at least in the case of poly ICLC.

Both the UV-induced autochthonous tumor in mice and the NMU induced mammary tumors in rats have been developed and implemented. This has provided not only a technical challenge but also required an extensive logistical and statistical developmental process. These experiments are approximately one year in duration following the induction of the primary tumors (1 year old). To date, the treatment of mice with UV-induced skin tumors with liposomes incorporating MTP-PE, poly ICLC, and OK-432 have been found to result in significant prolongation of survival. In contrast, the treatment of these animals with thymosin fraction five or Nor-MDP has not revealed any therapeutic activity. A large number of

additional BRMs and therapeutic schedules are ongoing in these models. In the NMU-induced autochthonous rat mammary tumor models, poly ICLC and liposomes incorporating MTP-PE have been found to significantly prolong survival. These studies have revealed that the chronic administration of immunomodulators in these older "tumor conditioned" animals requires the administration of significantly lower doses of the immunomodulator than that required in either normal animals or younger animals bearing transplantable tumors.

In summary, correlations have been noted between the optimal immunomodulatory protocol and optimal therapeutic protocol in tumor bearing animals, both of which were disparate from the maximum tolerated dose. It has been reported as a result of screen results that many BRMs express a bell-shaped curve not only for immunomodulation but also therapeutic activity. Differences were also noted in the immunomodulatory properties of BRMs in normal animals verses tumor bearing animals. Furthermore, the optimal therapeutic protocol and/or optimal therapeutic dose appears to vary dependent on the tumor burden, tumor site (ascites verses systemic metastasis), and the extent of "tumor conditioning" or age; i.e., autochthonous tumor bearing animals have increased toxicity at BRM doses and schedules that were nontoxic in young healthy hosts bearing transplantable tumors.

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 (Table VI) consists of respresentatives from the Division of Cancer Treatment, Division of Cancer Biology and Diagnosis, National Institute of Allergy and Infectious Diseases, Frederick Cancer Research Facility (FCRF), and Office of Biologics, FDA. Four of the ten rotating member slots are reserved for extramural representation. Members have been included who have expertise in: 1) basic research; 2) clinical medicine; 3) pharmacology; 4) toxicology; 5) regulatory policies; and 6) basic and clinical immunology. 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. Meetings are held on a quarterly basis or more frequently if so required.

Biological Response Modifiers Program Operating Committee (BRMPOC)

The BRMPOC acts as the steering committee for the BRMPDNC. Essentially, the BRMPOC reviews and prioritizes agents as to evaluation in the preclinical screen. The recommendations of the BRMPOC are subsequently presented to the DNC. Members of the BRMPOC are asked, on a rotating basis, to review and summarize packages of data from pharmaceutical firms and present their recommendations on any candidate agent to the Committee.

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TABLE I

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
Molecular Immunology		
INTERFERON		
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"
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"
36076-01	Merigan, T.	Stanford University "Clinical and Laboratory Studies of Interferon in Lymphoma"
38661-01	Rubin, B.	Research Institute of the NY Blood Center "Interferons Properties, Action and Patient Prescreening"
39039	Friedman, R.M.	Uniformed Services University of the Health Sciences "A Mechanism of Interferon Action"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
THYMIC FACTOR		
29943-03	Goldstein, A.L.	George Washington University "Role of Thymosin Peptides in T-Cell Differentiation"
LYMPHOKINES		
33429-01	Fink	Cleveland Clinic "Lymphokine Immunotherapy for Cancer Treatment"
33449-01	Rinehart, J.L.	Ohio State University Hospital "Liposome-Macrophage Activating Factor for Clinical Use"
33484-01	Welte, K.	Sloan-Kettering "Lymphokine in Antibody-Dependent (ADCC) Cancer Therapy"
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"
38043	Lachman, L.	M.D. Anderson Hospital and Tumor Institute "Biological Studies of Human IL-1"
38842	Pauly, J.L.	Roswell Park Memorial Institute "Interleukin 2-Mediated Regression of Mouse Breast Tumors"
38937	Mazumder, A.	Baylor College of Medicine "Immunotherapy of Cancer by Lymphokine-Activated Cells"
39286	Slovin, S.F.	Thomas Jefferson University "Developing T Cell Clones Cytotoxic to Sarcomas"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
LYMPHOKINES (CON'T)		
39441	Jones, M.	University of Texas Medical School "Studies of a Human Monocyte Cytotoxicity Factor"
39489-01	Mier, J.W.	New England Medical Clinic Hospital "The In Vivo Effects of Recombinant Human Interleukin-2"
41063	Remold, H.G.	Harvard Medical School "Development of Cell Lines for the Production of MIF and MAF"
Cell Surface Immunology		
ADJUVANTS		
P01 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.	Beilinson Medical Center "Intralesional BCG Immunotherapy in Malignant Melanoma"
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"
37113	Wolmark, N.	University of Pittsburgh "Murine Studies in Surgical Adjuvant Adoptive Immunotherapy"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
ANTIBODIES		
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"
19043-01	Preston, J.F.	University of Florida "Amanitin-Protein Conjugates as Potential Inhibitors"
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 Center "Monoclonal Antibody Therapy of Cancer"
28740-03	Bast, R.C.	Sidney Farber Cancer Institute "Specific Immunotherapy with Monoclonal Antibodies"
29125	Gillespie, G.Y.	University of North Carolina, Chapel Hill "Monoclonal Antibodies to Glioma Cell Surface Antigens"
29544-02	Wang, M.C.	Roswell Park Memorial Institute "Immuno-Specific Chemotherapies of Prostate Cancer"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
ANTIBODIES (CON'T)		
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"
31525-01	Merluzzi, V.J.	Sloan-Kettering "In Vivo Modification of Host Immunity after Chemotherapy"
31699-01	Lee, F.H.	Univ. of Southern California "Adriamycin and Antibody Conjugates in Cancer Therapy"
31753-01	Hawthorne, M.F.	Univ. of California, Los Angeles "Boren-10-Labeled Antibodies in Cancer Therapy"
33280-01	Hammerling, U.	Sloan-Kettering "Antibody Therapy of Transplants and 1° Leukemias"
33361-01	Preston, J.F.	University of Florida "Cell Specific Cytotoxicity of Amanitin Antibody Conjugates"
33397-01	Mendelsohn	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"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
ANTIBODIES (CON'T)		
33462-01	Bankert, R.B.	Roswell Park Memorial Institute "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"
33491	Sears, H.F.	The Wistar Institute "Monoclonal Antibody in Cancer Therapy"
34536-01	Lee, C.	Roswell Park Memorial Institute "Monoclonal Antibody in Treatment of Prostatic Tumor"
36233-01	Mitchell, M.	USC Cancer Center "Specific Active Immunotherapy of Human Melanoma"
36847-01	Goldenberg, D.	Center for Molecular Medicine and Immunology "Chemoimmunotherapy of Transplanted Colon Cancer"
37405-01	Primus, J.F.	Center for Molecular Medicine and Immunology "Tumor Localization and Therapy with Immunolipsomes"
37497	Royston, I.	University of California, San Diego "Monoclonal Antibodies in Cancer Detection and Treatment"
37646	Papsidero, L.	Roswell Park Memorial Institute "Targeting and Therapy of Tumors with Monoclonal Antibody"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
ANTIBODIES (CON'T)		
38011	Hellstrom, I.E.	Oncogen "Monoclonal Antibodies to Melanoma-Associated Antigens"
38845-01	Grant, C.	Pacific Northwest Research Foundation "Monoclonal Antibody Therapy of Feline Leukemia"
39051	Dennert, G.	Salk Institute "Immune Response to Cell Surface Antigens"
39930	Bast, R.C., Jr.	Duke University Medical Center "Specific Immunotherapy with Monoclonal Antibodies"
ANTIGENS		
24628-01	Nowotny, A.	University of Pennsylvania "Relation of Structure to Function in Endotoxins"
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	Niederkorn, J.Y.	University of Texas "Immunological Modulation of Ocular Tumor Metastasis"
36021	Matta, K.L.	Roswell Park "Novel Synthetic Carbohydrate Antigens as Immunogens"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
ANTIGENS (CON'T)		
36120-01	Livingston, P.	Sloan-Kettering "Use of Tumor Associated Antigens as Immunogens"
39748	Griffin, T.W.	University of Massachusetts Medical Center "Anti-CEA Immunotoxins"
39870-01	Huston, J.S.	Creative BioMolecules, Inc. "Engineering of Antibody Binding Sites to Tumor Antigen"
Cellular Immunology		
LYMPHOID CELLS		
25184-03	Klein, E.	Karolinska Institute "Autoreactive Cells in Tumor Patients"
25608-03	Merluzzi, V.J.	Sloan-Kettering "Specific Repair of Drug-Induced Immune Cellular Deficits"
30559-02	Cheever, M.A.	University of Washington "Specific Immunotherapy of Murine Tumors"
31544-01	Hersh, E.M.	M.D. Anderson "Monocyte and RES Functions in Human Cancer"
33035-01	Wing, E.J.	Montefore Hospital Pittsburgh "Anti-Tumor Activity of Colony Stimulating Factor"
33491-01	Koprowski, H.	The Wistar Institute "Monoclonal Antibody in Cancer Therapy"
36669-01	Choi, Y.S.	Sloan-Kettering "Human B-Cell Growth Factors"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
LYMPHOID CELLS (CON'T)		
37131-01	Seon, B.	Roswell Park Memorial Institute "Monoclonal Antibodies for Therapy of Human Cancer"
37218-01	Primus, J.	Center for Molecular Medicine and Immunology "Experimental Immunotherapy of Cancer"
39093	Granger, G.A.	University of California, Irvine "Lymphocyte Released Cell-Toxin"
39248-01	Berd, D.	Jefferson Medical College of Thomas Jefferson University "Augmentation of Human Immunity by Cyclophosphamide"
39675-01	Badger, C.	Fred Hutchsinson Cancer Research Center "Radiolabeled Antibody Therapy of Lymphoma"
40359	Mannick, J.	Peter Bent Brigham Hospital "Adoptive Immunotherapy in Renal Cell Carcinoma Patients"
GROWTH AND MATURATION FACTORS		
19043	Preston, J.F.	University of Florida "Amanitin-Protein Conjugates as Potential Inhibitors"
27765	Hiramoto, R.N.	University of Alabama "Exploitable Mechanisms in Combination Cancer Therapy"
28835	Cohen, S.A.	Research Foundation of State University of New York "The Natural Anti-Tumor Defense System of the Liver"

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

CATEGORY GRANT NUMBER	PRINCIPAL INVESTIGATOR	INSTITUTION AND GRANT TITLE
GROWTH AND MATURATION FACTORS (CON'T)		
33637-01	Gaffney, E.	Pennsylvania State University "Growth Modifiers from Activated Human Leukemia Cells"
33932	Deodhar, S.	The Cleveland Clinic "Therapy of Cancer Metastases with C-Reactive Protein"
38024	Sirbasku, D.A.	University of Texas Medical School "Monoclonal Antibodies to Mamary Tumor Growth Factors"
38100	Levine, A.E.	Baylor College of Medicine "Tumor Inhibiting Factor Purification and Characterization"
38768	Pantazis, C.	Medical College of Georgia "Use of Growth Factors and Anti-Growth Factors in Animal Tumors"
39051	Dennert, G.	University of Southern California "Immune Response to Cell Surface Antigens"
MISC. APPROACHES TO BIOLOGICAL RESPONSE MODIFICATION		
17393-08	Donahoe, P.	Massachusetts General Hospital "Mullerian Inhibiting Substance"
18105-07	Applebaum, F.R.	Fred Hutchinson Cancer Research Ctr. "Immunotherapy Studies of Spontaneous Malignancy"
P01 18221-06	Storb, R.F.	Fred Hutchinson Cancer Research Ctr. "Marrow Grafting in Treatment of Hematologic Malignancies"

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)		
P01 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 In Vivo"
27330-02	Nishioka, K.	University of Texas "Antitumor Activity of Tuftsin"
27579-01	Cohen, E.P.	Univ. of Illinois Medical Center "Use of Syngeneic Hybrid Cells in Leukemia Therapy"
28630-02	Hoffman, M.	Sloan-Kettering "Activation and Function of QA5 + NK Cells"
28835-02	Cohen, S.	State Univ. of N.Y. at Buffalo "Cancer Chemotherapy and Murine Natural Killer Cells"
28891-01	Regen, S.	Marquette University "New Synthetic Carriers for Antitumor Drugs"
32809-01	Butler, G.B.	University of Florida "Molecular Weight Effects on Polymeric Anti-Tumor Agents"
P01 33049-01	Oettgen, H.F.	Sloan-Kettering "Biological Approaches to the Treatment of Cancer"

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)		
34689-01	Bregman, M.D.	Univ. of Arizona Health Sciences Ctr. "Prostaglandin - Biological Modifier of Human Cancers"
35340-01	Papahadjopoulos, D.	University of San Francisco "Liposome-Mediated Intracellular Delivery In Vitro"
35761-01	Mokyr, M.	University of Illinois, Chicago "Mechanism of Melphalan-Mediated Tumor Eradication"
36702	Balint, J.P., Jr.	IMRE Corporation "Immunoabsorption for Treatment of Malignancy"
39448-01	Matthay, K.K.	University of California, San Francisco "Treatment of Bone Marrow by Antibody Directed Liposomes"
39942	Mix, T.W.	Mixix Corporation "A New Large-Scale Affinity Separation Technique"
COMPLICATIONS, ADVERSE EFFECTS AND RELATED PHENOMENA ATTENDING THE USE AND EVALUATION OF BRM IN CANCER THERAPY		
27598-03	Harris, J.	Rush University "Cancer Drug Effects on Patient Suppressor Cells"
39690-01	Webb, K.	Duke University Medical Center "Chemoimmuno (Toxin) Therapy of Metastatic Prostate Carcinoma"
39864-01	Goss, S.A.	URI Therm-X, Inc. "Heat Targeted Anticancer Drug Delivery Using Liposomes"

TABLE II

BIOLOGICAL RESPONSE MODIFIERS PROGRAM
BIOLOGICAL RESOURCES BRANCH

CONTRACTS PROJECTED FOR FUNDING IN FY 1985

CONTRACTS	Estimates ($K)
Chemical Coupling of Cytotoxic Agents to Monoclonal Antibodies	$ 400
Characterization and Analysis of Proteinaceous Materials	138
Production of Hybridomas Secreting Monoclonal Antibodies to Lymphokines	230
Collection, Storage, Distribution, and Quality Assurance of BRM	400
Animal Lymphokine Evaluation	137
Technical Support for the Review and Evaluation of BRM	369
Preclinial Assessment of Monoclonal Antibodies	500
Antitumor Activity of Human Lymphokines on Human Cells	150
Distribution of Biologics	0
Purchase of Biologicals	50
Task Orders	1200
Harlan/Sprague-Dawley	280
Total	$3854

TABLE III

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

INSTITUTIONS CURRENTLY HOLDING BRMP CLINICAL TRIALS MASTER CONTRACTS

Cleveland Clinic Foundation

Dartmouth-Hitchcock Medical Center

Duke University Medical Center

Fred Hutchinson Cancer Research Center

Georgetown University

George Washington University

Hahnemann University

Illinois Cancer Council

Ohio State University Research Foundation

Sloan-Kettering Institute for Research

University of Alabama in Birmingham

University of California, Los Angeles

University of California, San Diego

University of Southern California

University of Wisconsin Clinical Cancer Center

Vanderbilt University

Yale University School of Medicine

TABLE IV

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

CLINICAL TASK ORDERS FUNDED UNDER THE ORIGINAL CLINICAL MASTER AGREEMENT

PHASE I TASK ORDERS

Institution	Agent
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*
Univeristy 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
Illinois Cancer Council	Anti-T Cell Monoclonal Antibody Radio-Labeled Conjugate
Fred Hutchinson Cancer Research Center	Anti-T Cell Monoclonal Antibody Conjugate
University of California, San Diego	Anti-T Cell Monoclonal Antibody Conjugate
University of Wisconsin	IL-2 (Recombinant)
Illinois Cancer Council	IL-2 (Recombinant and Natural)

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

AGENTS ENTERED INTO THE PRECLINICAL SCREENING LABORATORY SINCE OCTOBER 1981

1. MVE-2
2. Nor-MDP
3. Azimexon
4. Thymosin alpha 1
5. Thymosin fraction 5
6. Interferon, human natural alpha
7. Interferon, human recombinant alpha clone A
8. Interferon, human hybrid recombinant alpha A/D Bgl
9. Interferon, murine recombinant gamma
10. Interferon, murine natural alpha/beta
11. N 137
12. Poly ICLC
13. Poly A-poly U
14. Lentinan
15. Lentinan + 5FU
16. Picibanil (OK-432)
17. Tuftsin (natural, synthetic)
18. Bestatin
19. rHuman tumor necrosis factor (TNF)
20. Alkyl-lyosphospholipid (ALP)
21. FK-565
22. MTP-PE
23. MTP-PE/liposomes
24. Levamisole + 5FU
25. IL-2 + Cytoxan
26. IL-2 + gamma IFN
27. nHu IL-2
28. CL-256, 738
29. CL-259, 763
30. Ampligen
31. Therafectin
32. Isoprinosine
33. rM IFN-beta
34. rM IFN-alpha
35. rM IL-3
36. rM IL-1
37. rHu IL-2
38. rHu IL-1
39. Thymosin beta 4
40. Thymosin alpha 7
41. Thymopoietin II
42. Factor thymique serique (FTS)
43. Thymic humoral factor (THF)
44. Thymic polypeptide extract (TP5)
45. Prealbumin dodecapeptide
46. Thymic factor X (TFX)

TABLE VI

BRMP DECISION NETWORK COMMITTEE

CHAIRMAN:

Dr. Carl Pinsky, Chief
Biological Resources Branch
BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 426
Frederick, Maryland 21701
(301) 695-1098

EXECUTIVE SECRETARY:

Mr. James D. Doyle, Program Analyst
Biological Resources Branch
BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 426, Room 6
Frederick, Maryland 21701
(301) 695-1098

STANDING MEMBERS:

Dr. Gregory Curt
Deputy Director, DCT
NCI, NIH
Building 10, Room 12C206
Bethesda, Maryland 20205
(301) 496-5374

Dr. Daniel Hoth
Chief, Investigational Drug Brnach
CTEP, DCT, NCI, NIH
Landow Building, Room 4C09
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-1196

Dr. Charles Grieshaber
Chief, Toxicology Branch
DTP, DCT, NCI, NIH
Landow Building, Room 3A22
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-8777

Dr. Steven Larson
Chief, Department of Nuclear Medicine
Clinical Center, NIH
Building 10, Room 1C401
Bethesda, Maryland 20205
(301) 496-6455

Dr. Michael Hawkins
Drug Regulatory Affairs Section
Investigational Drug Branch
CTEP, DCT, NCI, NIH
Landow Building, Room 4C17
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-7912

Dr. Cedric Long
Chief, Preclinical Trials Section
BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 426, Room 1
Frederick, Maryland 21701
(301) 695-1098

Dr. Ronald B. Herberman, Chief
Biological Therapeutics Branch
BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 560, Room 31-93
Frederick, MD 21701
(301) 695-1323

Dr. Daniel Longo
Associate Director
BRMP, DCT, NCI, NCI
Frederick Cancer Research Facility
Building 567
Frederick, MD 21701
(301) 695-1416

TABLE VI (CONTINUED)

BRMP DECISION NETWORK COMMITTEE

Dr. Freddie Ann Hoffman
Chief, Clinical Trials Section
BRB, BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 426, Room 1
Frederick, Maryland 21701
(301) 695-1098

Dr. Howard Holden
Investigational Drug Branch
CTEP, DCT, NCI, NIH
Landow Building, Room 4C17
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-7912

Dr. David Nelson
Metabolism Branch
DCBD, NCI, NIH
Building 10, Room 4N117
Bethesda, Maryland 20205
(301) 496-5387

Dr. Joost Oppenheim
Chief, Laboratory of Molecular Immunoregulation
BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 560, Room 31-71
Frederick, Maryland 21701
(301) 695-1551

Dr. Robert E. Wittes
Associate Director
Cancer Therapy Evaluation Program
DCT, NCI, NIH
Landow Building, Room 4C37
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-6138

Dr. J. A. R. Mead
Deputy Associate Director
Developmental Therapeutics Program
DCT, NCI, NIH
Landow Building, Room 5A21
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-8720

Dr. Maureen Myers
MIDP, NIAID, NIH
Westwood Building, Room 753
533 Westbard Avenue
Bethesda, Maryland 20205
(301) 496-7051

Dr. Matthew Suffness
Chief, Natural Products Branch
DTP, DCT, NCI, NIH
Landow Building, Room 5C09
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-8762

Dr. James E. Talmadge
Head, BRMP Preclinical Screen
Frederick Cancer Research Facility
P.O. Box B, Building 539
Frederick, Maryland 21701
(301) 695-1478

TABLE VI (CONTINUED)

BRMP DECISION NETWORK COMMITTEE

ROTATING MEMBERS:

Dr. Samuel K. Ackerman
Director, Division of Biological
Investigational New Drugs
Office of Biologics, Research and Review
HFN-823
Center for Drugs and Biologicals
8800 Rockville Pike
Bethesda, Maryland 20205
(301) 443-4864
Term: 1/85 - 1/87

Dr. Robert C. Bast, Jr.
Box 3843
Duke University Medical Center
Durham, North Carolina 27710
(919) 684-3377
Term: 5/84 - 5/86

Dr. Ernest C. Borden
Wisconsin Clinical Cancer Center
600 Highland Avenue
Madison, Wisconsin 53792
(608) 263-5354
Term: 1/84 - 1/86

Dr. Hilton Levy
Chief, Molecular Virology Section
LVD, IRP, NIAID, NIH
Frederick Cancer Research Facility
(301) 695-1196
Term: 1/85 - 1/87

Dr. Brian Leyland-Jones
Head, Drug Evaluation
and Reporting Section
IDB, CTEP, NCI, NIH
Landow Building, Room 4C09
7910 Woodmont Avenue
Bethesda, Maryland 20205
(301) 496-5223
Term: 1/84 - 1/86

Dr. Michael Chirigos
Head, Immunopharmacology Section
BTB, BRMP, DCT, NCI
Frederick Cancer Research Facility
Building 560, Room 31-76
Frederick, Maryland 21701
(301) 695-1488
Term: 1/85 - 1/87

Dr. Julie Djeu
University of Florida
Department of Medicine, Microbiology
12901 N. 30th Street
Tampa, Florida 33612
(813) 974-2178
Term: 1/84 - 1/86

Dr. Jules E. Harris
Principal Investigator
BRMP - Illinois Cancer Council
Comprehensive Cancer Center
for the State of Illinois
36 South Wabash Avenue, Suite 700
Chicago, Illinois 60603
(312) 942-5906
Term: 1/85 - 1/87

Dr. Enrico Mihich
Grace Cancer Drug Center
Roswell Park Memorial Institute
666 Elm Street
Buffalo, New York 14263
(716) 845-5860
Term: 1/84 - 1/86

Dr. Jay Siegel
CDB, DIR, DV
Building 29A, Room 2A11
Bethesda, Maryland 20205
(301) 496-4792
Term: 2/85 - 2/87

TABLE VI (CONTINUED)

BRMP DECISION NETWORK COMMITTEE

Dr. Annette E. Maluish
Head, Clinical Immunology Services
Frederick Cancer Research Facility
Building 560, Room 31-18
Frederick, Maryland 21701
(301) 695-1491
Term: 1/84 - 1/86

Dr. Robert Wiltrout
Immunobiology Section
LMI, BRMP, DCT, NCI, NIH
Frederick Cancer Research Facility
Building 560
Frederick, Maryland 21701
(301) 695-1258

CONSULTANTS:

Dr. Ole Henriksen
Koba Associates
200 Florida Avenue, NW
Washington, DC 20009
(202) 328-5700

Dr. Paul Walter
Koba Associates
200 Florida Avenue, NW
Washington, DC 20009
(202) 328-5700

SUMMARY REPORT

BIOLOGICAL THERAPEUTICS BRANCH

October 1, 1984 to September 30, 1985

INTRODUCTION

The Biological Therapeutics Branch (BTB) of the 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 (Chief, Dr. Ronald B. Herberman). Subsequently, the BTB was divided and 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 became the Laboratory of Molecular Immunoregulation. The BTB (Chief, Dr. Ronald B. Herberman) remains with four sections: Clinical Investigations Section (Kenneth A. Foon, M.D., Head), Monoclonal Antibody/Hybridoma Section (John Pearson, Ph.D., Acting Head), Natural Immunity Section (John R. Ortaldo, Ph.D., Head), and Immunopharmacology Section (Michael A. Chirigos, Ph.D., Head).

The BTB 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 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, headed by Dr. Kenneth Foon, is responsible for the investigation of the therapeutic efficacy of biologicals and BRMs 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 cytapheresis 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 BRMs involving small numbers of patients. Optimal immunomodulatory doses, as well as maximum tolerated doses, of these new agents are being determined.

Various recombinant and nonrecombinant α and γ 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 and have been previously reported. We have recently completed 3 Phase I studies with recombinant

and nonrecombinant γ interferons. Toxicity for each of these preparations was similar to α interferons, with a flu-like syndrome as well as minor hematologic toxicity, primarily decreased circulating leukocytes. Dose-dependent serum of IFN levels were detected, using both a biologic assay and enzyme-linked immunosorbent assay for the recombinant γ interferon. No antitumor responses were seen. We are also completing a Phase I trial with recombinant leukocyte interferon to determine the optimal immunomodulatory dose. Doses studied ranged from 10^3 to 10^6 units injected daily or biweekly. We have also begun a Phase I trial with recombinant interferon-γ in melanoma patients with minimal tumor burdens, to determine the optimal immunomodulatory dose.

In our initial Phase I trial of recombinant leukocyte interferon in patients with a variety of disseminated cancers, we demonstrated that this agent could be administered up to doses of 50 x 10^6/meter^2 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. Since the protocol for immunologic monitoring of patients receiving this agent failed to determine the optimal immunomodulatory dose, and since it was thought likely that the antitumor effects might be a result of the direct action of interferon on the tumor cells, it was decided to initiate a Phase II efficacy trial of recombinant leukocyte interferon at the 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 cutaneous T-cell lymphoma, as well as patients with refractory metastatic breast cancer. Eighteen patients with metastatic breast cancer were treated with recombinant leukocyte A interferon, with 16 patients progressing while on therapy and 2 remaining stable. Eighty-four patients with a variety of lymphomas have been treated, with a 56% response rate in 24 patients with favorable-histology lymphoma (9 partial responses, 5 complete responses) and a 45% response rate in 20 patients with cutaneous T-cell lymphoma (9 partial responses). Only 2 of 19 patients with chronic lymphocytic leukemia and 3 of 16 patients with unfavorable-histology lymphoma responded. Our currently active studies include randomizing favorable-histology lymphoma patients to low dose daily recombinant interferon-α ($3x10^6$) versus $50x10^6$ twice per week to see if the well tolerated low-dose regimen is as efficacious as the high dose schedule. We are also treating patients with hairy cell leukemia with daily doses of recombinant interferon-α ($3x10^6$) with excellent clinical results. Complete or partial responses have been observed in all but 2 of 25 evaluable patients, with marked improvement in hematologic and immunologic parameters.

We have evaluated the biological response modifying effects, as well as the anticancer and toxic effects, of poly ICLC (a synthetic double-stranded RNA which is added to a complex of poly-L-lysine and carboxymethylcellulose). This agent is an interferon inducer that in vitro augments natural killer activity, macrophage activity, cytotoxic T-cell activity, and provides anticancer activity in some animal models. Poly ICLC was infused intravenously twice weekly to patients with biopsy proven malignancy, who have received standard therapies, at 1 mg/m^2 or 4 mg/m^2. We have entered 20 patients with a variety of metastatic solid tumors. We have not demonstrated an antitumor effect in any patients and have observed toxicity that included mild fevers, fatigue, nausea, and hypo-

tension. Interferon levels have been measured in all patients studied and were consistently higher in patients treated with 4 mg/m^2. Immunologic monitoring has demonstrated a consistent enhancement of monocyte-mediated cytostasis, depression of mitogenstimulated proliferative responses in vitro, and usually decreased or unchanged natural killer cell activity.

A protocol has been designed to carefully compare the natural and recombinant forms of interleukin 2 and analyze their pharmacokinetic properties when given either subcutaneously, intramuscularly, or by intravenous infusion. After determining the toxicity and immunologic data from single escalating doses, patients will receive interleukin 2 in various subtoxic doses for prolonged periods of time to evaluate its possible antitumor effects.

Phase I trials of antitumor monoclonal antibodies have been performed with 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-idiotype monoclonal antibody in patients with malignant lymphoma and CLL. We have treated 13 patients with CLL with the T101 monoclonal antibody and we have witnessed transient reductions in circulating leukemia cells but have not seen reductions in the size of enlarged organs or lymph nodes. Of 12 patients with CTCL five patients had minimal improvement in their skin lesions. Toxicity has included mild fever and minimal shortness of breath. Twenty 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 excellent in vivo localization of antibody in cutaneous lesions. We have also successfully imaged patients by radionuclide scans using ^{111}Indium-T101 and ^{111}Indium-9.2.27.

An adoptive immunotherapy clinical protocol has been initiated to test the efficacy of activated elutriator-purified autologous monocytes in the treatment of peritoneal colorectal carcinomatosis. These patients have received weekly administration of their own elutriator purified monocytes (activated with γ-interferon) into their peritoneal cavity via an indwelling Tenckhoff catheter. We have shown this therapy to be relatively nontoxic and feasible on an outpatient basis. The infused monocytes were shown to remain in the peritoneal space for prolonged periods of time and appeared to infiltrate into the peritoneum. The first two patients to complete this trial are currently without evidence of malignancy.

In laboratory studies, molecular biology techniques have been utilized to study the various activation states of human monocytes in order to gain more detailed insight into the genetic control mechanisms operative in these cells. Low molecular weight messenger RNA forms for α-IFN were found to be produced in human monocytes secreting IFN and, in addition, a high molecular weight form of α-IFN messenger RNA was detected in stimulated human monocytes that do not secrete IFN.

MONOCLONAL ANTIBODY/HYBRIDOMA SECTION

The Monoclonal Antibody/Hybridoma Section (Acting Head, Dr. John Pearson) pursues investigations into the use of monoclonal antibodies alone, or as

conjugates with drugs, radionuclides or other toxic substances, directed at the tumor cell in order to achieve more effective therapy of cancer.

Several new animal model systems have been developed to evaluate the efficacy of immunoconjugates. A human melanoma (FeMX) and a human colon (Ht-29) xenografted either in nude mice or rats were found to induce progressively growing tumors at the site of inoculation (sc) or induce pulmonary foci (iv). A T-cell lymphoma has been adapted to grow in nude mice following the sc inoculation of $4x10^6$ cells. Specific MoAb's exist for each of these animal model systems. The administration of 50 µg of a gelonin or pokeweed antiviral protein (PAP) conjugate of 9.2.27 administered iv to nude mice bearing pre-palpable FeMX tumors demonstrated a 3 to 4 day delay in tumor emergence as compared to untreated animals. However, there was no difference in median survival time between the two groups when the study was terminated at 60 days. Localization studies have revealed that 1 to 2% of ^{125}9.2.27 localizes in FeMX tumors (7-8 mm) while 2 to 5% of the labeled MoAb was found in liver, kidney and spleen. Only .05% or less of a ^{125}I-labeled conjugate of gelonin or PAP conjugate of 9.2.27 (50 µg) reached the site of similar size FeMX-induced tumors. Two to 5% of the conjugates were taken up by the liver, kidney and spleen. Pretreatment of tumor-bearing animals with a nonspecific MoAb (RPC-5) followed by systemic administration of ^{125}I-9.2.27 failed to enhance the uptake of the labeled MoAb to the tumor site or decrease uptake of the specific MoAb by the reticuloendothelial system.

Several new conjugates of monoclonal antibodies with drugs or biological response modifiers have been synthesized. A major focus has been on the development of reproducible, simple, and efficacious methods for synthesis of these conjugates. Drug conjugates of 9.2.27, a monoclonal antibody to the 250K proteoglycan of human melanoma, and to a lesser extent D_3, a monoclonal antibody to the guinea pig line 10 hepatoma, have been made. The drugs utilized include actinomycin D, Daunomycin, Ara-C, 5-FU and methotrexate. We are now concentrating on actinomycin-D (ACT-D) conjugates of 9.2.27, to determine optimial yield, specificty, activity in vitro and in vivo, as well as in vivo tumor localization. Preliminary in vitro cytotoxicity studies have been carried out with conjugates of all the aforementioned drugs using antigen-positive melanoma cells (FEMX), and antisera-negative cells (A375). The ACT-D conjugates of 9.2.27 appear to have immunologic specificity as well as potency. Further efforts are now being made to determine the optimal conjugation ratios, using poly-L-lysine of various sizes as initial carriers of drugs to be conjugated to monoclonal antibodies. Further studies are being carried out to optimize in vivo delivery to tumor sites and to minimize non-specific uptake, especially in the reticuloendothelial system, by using the following approaches: the use of monoclonal antibodies with covalently modified PEG (polyethyleneglycol monomethyl ether); pretreatment of animals with heat-aggregated human gamma globulin; and the use of unrelated monoclonal immunoglobulins of the same subtype.

NATURAL IMMUNITY SECTION

The Natural Immunity Section, (Dr. John Ortaldo, Head) studies natural cell-mediated immunity to tumors in rodents and in man and analyzes the phenotypic, biochemical and functional characteristics of the effector cells; studies the

factors regulating the development and activation of these cells; analyzes the role of natural immunity in the immune response and the interaction of natural effector cells with other components of the immune system; evaluates patients with cancer for the correlation between immune parameters and clinical course of disease; investigates the effects of biological response modifiers on natural cell-mediated immunity; and studies the role of natural immunity in resistance against tumor growth and in other diseases.

A detailed study has been performed on the characteristics of mouse natural killer (NK) cells. NK cells from the spleen have been closely associated with large granular lymphocytes (LGL). Experiments with monoclonal anti-Qa5 indicate that the NK activity can be eliminated without affecting the NC activity in spleen, liver, or bone marrow populations. Attempts to eliminate NK or NC activity with the myelomonocyte specific anti-Gma-1 monoclonal antibody have indicated that the NK cells from spleen, liver, and bone marrow express this antigen only at low levels. Anti-Thy-1 elimination experiments also confirmed the Thy-1 expression on a large proportion of the isolated NK cells independent of the T lymphocyte subset markers Lyt2 and L3T4. Collectively, these data support the concept that spleen or bone marrow LGLs exhibiting NK activity are more related to the T-lymphocyte lineage than to the myelomonocytic subsets of hematopoietic cells. Liver LGL or highly enriched splenic LGL have been studied with a panel of 25 MoAb against cell surface markers. These results very clearly indicate that there are major quantitative differences in the surface expression of a number of these antigens between the liver-derived LGL and splenic LGL.

Studies have been performed to determine the mechanism of cytotoxicity by mouse natural cytotoxic (NC) cells and to contrast the information with the mechanism for cytotoxcity by natural killer (NK) cells. The NC-susceptible target cell, WEHI-164, has been found to be highly susceptible to both mouse and human recombinant tumor necrosis factor (TNF) and antibodies to mouse TNF strongly inhibited NC activity. In contrast, the NK-susceptible target cell YAC-1 was resistant to lysis by TNF and anti-TNF did not affect NK activity.

Further studies have also been performed to characterize rat NK cells. Results using a wide variety of target cells have shown that the naturally cytotoxic effector cells for both normal bone marrow and tumor targets are all included in the large granular lymphocyte (LGL) subpopulations. Studies with transplantable LGL leukemias in F344 rats have demonstrated close similarity with normal LGL. Similarities were also noted between these LGL tumors and previously reported cases of human Tγ-CLL. Biochemical analysis of these rat LGL leukemias has resulted in the purification of cytoplasmic granules containing a highly cytolytically active protein of approximately 60 Kd. Rabbit antibodies against these granules block both rat and human NK, antibody-dependent cellular cytotoxicity (ADCC), and inhibit the growth of the fungus, Cryptococcus neoformans. Molecular biologic studies have also shown the lack of β-chain rearrangement or mRNA expression, suggesting that the T cell receptor is not involved in the recognition of target cells by LGL. We have demonstrated that a cytoplasmic granule component is necessary for the NK and ADCC activity of LGL, and provide the first direct evidence that a secretory event involving these granules is involved in the lysis of both tumor cells and fungi.

Human NK cells and K cells mediating antibody-dependent cellular cytotoxicity have been shown to be LGL. The majority of LGL form lytic conjugates with a wide variety of NK-susceptible target cells. NK cytotoxic factors (NKCFs) are being examined for their specificity and mechanism of action. Three distinct steps have been defined for NKCF; a) production, b) binding to targets, and c) subsequent target lysis. With procedures able to independently measure these events, a variety of agents which have been reported to inhibit NK cell-mediated killing are being tested to determine their site of action. These NKCFs are produced by LGL and have a general specificity pattern similar to intact killer cells. Comparisons were made between NKCF and recombinant lymphotoxin (LT) and tumor necrosis factor (TNF). The results demonstrated that NKCF is distinct from both these cloned factors. In addition, LGL have been shown to produce IFN- α and β, interleukin 1 and 2, and B-cell growth factor in response to target cells or lectin. The effector cells for mediating antibody-dependent cellular cytotoxicity (ADCC) with various mouse monoclonal antibodies were shown to be associated with LGL. In addition, this ADCC effector cell was regulated in vitro by IFNs and IL-2 in a manner similar to the augmentation of NK activity.

NK progenitors in human bone marrow have also been studied. It has been possible to obtain NK cells in cultures of human BM cells in the presence of IL2, after eliminating all mature T cells, mature NK cells and CFU-C. NK cells cultured from progenitors were LGL, very similar to fresh human LGL, which have NK (K562) activity, surface phenotypes of $NKH1^+$, $OKM1^+$, $OKT10^+$, $3G8^+$ and $OKT3^+$. Thus NK progenitors in the BM are distinguishable from CFU-C and are not simply LGL expanded from mature contaminating T cells or NK cells.

A series of studies have been performed to study the regulation of human and mouse NK activity. Natural, recombinant and hybrid recombinant alpha, beta, and gamma interferon molecules have been shown to augment human NK activity but they varied 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; however, it was capable of augmentation of other leukocyte activities and demonstrated antiproliferative and antiviral activities similar to other IFN-α's. This finding has led to studies regarding 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. This IL-2-mediated augmentation appears to parallel production of IFN-γ by LGL, but abrogation of antiviral activity with anti-IFN-γ serum did not abolish NK boosting. Cultures and clones of highly purified LGL, grown in the presence of IL-2 have demonstrated morphology and cytotoxic patterns similar to fresh LGL. In addition to NK activity, cultured and clones of LGL have been shown to produce a variety of lymphokines (IL-1, IFN, CSF, BCGF).

Studies have continued on the regulation of mouse NK activity. The development of NK activity after birth was found to be appreciably accelerated by repeated inoculations of infant mice with interleukin 2 (IL2). Similarly, repeated inoculations of low doses of IL2 were able to induce more rapid reconstitution of NK activity by donor bone marrow cells in lethally irradiated mice. These results point to an important role of IL-2 in the in vivo differentiation or expansion of mouse NK cells.

Mouse model systems for induction of hyporesponsiveness to augmentation of NK activity, after multiple inoculations of natural or recombinant interferon, have been developed. This hyporesponsiveness was found to be generalized, not only in the blood or spleen but also in the liver and lungs. Subsequent inoculation with other biological response modifiers, such as IL2, poly ICLC or MVE-2, resulted in substantial augmentation of NK activity, pointing toward a combination treatment approach to overcoming the NK hyporesponsiveness.

Extensive studies have continued on the in vivo relevance of NK cells, especially for therapy of cancer. We have directly demonstrated an important in vivo antitumor role for large granular lymphocytes (LGL), the population of cells known to mediate NK and ADCC. The adoptive transfer of LGL into rats with depressed NK/ADCC 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 antitumor role for LGL and suggest that the adoptive transfer of highly enriched LGL populations should be further considered as one potential immunotherapeutic regimen in cancer patients. Studies with the BRM, OK432, have shown this agent to augment NK activity and increase survival of tumor-bearing rats.

We have found that highly lytic NK cells can also be induced in the tissue of both the lungs and liver of mice by the pyran co-polymer, MVE-2, and that these organ-associated NK cells are efficient in inhibiting the formation of metastases in the lungs and liver. Further, we have characterized the cells mediating this tissue resistance to metastasis as LGL. We have also studied the ability of rIL 2-stimulated cytotoxic lymphocytes and rIL 2 to enhance the antitumor effectiveness of the chemotherapeutic drug, doxorubicin hydrochloride (DOX). Chemoimmunotherapy of stage I murine renal carcinoma (Renca) cured 67% of Rencabearing mice, while adoptive immunotherapy or chemotherapy alone cured less than 20% of the mice. Further, effectiveness of chemoimmunotherapy alone was maximized against stage II or stage III Renca by a bicompartmental approach in which administration of treatment iv and ip cured >75% of Renca-bearing mice, whereas iv or ip treatment produced no cures. These results demonstrate that adoptive immunotherapy can enhance the effectiveness of chemotherapeutic drugs against tumors.

Anticoagulant drugs (PGI_2, heparin, warfarin) prevent interaction of tumor cells with the factors of the hemostatic system and also were found to help NK cells to eliminate tumor cells and inhibit metastasis formation. The antimetastatic effects of these drugs could be augmented or abrogated by stimulation or depression, respectively, of NK activity in mice.

The immunogenic and metastatic properties of the B16 and 3LL tumor cells treated with MNNG or UV light were further investigated. Tum^- clones from UV-treated 3LL cells have complete cross reactivity with non-homologous tum^-, tum^+ and parental 3LL tumor cells. Parental 3LL tumor cells were sensitive targets for specific cytotoxic T cells, and they were equally efficient as immunogenic tum^- cells in the cold target inhibition assay, but they were poorly immunogenic in in vivo and in vitro tests. These results indicate that parental 3LL cells possess tumor associated antigenic determinants but express them in non-immunogenic form. The primary antitumor response was highly sensitive to the

immunosuppressive action of x-irradiation (550R) or cyclophosamide (Cy) (200 mg/kg) treatment. However, antitumor resistance of the preimmunized mice remained fully expressed after these treatments. Cy treatment decreased the total number of spleen cells and the proportion of B-lymphocytes, but substantially increased the proportion of Ly 2.2^+ and L3T4 lymphocytes. Ly 1.1^+ lymphocytes were mostly responsible for the adoptive transfer of anti-3LL resistance. MNNG treatment of B16 melanoma cells increased the expression of class I MHC antigens and immunogenicity of the treated cells. Mice rejected Tum^- clone from B16T2 subline were resistant to the nonhomologous tum^- melanoma clones and to tum^+ clones which expressed high level of $H\text{-}2^b$ antigens, whereas tum^+ clones with low level expression of the $H\text{-}2^b$ complex or parental BL6 melanoma with little or no detectable MHC antigens, were able to grow in immune mice. Interferon treatment increased the expression of class I MHC antigens; however it did not influence the ability of B16 melanoma cells to grow in vivo, perhaps due to the transient effect of interferon on the $H\text{-}2^b$ antigen expression. The metastatic properties of MNNG-treated B16 melanoma cells was substantially reduced, probably due to the increase in their immunogenicity. Further understanding of the mechanisms responsible for the conversion of the non-immunogenic tumor cells into the highly immunogenic could open the way for the utilization of specific immune mechanisms for the immunotherapy of cancer patients.

The differentiation capacity of isolated subsets from normal mouse thymus has been examined in vivo and in vitro. In experimental transfer studies using congenic mice we have determined that the most immature intrathymic subset of adult mouse thymocytes consists of dull Lyl+ (dLyl) cells which are lacking both Lyt2 and L3T4 cell surface expression. This subset is seen early in thymus graft analyses. The dLyl cells isolated from adult thymus have been shown to be capable of repopulation in irradiated animals, but these cells are not capable of repopulating other hematopoietic compartments. Thus the dLyl cells are committed generative cells with limited capacity for repopulation. Some of the dLyl cells can also be shown to differentiate into Lyt2+,L3T4+ cells in short term in vitro culture.

IMMUNOPHARMACOLOGY SECTION

The Immunopharmacology Section, Dr. Michael Chirigos, Head, conducts studies to define: the host's humoral and cellular immune response to tumor growth; the specific changes in the host's immune response that occur as a result of tumor cytoreductive therapy; the mechanisms involved in enhancing specific cellular components of the immune system; and the role of specific agents capable of reconstituting and/or augmenting the immune response when used in concert with tumor cytoreductive therapy.

Immunopharmacokinetic studies of several biological response modifiers (BRMs) in vivo have been performed. The majority of BRMs (MVE-2, poly ICLC, IFN, glucan, Lentinan, Picibanil, $MnCl_2$, C. parvum, BCG) augment both NK cell and MØ tumoricidal activity. However, Imuthiol appeared to be selective for NK cells and Picolinic acid appeared selective for MØ. Multiple treatments with several BRMs maintained high levels of MØ activity but did not maintain elevated NK activity. Several possible mechanism(s) for this hyporesponsiveness to augmentation of NK cell activity were eliminated, i.e. suppresor lymphocytes or MØ and PGE production. The hyporesponsiveness to NK boosting by

multiple treatments with BRMs was consistantly found to be associated with a decrease in splenic large granular lymphocytes (LGLs), which are associated with NK cell activity, indicating a failure to maintain the expansion of LGLs in the spleen. Several BRMs were also examined in vitro and in vivo for their capacity to induce the production and secretion of regulatory factors (colony stimulating factor, CSF; Prostaglandin E_1 and E_2, PGE; Interferon, IFN). Poly ICLC, MVE-2, BM41332 and Picibanil induced the secretion of CSF and PGE. Poly ICLC and Picibanil also stimulated IFN secretion. Serum levels of CSF following injection with Poly ICLC or MVE-2 remained significantly elevated for 7 days compared to the 7 minute half-life of exogenously injected CSF. The increased CSF was found to be accompanied by increased bone marrow (BM) cells and stem cells (GM-CFU-C) developing from BM cells. MVE-2 and poly ICLC treatment following cytoreductive chemotherapy (cyclophosphamide) resulted in an earlier and elevated recovery of depressed NK activity and of bone marrow function.

Extensive studies with the MBL-2 lymphoma show that combined cyclophosphamide and BRM treatment (MVE-2 or Poly ICLC) leads to longer survival and an increased number of long-term survivors. The additive therapeutic effects of BRMs plus chemotherapy appears to be attributable to the ability of BRMs to reconstitute and/or enhance NK and MØ tumoricidal activity as well as to reconstitute bone marrow cellularity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09200-05 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. A. Foon	Head, Clinical Investigations Section	BTB, NCI
Others:	A. Alarif	Senior Staff Fellow	BTB, NCI
	H. C. Stevenson	Senior Investigator	BTB, NCI
	R. B. Herberman	Chief	BTB, NCI
	P. G. Abrams	Expert	BTB, NCI
	M. F. Fer	Visiting Fellow	BTB, NCI

COOPERATING UNITS *(if any)* Hoffmann-La Roche, Inc., Nutley, NJ; Burroughs-Wellcome Co., Research Triangle Park, NC; NCI-FCRF; Genentech, Inc., So. San Francisco, CA; Immunomodulatory Laboratories, Houston, TX; Meloy Laboratories, Springfield, VA

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	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 α and γ 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 and have been previously reported. We have recently completed 3 phase I studies with recombinant and nonrecombinant γ interferons. Toxicity for each of these preparations was similar to α interferons, with a flu-like syndrome as well as minor hematologic toxicity, primarily decreased circulatory leukocytes. Dose-dependent serum levels of IFN were detected, using both a biologic assay and enzyme-linked immunosorbent assay for the recombinant γ interferon. No antitumor responses were seen. We are also completing a phase I trial with recombinant leukocyte interferon to determine the optimal immunomodulating dose. Doses studied ranged from 10^3 to 10^6 units injected daily or biweekly. We have also begun a phase I trial to determine the optimal immunomodulatory dose with recombinant interferon-γ in patients with melanoma without metastasis but a high liklihood of recurrent disease.

PROJECT DESCRIPTION

PERSONNEL

Kenneth A. Foon	Head	CIS	BTB	NCI
Adhid Alarif	Senior Staff Fellow	CIS	BTB	NCI
Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Paul Abrams	Expert	CIS	BTB	NCI
Mehmet Fer	Visiting Fellow	CIS	BTB	NCI

OBJECTIVES

(1) To determine the toxicity of human gamma interferons (IFN-γ) when administered in escalating doses to patients with cancer.

(2) To determine the maximum tolerated dose of IFN-γ in patients with cancer.

(3) To determine the optimal biological response modifying doses of IFN-γ and IFN-α in patients with cancer.

(4) To determine the pharmacokinetics of IFN-γ administered by different schedules and different routes of injection.

(5) To determine the antitumor responses of IFN-γ.

(6) To compare different preparations of natural and recombinant IFN-γ in regard to all of the above properties.

METHODS EMPLOYED

Patients were accepted for these trials only if they had failed conventional therapy or if no effective established treatment existed for their disease, and only if they signed informed consents. Prior chemotherapy or radiation therapy must have been completed for at least 4 weeks before entry. No patient could receive corticosteroids or require anti-arrhythmic medications. Granulocyte counts could be no less than 1500/mm^3, platelet counts no less than 100,000/mm^3, and hepatic and renal functions had to be preserved. No patient was acceptable whose performance status was less than 60% (Karnofsky). All patients were monitored carefully for toxicity by repeated physical examinations, complete blood counts, and serum chemistry profiles. All patients were also monitored for NK activity, monocyte-mediated growth inhibition of a tumor cell line, lymphoproliferative responses to concanavalin A, and changes in leukocyte cell surface markers. The actual study designs varied somewhat for each of the different IFN-γ preparations and are detailed below.

Recombinant gamma interferon (Genentech, Inc., San Francisco, CA). Recombinant IFN-γ was administered on Mondays and Thursdays either intramuscularly or by 5 minute intravenous infusions of escalating doses of 0.05, 0.1, 0.25, 0.5, 1, 2, 5, or 10 mg/m^2. Dosage escalation continued in the absence of unacceptable toxicity. This trial began on January 2, 1984 and ended in March, 1984.

Determining the optimal immunomodulating dose of recombinant-α and recombinant-γ interferon. Two separate trials were designed to study the optimal immunomodulating doses of IFN-α and IFN-γ. In the IFN-α study patients with solid tumors only were treated with doses ranging from 10^3 to 10^6 units i.m. biweekly or daily. Extensive immunologic monitory including cell surface markers, 2'5'oligo-A synthetase, monocyte mediated cytostatic activity, mitogen and antigen-induced proliferative responses, and NK activity. These immunologic results have not yet been analyzed. If an optimal dose is determined, patients will return for 3 months of therapy at that dose.

We have recently begun a trial with recombinant IFN-γ (Genentech, South San Francisco, CA) in patients with melanoma without metastatic or residual disease but a high liklihood of recurrence. These patients will receive a two week course of i.m. daily rIFN-γ (.0001 mg/m^2, .001mg/m^2 or .1mg/m^2), will have a three week interval and then a second two week course at a different dosage (same range). Patients will be monitored for NK activity, proliferative responses to mitogens and antigens, 2'5'oligo-A synthetase, monocyte Fc receptors and cell surface markers. If an optimal dose is determined, a large extramural trial will be designed for this unique group of patients and perhaps for patients with other tumors with a high liklihood of recurrent disease.

MAJOR FINDINGS

A. Recombinant IFN-γ (Genentech)

Six patients were entered on each arm of the study in which the IFN-γ was administered either intramuscularly or intravenously. Two of the patients had to be taken off study for reasons unrelated to treatment, and are unevaluable because they received only one dose. Fever to 102.5°C was observed even with the lowest dose by either route. The major toxicity, however, appeared to be granulocytopenia with the absolute granulocyte counts falling to less than 1000/mm^3 by the 3rd to 8th doses in 3 patients. Dose-dependent serum levels have been measured by both an ELISA and biologic assay. The highest serum levels detected following the intravenous dose were 600 ng/ml and 200 ng/ml at 5 mg/m^2 and 1 mg/m^2 respectively. Peak levels following 5 mg/m^2 and 1 mg/m^2 intramuscular doses were 50 ng/ml and 10 ng/ml respectively.

Increased natural killer cell activity was observed 24 hours following the lowest doses of IFN-γ in 3 patients; however, none of the patients showed changes outside the limits of variability at the borderline levels of this function. Similarly, no consistent changes were observed in monocyte-mediated growth inhibition activity or cell surface markers on lymphocytes. An increase in HLA-DR expression on monocytes was observed. From these data, a biological response modifying dose could not be identified at the doses and schedule used in this phase I trial.

B. Optimum Immunomodulating Dose.

The rIFN- α trial is near completion and the data will be analyzed. The rIFN-γ trial is just underway.

SIGNIFICANCE

On Phase I trial rIFN-γ has led to a determination of the 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 rIFN-γ in a given malignancy.

Phase I trials to determine the optimum immunomodulating doses of rIFN-α and rIFN- γ should lead into phase II trials for diseases that are likely to be responsive to these agents. In addition, trials for patients without disease but high liklihood of recurrent disease with the optimum immunomodulatory dose would be designed as large extramural trials.

PROPOSED COURSE

Phase I trials of natural and recombinant IFN-γ to determine the maximum tolerated dose have been completed and we have determined the pharmacokinetics, toxicity, and feasibility of administering these agents to patients. We currently are planning phase II trials. Based on results of other trials or with other forms of interferon, it is possible that the administration of multiple recombinant interferons (for example,, IFN-α and -β, plus IFN-γ) might be undertaken to determine feasibility, immunomodulatory effects, and antitumor effects to these agents administered together.

In contrast to the above phase I trials, where we have determined the maximum tolerated dose, we are currently carrying out phase I trials with both recombinant α and γ interferons at very low doses (1000 U--1,000,000) to determine the optimum immunomodulatory dose. Phase II trials could then be designed at the optimum immunomodulatory doses. This dose could also be used in patients without metastatic disease but a high liklihood of recurrence to determine whether it might be effective in irradicating micrometastatic disease leading to prolonged disease-free intervals and possibly cures.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 09233-04 BTB

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Trials with Recombinant Leukocyte Interferon in Patients with Lymphoproliferative Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. A. Foon	Head, Clinical Investigations Section	BTB, NCI
Others:	H. C. Stevenson	Senior Investigator	BTB, NCI
	A. Alarif	Senior Staff Fellow	BTB, NCI
	R. B. Herberman	Chief	BTB, NCI
	P. G. Abrams	Expert	BTB, NCI
	M. F. Fer	Visiting Fellow	BTB, 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	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.)*

In our Phase I trial of recombinant leukocyte interferon (1981-82) in patients with a variety of disseminated cancers, we demonstrated that this agent could be administered up to doses of 50 x 10^6/meter^2 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. We 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 cutaneous T-cell lymphoma, as well as patients with refractory metastatic breast cancer. Eighteen patients with metastatic breast cancer were treated in 1982 with recombinant leukocyte A interferon, with 16 patients progressing while on therapy and 2 remaining stable. Eighty-four patients with a variety of lymphomas have been treated, with a 56% response rate in 24 patients with favorable-histology lymphoma (9 partial responses, 5 complete responses) and a 45% response rate in 20 patients with cutaneous T-cell lymphoma (9 partial responses). Only 2 of 19 patients with chronic lymphocytic leukemia and 3 of 16 patients with unfavorable-histology lymphoma responded. Our currently active studies include randomizing favorable-histology lymphoma patients to low-dose daily recombinant interferon-α ($3x10^6$) versus $50x10^6$ twice per week to see if the well tolerated low-dose regimen is as efficacious as the high dose schedule. We are also treating patients with hairy cell leukemia with daily doses of recombinant interferon-α ($3x10^6$) with excellent clinical results (13 responses among our first 14 patients).

PROJECT DESCRIPTION

PERSONNEL

Kenneth A. Foon	Head	CIS	BTB	NCI
Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Adhid Alarif	Senior Staff Fellow	CIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Paul G. Abrams	Expert	CIS	BTB	NCI
Mehmet F. Fer	Visiting Fellow	CIS	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 x 10^6 units/m^2. At higher doses, unacceptable myelotoxicity and hepatic toxicity were encountered. No evidence is available at this time with regard to the efficacy of 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 nonHodgkin'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 x 10^6 units/m^2 i.m. 3 times weekly. These trials provided more precise information as to the antitumor efficacy of recombinant leukocyte interferon in these specific malignancies. We determined that favorable-histology non-Hodgkin's lymphoma was very responsive to interferon but the doses used were too toxic. A second Phase II trial was initiated to randomize favorable-histology non-Hodgkin's lymphoma patients to daily low-dose interferon (3x10^6 units/day) versus 50x10^6 units per m^2 twice weekly. A phase II trial for patients with hairy cell leukemia treated with 3x10^6 units/day was also initiated.

METHODS EMPLOYED

Our first phase II trial has been completed. It involved patients with various lymphoproliferative disorders, including previously treated patients with non-Hodgkin's lymphoma (NHL) (both favorable and unfavorable histologies), chronic lymphocytic leukemia (CLL), and cutaneous T-cell lymphoma (CTCL). Patients received 50 x 10^6 units/m^2 i.m. 3 times weekly for a period of 3 months. Patients were monitored carefully for hematologic and hepatic toxicity as well as systemic toxicities known to be associated with interferon. These systemic toxicities consisted primarily of fatigue and anorexia. Doses were reduced to 50% and then 10% of their intial level if unacceptable toxicity in any of these categories occurred. Patients were monitored carefully for antitumor effect by periodic physical examinations and appropriate radiologic studies. Patients were also 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.

Our most recent phase II trial for patients randomizes them to either $3x10^6$ units/m^2 per day (i.m.) or $50x10^6$ units/m^2 twice weekly (i.m.). Another phase II trial is for patients with hairy cell leukemia treated with $3x10^6$ units per day (i.m. or subcutaneous).

MAJOR FINDINGS

Eighty-four patients were entered on the phase II lymphoma trial (44 with NHL, 20 with CLL, and 20 with CTCL). Major toxic reactions observed were fever, chills, fatigue, and anorexia. The average duration of therapy at 100% dosage was 2.5 weeks and 6.5 weeks at the 50% dose. Fatigue was the most common reason for dose reduction.

Our results indicate significant antitumor activity for rIFN-αA in patients with favorable histology NHL and CTCL. Fifty-six percent of patients with favorable-histology NHL and 45% with CTCL responded with either a partial or complete remission (Table 1). All responding patients were maintained on rIFN-αA therapy; the median duration of response is currently in excess of 8 months for favorable NHL and in excess of 5 months for CTCL. The five complete responders with NHL were shown to be tumor-free in sites of previous disease. The responses in patients with CTCL have included reductions of the size of skin plaques and tumors only, skin lesions and lymph nodes, and skin lesions and levels of circulating Sezary cells. It was particularly interesting that all of the responding patients had very advanced disease and had failed multiple courses of combination chemotherapy.

Table 1. Clinical Responses in Phase II IFN-αA Trial

Disease	Evaluable patients	Complete responses	Partial responses	No response	Progression
Favorable NHL	30	5	10	7	7
Unfavorable NHL	7	0	1	1	5
CLL	18	0	2	5	11
CTCL	20	0	9	7	4

Detailed immunological monitoring was not performed on these patients. Blood from patients with lymphoproliferative diseases receiving IFN was used to investigate the mechanism of depressed NK activity observed previously in the phase I trial of patients receiving IFN. Using single cell assays and cell separation techniques, it was found that the large granular lymphocytes (the cell subpopulation responsible for NK activity) were present in normal numbers, able to bind to the target cells but defective in their ability to kill the target cells.

In our most recent phase II trials 8 patients with favorable-histology NHL have been randomized to $3x10^6$ units daily and 7 patients to $50x10^6$ units/m^2 twice weekly. We have clearly demonstrated that patients respond at the

lower dose but it is too soon to assess these numbers for significance or durability of response.

Patients with hairy cell leukemia are very responsive to recombinant interferon-α with responses observed in all but one of our first 14 patients. Responding patients had a normalization of their CBC and 2 patients had complete clearance of hairy cells from their bone marrow. Most of the responding patients had recovery of their natural killer activity and normalization of their peripheral blood surface marker antigens.

SIGNIFICANCE

These trials have demonstrated that approximately 50% of patients with refractory NHL and CTCL had excellent partial responses and some complete responses. It is too soon to assess the response of NHL patients to low-dose interferon. Patients with hairy cell leukemia are very responsive to interferon with nearly all patients having a normalization of their peripheral blood counts and immunologic parameters. Future 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 BRM in patients with cancer. Further trials should include either interferon alone or in combination with drugs as initial therapy for patients with favorable-histology NHL and CTCL. In addition, randomization of hairy cell leukemia patients to splenectomy versus interferon as initial therapy should be carried out to determine whether IFN might be the preferred initial therapy for this disease.

PROPOSED COURSE

At the completion of our current phase II trials we should be able to determine whether low-dose daily interferon-α is as efficacious as high dose interferon-α for favorable-histology non-Hodgkin's lymphoma. The low dose regimen is very well tolerated and if it is as effective as the high-dose protocol, it should be used in phase III trials. A high proportion of hairy cell leukemia patients respond to interferon-α and future studies should focus on interferon versus splenectomy as initial therapy. Another planned phase II interferon-α trial will be for patients with metastatic melanoma. A 20% response rate has ben reported for melanoma and in this trial we will compare interferon therapy with and without indomethacin to determine whether it will reduce toxicity without interfering with the efficacy of interferon.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09235-04 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. A. Foon	Head, Clinical Invest. Section	BTB, NCI
Others:	A. Alarif	Senior Staff Fellow	BTB, NCI
	R. B. Herberman	Chief	BTB, NCI
	H. C. Stevenson	Senior Investigator	BTB, NCI
	P. Abrams	Expert	BTB, NCI
	C. Morgan	Expert	BTB, NCI
	R. Schroff	Senior Staff Fellow	BTB, NCI
	C. Woodhouse	Visiting Fellow	BTB, NCI
	~~M. Fer~~	~~Visiting Fellow~~	~~BTB, NCI~~

COOPERATING UNITS *(if any)*

NCI-FCRF; NCI-Navy MOB, NCI; MB, NCI; Nuclear Medicine Branch, NIH.

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.5	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 included 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-idiotype monoclonal antibody in patients with malignant lymphoma and CLL. We have treated 13 patients with CLL with the T101 monoclonal antibody and have witnessed transient reductions in circulating leukemia cells but have not seen reductions in the size of enlarged organs or lymph nodes. Of 12 patients with CTCL, five patients had minimal improvement in their skin lesions. Toxicity has included mild fever and minimal shortness of breath. Twenty 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 excellent in vivo localization of antibody in cutaneous lesions. We have also successfully imaged patients by radionuclide scans using ^{111}Indium-T101 and ^{111}Indium-9.2.27. We have successfully developed anti-idiotype antibodies for 6 patients with B-cell lymphomas and one with CLL. The patient with CLL was treated with 1.5 grams of anti-idiotype antibody without a clinical response. The other patients have not been treated.

PROJECT DESCRIPTION

PERSONNEL

Kenneth A. Foon	Head	CIS	BTB	NCI
Adhid Alarif	Senior Staff Fellow	CIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Paul Abrams	Expert	CIS	BTB	NCI
Charles Morgan	Expert	MAHS	BTB	NCI
Robert Schroff	Senior Staff Fellow	MAHS	BTB	NCI
Clive Woodhouse	Visiting Fellow	MAHS	BTB	NCI
Mehmet Fer	Visiting Fellow	CIS	BTB	NCI

OBJECTIVES

(1) To evaluate the toxicity, clinical response, and the optimal dose needed to in vivo saturate tumor cells of multiple dose administration of monoclonal antibodies in patients with lymphoma/leukemia and malignant melanoma.

(2) To monitor the patients for the presence of free antigen, free antibody, antigenic modulation and antimurine antibody formation following administration of monoclonal antibody.

(3) To determine the effect of these monoclonal antibodies on various components of the immune system.

(4) To determine the utility of these antibodies conjugated to radionuclides for tumor imaging studies.

METHODS EMPLOYED

We have completed 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 those with chronic lymphocytic leukemia and cutaneous T-cell lymphoma. 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. Patient's cells 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-idiotype monoclonal antibody trial for patients with malignant lymphoma involved the administration of anti-idiotype antibodies prepared against immunoglobulins specifically being secreted by the patient's malignant cells. Specific hybridomas are prepared against the tumor idiotype of each patient treated. The first step in developing an anti-idiotype 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-idiotype monoclonal antibodies are generated.

Both the 9.2.27 antibody and T101 have been conjugated to ^{111}In for imaging trials which are ongoing.

MAJOR FINDINGS

Twenty-five patients have been treated with the T101 monoclonal antibody. This includes 13 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, 2 patients treated with 100 mg of antibody over 50 hours and 1 patient with 140 mg of antibody per 12 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, circulating leukemia cells, and lymph node cells. None of the patients with chronic lymphocytic leukemia demonstrated anti-mouse antibody responses.

Twelve 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. The last 3 patients were treated with a weekly dose escalation through 100 mg, 200 mg and 500 mg. 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 such as liver or spleen. 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 1 patient at 10 mg.

Twenty 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 initially treated with 1, 10, 50, 100, and 200 mg of antibody, twice weekly with dosages given intravenously over 2 hours. Another group of patients were treated with 100 mg daily for 5 days followed in 4 weeks by one dose of 500 mg. 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 developed a series of anti-idiotype antibodies to patients with B-cell malignancies. We have treated 1 patient with chronic lymphocytic leukemia and did not witness any significant toxicity or responses most likely due to circulating idiotype in the patient's plasma. Two patients expired prior to our beginning therapy and two patients have a high level of circulating idiotype which would likely prevent successful therapy and cause toxicity due to antigen-antibody complexes.

Imaging trials with ^{111}In-T101 (11 patients with CTCL) and ^{111}In-9.2.27 (9 patients with melanoma) injected intravenously have demonstrated excellent tumor localization but also nonspecific uptake in the liver, spleen and bone marrow. Specificity of localization was demonstrated by infusing ^{111}In-9.2.27 into a patient with CTCL and observing no specific tumor localization. In addition, we have injected the ^{111}In-T101 either intralymphatically or into the webbing of the toes to be picked up in the lymphatics. This has led to excellent tumor localization with minimal non-specific labeling in the liver.

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. 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

We continue to study imaging with T101 and 9.2.27 with ^{111}In and most recently ^{131}Iodine. We will eventually study these same antibodies conjugates as Fab and F(ab)$'_2$ fragments to see if this will improve tumor imaging. We are also anticipating studies with T101 and 9.2.27 conjugated to yttrium-90 which is a

beta emitter and might be useful therapeutically. We are also planning to begin a serotherapy and imaging trial with a B cell antibody that reacts with B lymphocytes and B cell leukemias and lymphomas. Finally, we anticipate beginning a serotherapy and imaging trial with a human monoclonal antibody reactive with a colorectal carcinoma-associated antigen.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09276-02 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Phase I Trial of Poly ICLC in Cancer Patients

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	K. A. Foon	Head, Clinical Investigations Section	BTB, NCI
Others:	A. Alarif	Senior Staff Fellow	BTB, NCI
	H. C. Stevenson	Senior Investigator	BTB, NCI
	R. B. Herberman	Chief	BTB, NCI
	P. G. Abrams	Expert	BTB, NCI
	M. F. Fer	Visiting Fellow	BTB, NCI

COOPERATING UNITS *(if any)*

NCI-FCRF; Portsmouth Naval Medical Center

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	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.)*

We have entered 20 patients with a variety of metastatic solid tumors on a phase I trial of the interferon inducer poly ICLC, twice weekly IV, at doses of 1 mg/m^2 or 4 mg/m^2. We have not demonstrated an antitumor effect in any patient and have observed toxicity that included mild fevers, fatigue, nausea, and mild hypotension. Interferon levels have been measured in all patients studied and were consistently higher in patients treated with 4 mg/m^2. Immunologic monitoring has demonstrated a consistent enhancement of monocyte-mediated cytostasis, depression of mitogen-stimulated proliferative responses in vitro, and usually decreased or in changed natural killer cell activity.

PHS 6040 (Rev. 1/84)

GPO 914-918

PROJECT DESCRIPTION

PERSONNEL

Kenneth A. Foon	Head	CIS	BTB	NCI
Adhid Alarif	Senior Staff Fellow	CIS	BTB	NCI
Henry C. Stevenson	Senior Investigator	CIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Paul G. Abrams	Expert	CIS	BTB	NCI
Mehmet F. Fer	Visiting Fellow	CIS	BTB	NCI

OBJECTIVES

1. To determine the biological response modifying effects of poly ICLC administered intravenously (IV) twice weekly at 1 mg/m^2 and 4 mg/m^2.

2. To determine the anticancer and toxic effects of poly ICLC at these doses.

METHODS EMPLOYED AND MAJOR FINDINGS

All the patients had to have a biopsy-proven diagnosis of disseminated cancer and have received standard curative therapy, if such therapy exists, and not received palliative treatment for their cancer in the form of radiotherapy, chemotherapy, or hormonal therapy for at least 4 weeks prior to entering the study. All of the patients had to be willing and able to give written informed consent. Patients must have a performance status of 70% or better and be 18 years of age or older.

Patients were treated with poly ICLC, administered intravenously at a dose of either 1 mg/m^2 or 4 mg/m^2, infused over a period of 1 hour, twice weekly, for 8 weeks. Our study is being carried out collaboratively with Dr. James Reid of the Naval Regional Medical Center in Portsmouth, Virginia. In parallel with our twice weekly IV dose (1 mg/m^2 and 4 mg/m^2), he had carried out a once weekly IV dose at the same dose levels. Comparison with immunomodulating effects associated with the intramuscular route, as well as the once and twice weekly IV routes, were sought, and the optimal immunomodulating dose and route ascertained. Such a dose can then be used in phase II trials. Antitumor effects of each dose were also assessed.

The off-study criteria include unacceptable grade 4 toxicity, tumor progression, or patient noncompliance or refusal of therapy. Patients were monitored carefully, both clinically and immunologically.

Immunological monitoring was performed on all patients. Natural killer cell activity was increased in 11% of time points following i.m. injections of 1 mg/m^2 and decreased in 3%. This contrasted sharply with the 4 mg/m^2 i.v. doses (either 1/week or 2/week) when 8% and 0% of values were increased and 38% and 37% were decreased. Monocyte-mediated growth inhibition activity was augmented in 90% of the patients, with no apparent dose relationship being observed. Some consistent changes were observed in cell surface marker studies. OKT4 positive cells were increased while OKT8 positive cells were decreased resulting in an increased T4/T8 ratio. 2'5' oligo-A synthetase (an interferon-

induced enzyme) was measured and found to be markedly increased even in patients receiving i.m. poly ICLC, who had little or no detectable circulating interferon.

Lymphoproliferative responses were depressed initially and the responses were even lower following poly ICLC. These data suggest that augmented immunological effects may be observed at very low doses of poly ICLC, but an optimal immunomodulatory dose schedule was not determined.

The poly ICLC was formulated by the University of Iowa under contract with the Division of Cancer Treatment according to the original procedure described by Levy et al.

We have entered 20 patients with a variety of metastatic solid tumors on the twice weekly IV dose of 1 mg/m^2 and 4 mg/m^2 of poly ICLC. We have not demonstrated an antitumor effect in any patient. At 1 mg/m^2 we have witnessed minimal toxicity, with mild fevers up to 38.5°C, fatigue, nausea, and mild hypotension. Two patients treated at 4 mg/m^2 developed severe hypotension (systemic BP < 80) lasting 2 to 3 hours. One required fluid support and the other required dopamine. Following this, we modified our protocol so that 11 patients received at least one dose of 1 mg/m^2 prior to receiving the 4 mg/m^2 dose. Following this altered schedule, we have treated 4 patients with 4 mg/m^2 without any repetition of the severe hypotension, although we have witnessed fevers up to 39°C, nausea, vomiting, and fatigue at the 4 mg/m^2 dose. IFN levels have been demonstrated in all of patients studied to date, which peaked at 4 to 6 hours following the poly ICLC infusion (ranged from 5 to 200 U/ml for the 1 mg/m^2 patients and 5 to 600 U/ml for patients treated with 4 mg/m^2).

SIGNIFICANCE

While poly ICLC has been studied in a number of clinical trials, the optimal immunomodulating dose and optimal route of administration have not been determined. In this study, we would expect to determine the optimal dose and route for future phase II trial.

PROPOSED COURSE

At the completion of this study, we will evaluate the immunologic data from from all of the patients treated at the BRMP and Portsmouth. This evaluation will include a large cohort of patients treated at 1 mg/m^2 and 4 mg/m^2 once and twice weekly by both the IM and IV routes. We would hope to be able to determine the optimum immunomodulating dose for poly ICLC which can then be used to design phase II trials. The possibility exists that we will have to extend dose range examined and examine immunomodulation and toxicity of doses of <1 mg/m^2 IM and IV.

PUBLICATIONS

Stevenson, H. C., Abrams, P. G., Schoenberger, C. S., Smalley, R. B., Herberman, R. B., and Foon, K. A.: A phase I evaluation of poly ICLC in cancer patients. J. Biol. Resp. Modif., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09278-02 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Gene Expression Studies of Human Monocytes: Potential Clinical Applications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. C. Stevenson	Senior Investigator	BTB, NCI
Others:	P. J. Miller	Biologist	BTB, NCI

COOPERATING UNITS *(if any)*

Ingene Laboratories, Santa Monica, California, Genentech Corp., South San Francisco, California and Harvard University Medical School, Boston, Massachusetts.

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.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 monocytes have been employed in in vitro assay systems to determine the macromolecular basis for their function. Monocytes have been studied in their unactivated state, following muramyl dipeptide activation, and following activation with poly ICLC; only the poly ICLC stimulated cells are capable of releasing interferon, whereas the other activation states of monocytes are characterized by possessing other distinctive functions. When total RNA metabolism and total messenger RNA synthesis was analyzed in these three distinct activation states of human monocytes, it was found that only monocytes stimulated with muramyl dipeptide displayed a reproducible increase in RNA metabolism; this increase in total and messenger RNA synthesis peaked at 4 hrs. following muramyl dipeptide activation. To evaluate whether the poly ICLC stimulated monocytes were indeed secreting interferon specific messenger RNA, Northern blotting analysis of messenger RNA obtained from these three monocyte activation states was performed with a cDNA probe for alpha interferon. It was found that poly ICLC stimulated monocytes synthesized three molecular forms of the alpha interferon message whereas unstimulated monocytes do not synthesize detectable amounts of alpha interferon messenger RNA. Muramyl dipeptide stimulated monocytes synthesize only a high molecular weight form of the interferon messenger RNA; synthesis of this message appears to be associated with an intracytoplasmic form of alpha interferon activity. This molecular biology technology has potential clinical application for monitoring the gene expression events associated with cancer immunotherapies operative on the blood monocytes of cancer patients.

PROJECT DESCRIPTION

PERSONNEL

Henry C. Stevenson	Senior Investigator	BTB	NCI
Paul Miller	Biologist	BTB	NCI

OBJECTIVES

The objectives of the current project are to analyze the gene expression regulatory mechanisms operative during the many distinct activation stages of human monocytes. The information gleaned from this project will be utilized to better understand the gene expression basis for any clinical responses observed in patients receiving immunotherapy that is operative on their blood monocytes.

METHODS EMPLOYED

The methods employed for this study represent a fusion of the unique methodologies developed by our group for purifying and culturing human monocytes along with classical molecular biology techniques. We purify blood monocytes from normal human volunteers and cancer patients by a combination of cytapheresis coupled with counter-current centrifugal elutriation. The purified monocytes thus obtained are cultured in suspension in serum-free media utilizing specially developed Teflon labware; no antibiotics are utilized to keep these cells sterile. We have utilized several distinct monocyte activators to stimulate the release of the desired cytokine; poly ICLC has been our best stimulus for α-interferon release, muramyl dipeptide has been our best stimulator for monocyte-derived fibroblast growth factor secretion and LPS than the best stimulator for tumor necrosis factor release. The interferon assay that we employ is measurement of the antiviral activity of monocyte culture supernatants of cultured human foreskin cells. The assay for fibroblast growth factor activity that we employ centers on measuring the proliferative activity of monocyte culture supernatants on standardized human fibroblast cell lines. The tumor necrosis factor assay that we employ centers around measuring the cytotoxic activity of monocyte supernatants against mitomicin-C-treated L929 cells. Specificity of our tumor necrosis factor containing supernatants is confirmed with the use of a rabbit antitumor necrosis factor antibody which completely neutralizes the cytotoxic activity in our supernatants. The molecular biology techniques employed to date include the isolation of total RNA using cesium chloride gradients and the isolation of messenger RNA from the total RNA using oligo-DT column chromotography. Electrophoresis of messenger RNA specimens is accomplished using a .8% arose gel with formaldehyde and the electrophoresed messenger RNA is fixed to nitrocellulose sheets using the Northern transfer blotting technique. The cDNA probes employed todate are ^{32}P labeled by the Nick translation procedure; hybridization experiments to probe total messenger RNA for the existence of message specific messenger RNAs is accomplished under moderately stringent conditions.

MAJOR FINDINGS

Our molecular biology and biochemical studies are based on the observation that monocyte activation is not a single state in the human blood monocyte but rather is composed of multiple activation subset states; each subset state focusing around a particular function (or group of functions.) For example, we have found that poly ICLC in concentration ranges from 10 to 200 µg per ml stimulates a dose dependent release of α-interferon from human monocytes; maximal secretion is observed at 36 hours. In contrast, other known monocyte activators such as bacterial lipopolysaccharide (LPS), C. parvum, BCG or muramyl dipeptide (MDP) did not stimulate detectable release of α-interferon by monocytes. In contrast, the secretion of fibroblast growth factor by blood monocytes displayed a different pattern, with poly ICLC being relatively ineffective and muramyl dipeptide inducing a distinctive dose dependent release of fibroblast growth factor. Similarly, it appears that bacterial lipopolysaccharide (LPS) is the most potent stimulator of tumor necrosis factor release by human blood monocytes although more detailed investigation into possible synergism between these activating agents is still required. Our initial studies have focused on the gene expression basis for these distinctive activation states of human blood monocytes centering around an analysis of overall RNA metabolism and message-specific RNA metabolism by these cells.

Of these various monocyte activation subset states, only monocytes stimulated with muramyl dipeptide showed an increase in overall total messenger RNA synthesis and overall messenger RNA synthesis. Specifically, muramyl dipeptide caused a 40% increase in ^{3}H uridine incorporation which peaked at 4 hrs. following activation, with a return to baseline within a 12-hr. period. The pattern for overall RNA synthesis and messenger RNA synthesis following MDP stimulation were virtually superimposeable. In contrast, monocytes activated with poly ICLC (for α-interferon synthesis) or lipopolysaccharide (for tumor necrosis factor production) did not display any change in the rates of treated uridine incorporation into total or messenger RNA. Thus human monocytes display different patterns of overall RNA metabolism when activated to one function versus another.

To gain more detailed insight into the control mechanisms operative at the RNA level for human monocyte activation, we obtained two cDNA clones for α-interferon, PAS1 and HUIFN α-2; these two cDNA's code for two of the fifteen subspecies of αinterferon. Monocytes were cultured under serum-free conditions in suspension for 18 hrs. without stimulation, with muramyl dipeptide (from 10 to 200 µg per ml) or with poly ICLC (from 10 to 200 µg per ml). Autologous elutriator purified lymphocytes were cultured under identical conditions. Only the poly ICLC stimulated monocytes were shown capable of releasing α-interferon; however, we were able to identify α-interferon specific messenger RNA's in several of our experimental conditions. Unstimulated monocytes and unstimulated lymphocytes did not synthesize any messenger RNA which was hybridizable with our α-interferon cDNA probes. In contrast, poly ICLC stimulated monocytes synthesized a messenger RNA which has a size (1.0 kb) compatible with a molecular weight of the α-interferon protein. In addition, poly ICLC stimulated monocytes synthesized two higher molecular weight forms of the interferon message (at 2.8 and 5.5 kb). In contrast,

muramyl dipeptide stimulated monocytes (which did not secrete detectable amounts of α-inteferon) synthesized only the 2.8 kb molecular weight form of the α-inteferon messenger RNA. This pattern of messenger RNA synthesis in muramyl dipeptide stimulated monocytes was found to be associated with detectable levels of intracytoplasmic interferon activity. Using dot plot hybridization techniques, we were able to show the expression of the α-interferon message in the poly ICLC stimulated monocytes followed dose response kinetics. Interestingly, although unactivated lymphocytes did not synthesize any messenger RNA that was hybridizable with our α-interferon cDNA probes, poly ICLC stimulated lymphocytes (though they also did not secrete detectable amounts of α-interferon) did secrete the 2.8 kb form of messenger RNA. However, when these poly ICLC stimulated lymphocytes were analyzed for intercytoplasmic interferon activity, none was detected.

We recently have been comparing two human blood monocyte subsets with regard to differences that might exist at the macromolecular synthetic level with regard to α-interferon secretion. We have recently described that one of these subsets (IM) is approximately 4 to 5 times more sensitive than is the other subset (RM) with regard to α-interferon secretion in response to standardized doses of poly ICLC (from 10 to 200 μg per ml). Using dot plot hybridization technology along with a cDNA probe for α-interferon (PAS-1) we have shown that at 18 hrs, the RM monocytes appeared to actually be synthesizing more α-interferon specific RNA when compared to the IM cells. This may actually reflect a difference in the time course of secretion of α-interferon specific messenger RNA's; experiments to confirm this hypothesis are ongoing. Similarly, it appears that monocytes and macrophages differ with regard to their ability to secrete a wide number of biological response modifiers including α-interferon. Blood monocytes appear to be more effective in this regard. We are currently examining the macromolecular synthetic basis for the differences in α-interferon secretion as cells mature along the monocyte and macrophage maturation axis.

SIGNIFICANCE

One of the major research thrusts of the Biological Response Modifiers Program is to develop new insights and understandings of the human immune system as an immunosurveillance weapon to prevent clinical malignancy or to reduce or eliminate established disease. The monocyte and its tissue counterpart vis-a-vis, the macrophage, play pivotal roles in immune system particularly vis-a-vis host defense. It is known that these cells are critical for antigen-processing, as accessory cells for a variety of T-lymphocyte and B-lymphocyte functions, and that can secrete a number of potent biological response modifiers (perhaps as many as 100) and have dramatic immunoregulatory activities. In addition these cells are capable of secreting a wide range of complement components and are major participants in the phenomenon of spontaneous as well as antibody-dependent cellular cytotoxicity. The regulatory mechanisms for this multifaceted cell have never been studied at the genetic level in humans. The goal of this project is to determine the gene expression events whereby monocytes are able to participate in this wide range of immunoregulatory functions. Since many of these monocyte activities have opposite effects on target tissues, it is readily apparent that very precise control mechanisms must be operative in vivo. The ability to understand at the gene expression

level how the human monocyte is able to regulate its various activities will necessarily allow us to more precisely define the interrelationship between the many different functions of monocytes and hopefully allow us to better modulate these functions. Our ultimate clinical goal is to define monocyte defects that might exist in patients (such as recently described monocyte defects in AID's patients and cancer patients) at the genetic level. Moreover, as we enter an age where monocytes are being used in an adoptive transfer setting, we would like to characterize any changes in their function both in vivo and in vitro at the level of gene expression. We feel that the ability to characterize monocyte immunosurveillance defects at the gene expression level should permit very focused attempts to "up-regulate" effective monocyte function through biological response modifier administration and adoptive immunotherapy strategies.

PROPOSED COURSE

Future studies in this project will center around four major areas: (1) Continuing to analyze α-interferon messenger RNA metabolism in the various monocyte subset states and differentiation states; (2) the performance of similar studies utilizing a cDNA probe for tumor necrosis factor obtained from the Genentech Corp; (3) continuing collaborative experiments with regard to C3 and properdin factor B synthesis in blood monocyte subsets and differentiation states in collaboration with Harvard University Medical School and (4) clinical applications of our monocyte molecular research.

As described in our project regarding the characterization of the elutriator purified monocytes, we will continue to characterize the difference in interferon production this between the two human monocyte subsets (IM and RM) at the molecular biology level. We will perform time course experiments to analyze in greater detail the differences in expression of α-interferon messenger RNA's and will particularly focus on differences in the size forms of the α-interferon messenger RNA's that are expressed in these two monocyte subsets. Similar studies will be performed to characterize the differences in interferon synthesis that exists between fresh blood monocytes and cultured macrophages. We will also apply this macromolecular biological approach to determine the gene expression basis for the secretion of tumor necrosis factor (TMF) and several of the complement components including C3 and properdin factor B.

We are interested in performing analyses of the gene expression events operative in human monocytes of patients being treated with biologicals which activate monokine release by monocytes. Currently for example, we are treating colon cancer patients with infusions of their own highly purified blood monocytes activated with γ-interferon. It is not clear as to how this treatment boosts the cytotoxic activity of these cytotoxic cells in vitro and hopefully in vivo. One experiment that is proposed for the upcoming year is to measure the amounts of tumor necrosis factor specific messenger RNA induced in these cells by this in vitro treatment and then recover the infused monocytes 24 hrs. after intraperitoneal infusion and reanalyze tumor necrosis factor specific messenger RNA synthesis following in vivo incubation. This information might be useful for the rational design of future trials and or for the

monitoring of the effectiveness of this and other related monocyte immunotherapy treatments.

PUBLICATIONS

Stevenson, H. C., Dekaban, G., Miller, P., Benajati, C., and Pearson, M.: Analysis of human monocyte activation at the level of gene expression. I. Expression of the alpha-interferon gene during different activation states of the human monocyte. J. Exp. Med. 161: 503-513, 1985.

Stevenson, H. C., Miller, P. J., Huffer, T. C., Oldham, R. K., Kanapa, D. J., and Sen, A.: Characterization of ^{3}H-Uridine incorporation and messenger RNA synthesis in human monocytes activated to secrete alpha interferon or fibroblast growth factor. J. Leuk. Biol. 37: 585-595, 1985.

Stevenson, H. C., Miller, P., and Dekaban, G.: Comparative analysis of the gene expression basis for human monocyte and lymphocyte function following activation to alpha interferon secretion. In Streilein, J., Black, S., Blomberg, B. and Voellmy, R. (Eds.): Advances in Gene Technology: Molecular Biology of the Immune System. Cambridge Universtiy Press, pp. 315.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09279-02 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Phase I Trials of Interleukin-2 in Patients with Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. C. Stevenson	Senior Investigator	BTB, NCI
Others:	K. A. Foon	Head, Clinical Investigations Section	BTB, NCI
	A. Alarif	Senior Investigator	BTB, NCI
	F. Ruscetti	Head, Lymphokines Section	LMI, NCI
	R. B. Herberman	Chief	BTB, NCI
	D. Longo	Associate Director	BRMP, NCI

COOPERATING UNITS *(if any)*

Frederick Memorial Hospital, Frederick, MD 21701 and Program Resources Inc., FCRF, Frederick, MD 21701.

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	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 clinical investigation trial to analyze the role of interleukin-2 in the treatment of patients with cancer has been designed. This trial will test the toxicity of recombinant interleukin-2 preparations given subcutaneously, intramuscularly, or by slow intravenous infusion in escalating doses. Concomitantly, we will study the pharmacokinetics and immunomodulatory dose properties of recombinant interleukin-2 when given by these three routes in escalating doses. Finally, in an attempt to evaluate the possible antitumor effects of interleukin-2 in cancer patients, we will administer the agent for three weeks on a daily basis at either the optimal immunomodulatory dose (as determined by our in vitro immunomonitoring assays) 1/10 the optimal immunomodulatory dose or 10 x the optimal immunomodulatory dose, provided this dose is below the maximum tolerated dose (defined from previous toxicity testing).

PROJECT DESCRIPTION

PERSONNEL

Henry Stevenson	Senior Investigator	CIS	BTB	NCI
Adhid Alarif	Senior Staff Fellow	CIS	BTB	NCI
Frank Ruscetti	Head	LS	LMI	NCI
Ronald B. Herberman	Chief		BTB	NCI
Kenneth Foon	Head	CIS	BTB	NCI
Dan Longo	Associate Director		BRMP	NCI

OBJECTIVES

The objectives of this study are:

(1) To determine the toxicity in cancer patients of recombinant interleukin-2 (IL-2) when given subcutaneously, intramuscularly or by slow intravenous injection in escalating doses.

(2) To study the pharmacokinetics and immunomodulatory properties of interleukin-2 when given by these three routes and in escalating doses.

(3) To evaluate the possible antitumor effects of interleukin-2 in cancer patients.

METHODS EMPLOYED

Recombinant interleukin-2 is produced by standard genetic engineering technology and expanded in E. coli. We have obtained an interleukin-2 preparation from the Hoffmann LaRoche Corp. which is felt to have the lowest contamination levels of endotoxins and the highest specific activity of the various interleukin-2 preparations that we have tested to date. This clinical grade material has passed FDA inspection including pyrogenicity testing. The patients eligible for this protocol will be cancer patients with histologically confirmed diagnoses for which there is no standard or effacious therapy. T-cell malignancies will be excluded (because of the possibility the interleukin-2 might stimulate the growth of these malignant cells). The patients must be between the ages of 18 and 70 and have a life expectancy of at least 3 months and a performance status greater than 60% of the Karnovsky scale. In addition to giving conformed consent these patients must have normal hemalogic, renal and liver function. Also, the omission of previous chemotherapy, radiation therapy or biological response modifier therapy for 4 weeks prior to entry onto the protocol is required. Two basic study plans (Schedule A and Schedule B) for interleukin-2 have been identified. On Schedule A, patients will be randomized to one of three infusion routes (i.v., i.m., or subcut) and patients will receive weekly escalating doses of interleukin-2 from 10^3 to 10^8 units per meter square. In addition to monitoring toxicity and defining the maximal tolerated dose (MTD), the optimal immunomodulating dose (OID) will be defined. Following a three week wash out period, each patient will be randomized to receive a .1 OID, 1 OID or 10 OID amounts of interleukin-2 i.m. on a daily basis for three weeks (Schedule B).

The immunomodulatory testing that will be performed throughout the study will include assessment of peripheral blood spontaneous lymphocyte proliferation in vitro and the proliferative responses of these cells to lectins and a mixed leukocyte response (MLC) stimulus. In addition, T-cell subsets, B cells and natural killer cells and monocytes will be enumerated by fluorescence activated cell sorter analysis. Also, natural killer cell activity, monocyte-mediated cytostasis and studies of interleukin-2 gene expression will be performed.

Patients on Schedule B will be randomized to receive either .1 X OID, 1 X OID or 10 X OID amounts of interleukin-2 on a daily basis for three weeks. Patients demonstrating a partial response to the interleukin-2 treatment at the end of three weeks will be eligible to continue receiving the agent according to their Schedule B protocol. If there is no further response, the patient will be taken off the study and observed. If there is a complete response, the patient will be treated for one more month at the same schedule and then taken off treatment. If there is a further regression but not a complete response, the patient may be continued on the same therapy until no further response is evident. If grade 4 toxicity is observed, interleukin-2 will be discontinued. Similarly patients demonstrating a growth in their tumor over the time of treatment will be taken off the study.

MAJOR FINDINGS

No patients entered to date. Patient accrual begins on June 15, 1985.

SIGNIFICANCE

One of the major research thrusts of the Biological Response Modifiers Program is to develop new insights and understandings into the role of the immune system as an immunosurveillance modality to prevent clinical malignancy or to reduce established disease. The acquisition of theoretical concepts and practical knowledge in this arena has been quite slow, particularly in applications of basic research and animal model research to clinically relevant situations. We have a major commitment to understanding more about the total spectrum of signals that could augment the human immune response to tumor antigens (biological response modifiers). As we understand more about each leukocyte subtype that participates in the human immune response, we strive to acquire a deeper understanding of the biological response modifiers signals whereby those human leukocytes subsets interact with one another as they participate in immune response; we are constantly struggling to place these new research understandings into a clinical setting whereby we may be able to "up-regulate" the inefficient immune system of certain cancer patients. Interleukin-2 is clearly one of the best characterized biological response modifiers to date. Human experimentation with interleukin-2 has been predominantly limited to patients with immunodeficiency diseases such as the Acquired Immune Deficiency Syndrome, although studies utilizing interleukin-2 in conjugation with lymphokine activated killer T cells have recently been initiated. No systematic evaluation of the pharmacokinetics of interleukin-2 with regard to noncytotoxic T lymphocytes, natural killer cell, B lymphocyte and monocyte functions has yet been undertaken. Given the immunoregulatory effects of interleukin-2 in animal studies, it appears likely that the immune status of cancer patients

receiving interleukin-2 might be significantly altered. The proposed clinical protocol has been designed to determine the toxicity and tolerance of Hoffmann LaRoche recombinant interleukin-2 and to determine the pharmacokinetics and immunomodulatory properties; the antitumor activities of this agent when given by three distinct administration routes will be assessed as well.

PROPOSED COURSE

As detailed in the Methods Employed section.

PUBLICATONS

None to date.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09280-02 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Characterization of Elutriator-purified Human Monocytes: Clinical Applications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	H. C. Stevenson	Senior Investigator	BTB, NCI
Others:	P. Miller	Biologist	BTB, NCI
	J. Beman	Research Nurse Specialist	BTB, NCI
	K. Foon	Head, Clinical Investigations Section	BTB, NCI
	P. Sugarbaker	Head, Colorectal Section	SB, NCI
	S. Larson	Chief	CC, NIH
	S. Rosenberg	Chief	SB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	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.)*

Human monocytes have been removed from the peripheral blood of normal volunteers and cancer patients for study in a wide range of immunologic assay systems. The technique of counter-current centrifugal elutriation has been applied to these cells that generate as many as 10^9 95%-pure monocytes. In addition, lymphocytes that are completely monocyte-depleted can be obtained by the same technology. Elutriation generates monocytes that are in suspension; we've developed techniques to maintain these cells in suspension culture using serum free media and specially developed Teflon labware. Thus, these cultured cells are thought to be most representative of the native state of monocytes in the blood stream. These cells have been studied with regard to their accessory cell function for human lymphocyte activation, their MIF activity, chemotactic activity, ability to release biological response modifiers (including colony stimulating factor, interferon, fibroblast growth factor, prostaglandins and tumor necrosis factor); in addition, the tumorcidal activity of these cells in antibody dependent cytotoxicity and spontaneous cytotoxicity assay systems has been measured. Having documented the tumoricidal activity of elutriator-purified monocytes and our ability to boost this tumor killing capability with γ-interferon, we have initiated a clinical protocol utilizing large numbers of these cells in an adoptive transfer setting in patients with peritoneal colorectal carcinomatosis. These patients have received weekly administration of their own elutriator-purified monocytes (activated with γ-interferon) into their peritoneal cavity via an indwelling Tenckhoff catheter. We have shown this therapy to be relatively nontoxic and have developed an outpatient treatment regiment that is very compatible with an active patient lifestyle. We have found that the infused monocytes not only remain in the peritoneal space for prolonged periods of time, but also become integrated into the peritoneal surface itself. The first two patients to complete this trial are currently without evidence of ongoing malignancy.

PROJECT DESCRIPTION

PERSONNEL

Henry Stevenson	Senior Investigator	CIS	BTB	NCI
Paul Miller	Biologist	CIS	BTB	NCI
JoAnn Beman	Research Nurse Specialist	CIS	BTB	NCI
Kenneth Foon	Head	CIS	BTB	NCI
Paul Sugarbaker	Head	CS	SB	NCI
Steven Larson	Chief		CC	NIH
Steven Rosenberg	Chief		SB	NCI

OBJECTIVES

The objectives of the present protocol are:

(1) To develop the technology for isolating large numbers of highly purified blood monocytes from normal volunteers and patients.

(2) The development of in vitro technology to allow for the suspension culture of human monocytes under conditions which most closely replicate the in vivo state of these cells.

(3) Characterization of the activation status, maturation status, metabolic and functional activity of human elutriator-purified monocytes from normal donors in cancer patients.

(4) The application of above technology to adoptive immunotherapy trials of cancer patients utilizing activated elutriator-purified monocytes as cytotoxic effector cells.

METHODS EMPLOYED

We've established a cytapheresis unit in the Biologoical Response Modifiers and have recruited a stable population of approximately 75 normal volunteers. In addition, this unit services the clinical needs for cytapheresis for cancer patients being currently treated in the Biological Response Modifiers Program. Utilizing a continuous flow machine cytapheresis technique, we can routinely obtain over 10^{10} leukocytes from each normal donor or patient. The mononuclear cells are purified by Hypaque-Ficoll gradients and are separated by counter-current centrifugal elutriation into highly purified populations of monocytes (up to 1×10^9 cells) and lymphocytes (up to 7×10^9 cells). Functional subsets of human blood monocytes [designated IM (intermediate monocytes) and RM (regular monocytes)] can also be purified by modification of the elutriation technique. Since one of the major advantages of the counter-current centrifugal elutriation technique is the ability to collect large numbers of these cells in their native suspension state, we've embarked on a series of studies in which the function of elutriator-purified monocytes cultured to standard polystyrene plastic labware and specially developed Teflon labware (to which monocytes won't adhere) have been compared. We have also eliminated the variable of fetal calf serum or human serum which has previously added a great deal of unnecessary complexity to our system by developing a chemically defined serum-free medium

for monocytes. Functional assays of human monocytes employed thus far include the release of monokines such as α-interferon, colony stimulating factor, interleukin-1, fibroblast growth factor and tumor necrosis factor. The ability of monocytes to spontaneously kill tumor targets is measured in an in vitro assay against human colon cancer lines as is the enhancement of this function by various macrophage activating factors (including γ-interferon). The antibody-dependent cellular cytotoxicity function (ADCC) of elutriator purified monocytes is also measured in vitro. The ability of monocytes to migrate randomly in vitro is measured as is the inhibition of this function by monocyte-migration inhibitory factors (MIF). The ability of monocytes to perform accessory cell functions in mitogen- and antigen-stimulated T-cell systems and mitogen- and antigen-stimulated B lymphocyte systems is also determined in vitro. Assessment of the maturation of human monocytes into macrophages is determined by measuring peroxidase activity, lysosyme activity, alkaline phosphodiesterase activity and 5'nucleotidase activity. Monocytes are further analyzed by a computer-monitor automated cell sizer and specific antigenic determinants on monocyte membranes are assessed by fluorescence activated cell sorter (FACS) analysis.

MAJOR FINDINGS

Refinements of the elutriation technique have allowed us to identify two functional subsets of human monocytes (named IM and RM), and to obtain large numbers of each of these subsets at greater than 90% purity. Originally, the IM cells were purified from contaminating lymphocytes by the use of albumin gradients; recently a novel modification of the counter-current centrifugal elutriation apparatus has allowed us to purify these two subsets of monocytes directly with the use of the elutriation apparatus alone. The IM subset of cells is found to have lower peroxidase activity, lower protein content and lower alkaline phosphatase activity than RM cells. The phagocytic activity, Fc receptor expression, and the percent of HLA-Dr_{α} positive cells was the same for the IM and RM subsets. The IM subset has been shown to secrete significantly higher levels of certain cytokines including set colony stimulating factor, interleukin-1, and α-interferon. Conversely, the IM subset is known to release significantly less prostaglandin E than do the RM subset cells. Since prostaglandin E can interfere with the effects of colony stimulating factor, interleukin-1 and α-interferon at the target cell level, the immunoregulatory differences between IM and RM cells are considerable. Following donor monocyte depletion by cytapheresis, the relative proportion of IM cells increases appreciably in the peripheral blood of normal volunteers; this suggests that the IM subset is more mobilizable from human monocyte reservoirs and/or that this subset form the bulk of human monocyte reservoirs.

Special Teflon labware was developed in our laboratory which was shown to be capable of sustaining long term cultures of monocytes while still maintaining them in suspension. Parallel experiments with regard to cytokine release revealed that adherence-cultured monocytes function identically to Teflon cultured monocytes with the exception that baseline levels of secretion of certain constituitively secreted factors (such as fibroblast growth factor) was significantly elevated in adherence cultured cells. Similarly, the ADCC activity of adherence-cultured monocytes was also increased above that of the suspension cultured monocytes indicating that significant activation of

these monocyte functions takes place during the adherence procedure. In parallel experiments with monocytes cultured in optimal concentrations of human serum, we also noted that the secretion of α-interferon and colony stimulating factor were both significantly enhanced with serum-free media. Detailed studies of the mechanisms for this observation revealed that monocytes cultured in human serum matured from monocytes into macrophages over the course of two to three days. In contrast, monocytes cultured in serum-free medium did not mature into macrophages over this period of time. Thus it appears that the enhanced ability of mononuclear phagocytes to release certain biological response modifiers is associated with being maintained in their monocytoid form. Of interest, was the finding that serum-free medium was deficient in a protein (or series of proteins) which promote macrophage maturation and culture. It was shown an addition of 5 mg per ml of highly purified monomeric human immunoglobulin G to serum-free medium could correct this defect. The exact mechanism for this phenomenon is not as yet understood; however, it appears that immunoglobulin G and a few other serum proteins which interact with monocyte membrane receptors may promote the monocyte to macrophage maturation process directly.

Elutriator-purified monocytes have been applied to wide range of immunologic assays including the human monocyte migration inhibition factor (MIF) assay, chemotaxis assays, secretion of a wide range of biological response modifiers, phagocytosis assays, and assays of lipid metabolism including prostaglandin E. Perhaps most intensively studied to date has been the ability of elutriated purified monocytes to kill tumor cells in culture. The antitumor cytolytic activity of elutriator purified monocytes has been tested in a 72 hr assay with IUDR labeled human breast, colon, melanoma and glioma cell lines. It is clear that monocytes have an appreciable spontaneous cytotoxicity against these targets but that these cytotoxicity levels can be boosted further by the addition of certain macrophage activating factor containing preparations. Perhaps most effective in boosting the cytotoxic capability of human elutriator-purified monocytes against colon cancer cell lines is the addition of γ-interferon (both the natural and recombinant forms); in contrast α or β-interferons have little effect in augmenting this function. In addition to examining the killing capability of human monocytes against colon cancer targets in vitro, we have developed a nude mouse peritoneal colorectal carcinomatosis model using the human colon cancer cell line, LS174. Inoculation of the peritoneum of these animals with this cell line causes peritoneal carcinomatosis within 2 weeks to 30 days. We are currently examining the ability of human elutriator-purified monocytes to limit or eliminate this tumor cell spread in the nude mouse model. Moreover, we are experimenting with various systemic regimens for prolonging the cytotoxic capability of adoptively transferred human-elutriator purified monocytes in vivo.

Within the last year, we have utilized the technology cited above in an effort to apply elutriator-purified monocytes in an adoptive immunotherapy clinical trial setting with colon cancer patients. This treatment protocol is termed "ex vivo leukocyte activation (EVLA)." In collaboration with Dr. Paul Sugarbaker of the NCI Surgery Branch, five patients with peritoneal colorectal carcinomatosis have been receiving weekly administration of their own elutriator-purified blood monocytes (activated in γ-interferon for 18 hrs.) into their peritoneal cavity via an indwelling Tenchoff catheter. This

therapy has been remarkably nontoxic; symptoms to date include a mild abdominal pain on the night of cell infusion accompanied with a low grade fever lasting approximately 12 hrs. Patients who have any depression of their leukocyte counts from repeated cytapheresis are placed on an every other week treatment schedule. Overall, this protocol has impacted on the active lifestyle of our five patients very little and the protocol overall has been very well tolerated. To date, two patients have finished the EVLA treatment protocol both were judged to be responders at the time of "second-look" surgery and both have completed a course of maintenance therapy. One patient has been off of maintenance therapy for approximately 11 months and continues to be without any evidence of disease. The second patient just recently completed maintenance therapy and continues to be without evidence of disease. Utilizing ^{111}indium labeled blood monocytes, we have recently shown that the cells that are infused into these patients remain in the peritoneal cavity for prolonged periods of time (up to at least five days); they appear to be distributed in a rather uniform fashion throughout the peritoneal space when infused. Autoradiographic analysis of biopsy specimens obtained from patients following intraperitoneal infusion with ^{111}indium labeled monocytes indicates that these cells appear to become intimately associated with the peritoneal lining. Since this anatomical location is the exact site of tumor burden in these patients, we feel that we are probably placing these highly cytotoxic cells in direct opposition to the tumor-bearing sites of the patients.

SIGNIFICANCE

One of the major research thrusts of the Biological Response Modifiers Program is to develop new insights and understandings into the role of the human immune system, both as an immunosurveillance weapon to prevent the development of clinical malignancy and to reduce or eliminate established disease. The monocyte and its tissue counterpart, the macrophage, have been documented to play pivotal roles in the immune system particularly with regard to host defense. They're major participants both in spontaneous tumor cytolytic activity and in the antibody-dependent cytotoxicity phenomena. However, application of current understandings about this multifaceted cell to the clinical arena have been quite slow. The development of the technology required to purify large numbers of human monocytes in a way which least affects their native function and to couple this purification technique with suspension culture technology which most closely replicates the in vivo state of these cells has allowed us to make rapid strides in the "translation" of valuable basic research data into clinically relevant human monocyte research protocols. This advance is perhaps most dramatically shown in the development of a clinical research protocol (the EVLA project) utilizing γ-interferon activated autologous monocytes in an adoptive immunotherapy setting with peritoneal colorectal carcinomatosis patients. We are the first laboratory internationally to attempt to treat cancer patients with purified cytotoxic monocytes; data obtained from our first five pilot patients indicate that this is indeed a very well tolerated procedure. The data obtained from the first two patients to complete the project indicates that the EVLA protocol may be of benefit in controlling the disease. In addition to learning about the clinical effects and side effects of this form of therapy, we are also able to monitor the trafficking of these cells in vivo and to assess the function of these activated monocytes in humans. This detailed basic in

vivo human research will allow us to definitively characterize the physiology and clinical effects of γ-interferon activated autologous monocytes and thus build upon these experiments with regard to the design of future adoptive immunotherapy trials. Further clinical trials utilizing activated autologous cytotoxic monocytes might include the use of other monocyte activators such as muramyl dipeptide, the use of these cells in conjunction with colon cancer specific monoclonal antibodies, and combined adoptive immunotherapy protocols utilizing other cytotoxic effector cells as well (cytotoxic T lymphocytes and/or natural killer cells).

PROPOSED COURSE

We have initiated an in-depth study regarding the metabolism, differentiation, functional characteristics and mechanism for control of the human monocyte activation process. We plan to continue defining the differences and inter-relationships between the two elutriator-purified monocyte subsets characterized so far (IM and RM). Studies will be performed to evaluate the functional differences between these subsets with regard to their tumoricidal activity, biological response modifier secretion capability and morphological differences. We will be examining these cells for differences with regard to their ability to secrete tumor necrosis factor. Patients will be studied with regard to identifying potential imbalances in the normal IM to RM cell ratio in various disease states, particularly in cancer patients.

We will continue to study the functional differences that exist between blood monocytes as they mature into macrophages in vitro. We will determine the precise timing for alterations and assays of accessory cell function, MIF responsiveness, chemotactic responses, antigen-presentation, a wide variety of cytotoxic functions, membrane antigen expression, and particularly biological response modifier production. Utilizing our serum-free suspension culture system, we will continue to further delineate the factors which control the monocyte-to-macrophage maturation process and thus influence the functional activity of these cells.

The goal of all of these basic research studies is to develop more refined clinically applicable insights with regard to the treatment potential of these cells particularly the cancer patient setting. Our phase I adoptive immunotherapy protocol with monocytes (the EVLA project) with activated elutriator-purified human monocytes will be continued with our natural human γ-interferon preparation until five patients have been completed. At that time, a second study will be initiated in which recombinant γ-interferon will be used as the monocyte activator. The goal of the second study will be to determine the degree of overlap between the natural and recombinant γ-interferon preparations with regard to in vitro cytotoxic function and in vivo clinical effects and side effects. We certainly expect that we will be able to utilize the information gleaned from the second generation study to treat patients with bulky established colorectal carcinomatosis; the exact details of this third generation study are currently being finalized.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09281-01 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

The Synthesis and Evaluation of Drug-Monoclonal Antibody Conjugates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Alarif	Senior Staff Fellow	BTB, NCI
Other:	J. Pearson	Acting Head, Monoclonal Antibody/ Hybridoma Section	BTB, NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc. (J. Nemec) Lilly Research Laboratories (T. Bumol) and Teijin Limited (T. Hara)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Clinical Investigations Section and Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

Several new drug conjugates of monoclonal antibodies and or response modifiers have been synthesized. A major focus has been on the development of reproducible, simple, and efficacious methods for synthesis of these conjugates. Drug conjugates of 9.2.27, a monoclonal antibody to the 250K proteoglycan of human melanoma, and to a lesser extent D_3, a monoclonal antibody to the guinea pig line 10 hepatoma, have been made. The drugs utilized include actinomycin-D, (ACT-D), 5-Fu, Daunomycin, Ara-C, and methotrexate. We are now concentrating on ACT-D conjugates of 9.2.27 to determine optimal yield, specificity, activity in vitro, and in vivo vivo, as well as in vivo tumor localization. Preliminary in vitro cytotoxicity studies have been carried out with conjugates of all the aforementioned drugs using antigen positive melanoma cells (FeMx), and antigen negative melanoma cells (A375). The ACT-D conjugates of 9.2.27 appear to have immunologic specificity as well as potency. Further efforts are now being made to determine optimal conjugation ratios, using poly-L-lysine of various sizes as initial carriers of drugs to be conjugated to monoclonal antibodies. Further studies are being carried out to optimize in vitro delivery to tumor sites and minimize non-specific uptake especially in the reticuloendothelial system (RES) by using the following approaches; the use of monoclonal antibodies with covalently modified polyethyleneglycol monomethyl ether (PEG); pretreatment of animals with heat aggregated human gamma globulin; and the use of monoclonal immunoglobulins of the same subtype.

PROJECT DESCRIPTION

PERSONNEL

Adhid Alarif	Senior Staff Fellow	NIS BTB NCI
Jack Pearson	Acting Head	MAHS BTB NCI

OBJECTIVES

The aim of this project is to develop effective methods for synthesizing drug-monoclonal antibody (MoAb) conjugates for the control of established and metastatic tumors. The technology developed will hopefully also be used to synthesize conjugates of MoAb with radiolabeled substrates, for imaging, as well as for therapeutic purposes, that are more chemically stable than currently labeled MoAb. Specifically this will involve the synthesis of I^{125} 6-Iodocholesterol, I^{131}, and $At(^{211})$ - 6-Astatinocholesterol (α-emitter). Following synthesis of these conjugates, evaluation, in vitro and in vivo will follow as described in detail in (see Dr. J. Pearson's report, Z01 CM 09226-05 BTB).

METHODS EMPLOYED

I. Monoclonal Antibodies (MoAB)

The MoAb's used in this project have been 9.2.27 and D_3. If the results in in these model systems are successful, an attempt will be made to apply the same technology to 2945 (colon MoAb), and T-101 (T-cell lymphoma MoAb). Following drug conjugation, the conjugate will be assessed by HPLC and SDS-PAGE, as well as by flow cytometry with antigen positive, and antigen negative tumor cells. Other in vitro methods include a competitive binding assay of different unlabeled conjugates with labeled, untreated monoclonal antibody to antigen positive cells. Also, conjugates are radiolabeled with I^{125} to assess the binding of a constant amount of labeled conjugates to varying numbers of antigen positive cells. These methods allow for direct measurements of damage done to the immunoreactivity of monoclonal antibodies by various conjugation procedures

II. Methods of Conjugation

The basic principles of drug conjugation relate to the fact that most drugs have a m.w. of 300-1000, while MoAb have a m.w. of 150,000. Addition of more than 6 moles of drug/MoAb will generally result in the destruction of the immune reactivity of the antibody. For this purpose we have recently developed the use of poly-L-lysine carriers which will be "loaded" with drugs since poly-L-lysine contains numerous free amino groups for conjugation, and thus in principle, one can overcome the problems associated with the molecular weights. Initially poly-L-lysine (poL-L) is derivatized with either SPDP (3-2-pyridyLdithiopropionic acid N-hydroxy succinimide ester) or MBS (maleimidobenoic N-OH succinimide ester). This is then followed by addition reaction of the activated drug to the poL-L followed by purification from unreacted drug, including extractions with organic solvents, dialysis, and gel filtration. The following are the steps for ACT-D conjugation with 9.2.27 MoAb.

1. ACT-D $\xrightarrow{10\%\ HCL}$ 2-Hydroxy-ACT-D $\xrightarrow[(thionyl\ chloride)]{SOCL}$ 2-chloro-ACT-D $\xrightarrow{SPDP\text{-}PoL\text{-}L}$ SPDP-ACT-D-Pol-L

2. 9.2.27 + MBS $\longrightarrow$ MBS-9.2.27

3. SPDP-ACT-D-poly-L + DTT $\rightarrow$ HS-ACT-D-poly-L

4. MBS-9.2.27 + HS ACT-D-poly-L $\longrightarrow$ ACT-D-poly-L-S-MBS-9.2.27

The conjugates are dialyzed vs. Amicon XM-membrane (exclusion limit of 50,000d). They are then tested for cytotoxicity, purity and immunoreactivity.

This technology was also applied in a preliminary fashion to methotrexate, Daunomycin, 5-Fu, and Ara-c. The technology seems adaptable to a variety of clinically useful drugs. The chemistry involved in conjugating the other drugs is different for each drug For example methotrexate has been activated using three methods: 1) use of a mixed anhydride of isobutyl chloroformate, and adding that to poly-L-lysine. Carbodiimide activation was found to be very unsatisfactory (polymerizations were noted). 2) N-hydroxosuccinimide ester of methotrexate. 3) The addition of methotrexate to glutathione disulfide, reducing the disulfide with DTT (dithiothreotol), then adding the sulfhydryl derivative to MBS-poL-L. This preserves the terminal glutamic acid residue in methotrexate, and mimicks a diglutamate derivative of methetrexate. Further efforts are needed to select the most active method for conjugation of this drug. Antibody conjugation is simlar to that used with ACT-D conjugates.

3. In Vitro Cytotoxicity

Cytotoxicity of conjugates is assessed in vitro by inhibition of ^{3}H-thymidine uptake into antigen positive, and antigen negative cells after a 3 day incubation. If conjugates appear promising on this assay, an 8 hr assay, followed by washing the cells free of conjugates is conducted to determine potential cytotoxicity and specificity.

4. Biochemical, Chemical, and Immunological Purity

All conjugates are assessed by the following methods initially:
a) Flow cytometry with antigen positive, and negative cells.
b) Competitive inhibition assay, using unlabeled conjugates and I^{125} labeled antibody on antigen positive, and negative cells.
c) Binding assay using ^{125}I-labaled conjugates to antigen positive and negative cells.

This will be later used for localization studies (see Dr. Pearson's report).
d) HPLC profile, and purification using high performance gel permeation chromatography and pooling appropriate fractions.

5. Radiolocalization

MoAbs and drug conjugates are labeled with ^{125}I, and injected i.v. into tumor bearing animals. The uptake in tumor/non-tumor tissues is measured after a variety of treatments including pretreatment with aggregated human gamma globulin, as well as pretreatment with "cold" MoAb's. The last approach to inhibit non-specific localization is to use the conjugation of MoAb's to polyethylene glycol monomethyl ether (PEG) which has been successfully performed in this laboratory with 9.2.27 and D_3.

MAJOR FINDINGS

1. Chemical conjugation of a variety of clinically useful drugs used in oncology has been demonstrated using the carrier poly-L-lysine which is then attached to MoAbs. In vitro cytotoxicity, binding data, and preliminary HPLC analysis confirmed the specificity and cytotoxicity of ACT-D conjugates to 9.2.27. For example our most recent ACT-D conjugates were found to be cytotoxic to antigen positive cells without affecting the antigen negative cells at a concentration of 0.5 µg/ml of conjugate. Further research is needed to confirm these observations, and to insure that it is not drug sensitivity that causes this apparent specificity. Currently we are upscaling our preparitive and purification efforts to obtain larger quantities of conjugates for further evaluation in vitro and in vivo.

Preliminary 8 hour incubation studies with ACT-D conjugates followed by washing of conjugates reflect both cytotoxicity and specificity when used with antigen positive, and antigen negative cells. Our strategy now is to concentrate on ACT-D. Upon completing this project, we will move on to the other drugs, i.e. methotrexate. The justification for using ACT-D are as follows: 1) it is the most potent (and toxic) on a molar basis, antineoplastic agent approved for clinical use, 2) it is water soluble, and 3) preliminary data indicate that conjugation with it is chemically possible, and cytotoxic in vitro, and have apparent specificity. We are now attempting to use smaller m.w. polylysines. We have used in all our work poly-L-lysines of m.w. 12,000-14,000, and we will we looking at smaller polylysines (i.e. 3000-6000k)

2. The 9.2.27-PEG conjugates have been examined by flow cytometry, and binding, and were found to have retained a major portion of their immunoreactivity. Experiments are in progress now with regards to their distribution in vivo in tumor bearing animals. A rather unusual finding is that the binding of labeled (I_{125}) 9.2.27-PEG conjugate (1:5 ratio) was found to bind more avidly to antigen positive cells than control I^{125} labeled 9.2.27 tested under similar conditions. None of the PEG conjugates tested showed any decrease in binding than control antibody alone when bound to antigen positive or negative cells. This is in contrast to flow cytometry where there was found to be a decrease in mean flourescence intensity of the PEG conjugates when compared to antibody alone. We have no explanation for these preliminary data except to suggest that flow cytometry is an indirect method as compared to a binding assay, and that these results are a reflection of assay methods used. Thus, in summary, PEG can be easily attached to monoclonal antibodies and with some loss of antibody binding activity, but not to a significant extent.

3. Aggregated human gamma globulin appears to decrease splenic uptake of labeled MoAbs in portions of the RES. Further data on this issue are pending.

SIGNIFICANCE

The significance of these studies is to develop technologies such that drugs, toxins, or radiolabeled substrates can be selectively targeted to tumor tissues in vivo, using MoAbs as the specific delivery vehicles, so as to improve 1) therapeutic specificity, 2) decrease toxicity and 3) increase remission duration in patients with malignant diseases.

PROPOSED COURSES

1. Refine the ACT-D conjugate work, and concentrate on it till all the problems are resolved, test it for in vivo effects and antitumor potential. Promising conjugates will be purified and characterized as described in the methods section. Upon completion of the ACT-D conjugation effort, a second drug, likely methotrexate will be evaluated since previous experience has shown that its chemistry is manageable and practical for conjugation to MoAb's.

2. Collaborate with Drs. Nemec and Welch on the synthesis of 6-iodocholesterol (I^{125}) and I^{131}, as well as A^{211}-6-astatinocholesterol (α-emitter) using the technology discussed above. The purposes of this project are as follows:

a) The conjugation of 6-I_{125}-cholesterol, I^{131} or A^{211} to monoclonal antibodies will be pursued. It has been established that 6-iodocholesterol is a very chemically, and biologically stable iodide that is chemically unreactive. This will avoid the problems assocaited with current iodination methodologies based on iodinatine tyrosine or imidazole in proteins that lead to a very chemically unstable phenolic and imidazole iodides. Such organic iodides are similar to thyroid hormones which are known to be dehalonegated in vivo (enzymatic?). Astatine is a halogen, and once the chemistry is worked out for iodine, it will likely be applicable to astatine, and it has been demonstrated as such by Vissar and his colleagues.

b) The Iodo or Astatino cholesterol conjugates will be tested using our assays described in the methods section.

4. Pursue the conjugation of PEG to MoAbs or MoAb conjugates as a source of inhibiting Fc receptor binding, as well as a solublizing agent for water insoluble drugs such as the anthracyclines and methotrexate, since such drugs become highly insoluble once they become conjugated.

PUBLICATIONS

None to date.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09226-05 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Preclinical Evaluation of Immunoconjugaes Against Established Tumors & Metastases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Pearson	Acting Head, Monoclonal Antibody/ Hybridoma Section (12/22/84 to Date)	BTB, NCI
Others:	A. I. Alarif	Senior Staff Fellow	BTB, NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc. (J. Nemec); Lilly Research Laboratories (T. Bumol); Teijin Limited (T. Hara)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	2.25	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.)*

Several new animal model systems have been developed to evaluate the efficacy of immunoconjugates. A human melanoma (FeMX) and a human colon (Ht-29) xenografted either in nude mice or rats were found to induce a progressively growing tumors at the site of inoculation (sc) or induce pulmonary foci (iv). A T-cell lymphoma has been adopted to grow in nude mice following the sc inoculation of $4x10^6$ cells. Specific MoAb's exist for each of these animal model systems. The administration of 50 µg's of a gelonin or pokeweed antiviral protein (PAP) conjugate of 9.2.27 administered iv to nude mice bearing pre-palpable FeMX tumors demonstrated a 3 to 4 day delay in tumor emergence as compared to untreated animals. However, there was no difference in median survival time (MST) between the two groups when the study was terminated at 60 days. Localization studies have revealed that 1 to 2% of ^{125}I-labeled 9.2.27 localizes in FeMX tumors (7-8 mm) while 2 to 5% of the labeled MoAb was found in liver, kidney and spleen. Only .05% or less of a gelonin or PAP conjugate of ^{125}I-labeled 9.2.27 (50 µg) reached the site of similar size FeMX induced tumors. Two to 5% of the conjugates were taken up by the liver, kidney and spleen. Pretreatment of tumor-bearing animals with a non-specific MoAb (RPC-5) followed by systemic administration of ^{125}I-labeled 9.2.27 failed to enhance the uptake of the labeled MoAb to the tumor site or decrease uptake of the specific MoAb by the reticuloendothelial system (RES).

PROJECT DESCRIPTION

PERSONNEL

John Pearson	Acting Head	MAHS, BTB, NCI
Adhid Alarif	Sr. Staff Fellow	MAHS, BTB, NCI

OBJECTIVES

The aim of this project is to develop effective protocols using monoclonal antibodies (MoAb) directed toward tumor associated antigens, alone or in combination with drugs or toxins for the control of established and metastatic tumors. The models currently under study include a human melanoma (FeMX) and colon (Ht-29) xenografted in either nude mice or rats and a transplanted syngeneic L10 hepatocarcinoma tumor in strain 2 guinea pigs which spontaneously metastasizes to draining lymph nodes and to visceral organs. The specific objectives of this project are as follows: 1) to develop and/or utilize appropriate animal model systems for testing the therapeutic effectiveness of MoAb-drug or toxin conjugates, 2) to produce drug conjugates with several MoAb's; 9.2.27 (reactive with human melanoma), 2945 (reactive with human colon), and D3 (reactive with guinea pig L-10 hepatocarcinoma), 3) to produce immunoconjugates consisting of whole toxins, or the A chain of ricin or abrin, with the above antibodies to compare with drug conjugates for efficacy, 4) to label MoAb's and their conjugates with radionuclides in order to evaluate both their biodistribution, therapeutic and diagnostic potential in animal models, 5) to assess modalities for inhibition of RES uptake of drugs or toxins conjugated to specific MoAb's, 6) to develop optimal treatment protocols, i.e. dose, route, schedule to maximize the therapeutic efficacy of specific conjugates toward local tumor growth, experimentally induced pulmonary foci and spontaneous metastasis, and 7) to test the feasibility of combination therapy, i.e. surgery, chemotherapy and radiotherapy with immunoconjugates.

METHODS EMPLOYED

1. Monoclonal Antibodies - 9.2.27, 2945 and D3 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, and the immunoglobulins are purified by 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 - such drugs as actinomycin D, methotrexate, 5 FU and adriamycin are attached directly to a ligand, poly-L-lysine, followed by subsequent conjugation by a thioether linkage to MoAb's 9.2.27, 2945 or D3 (see Dr. Alarif's report).

3. In vitro cytotoxicity - the cytotoxicity of a drug conjugate is assessed by inhibition of cellular protein synthesis or proliferation.

4. Immunochemical - the drug conjugates are assessed for the presence of free antibody or ligand by HPLC/FPLC chromatography (gel filtration, ion exchange)

and SDS-PAGE. Immunoreactivity of drug conjugates is assessed by titration via flow cytometry and ^{125}I binding assays.

5. Radiolocalization - MoAb's or drug conjugates are labeled by either ^{125}I or ^{111}In and injected iv into tumor-bearing animals. The uptake of MoAb or drug conjugates by tumors and tissues is determined either by scintigraphic imaging using a gamma camera or by direct counting of radioactivity in organs.

6. Immunoperoxidase technique - retention of antigenicity and antibody localization is assessed by immunoperoxidase staining.

7. Surgery and/or chemotherapy - these two procedures are utilized to debulk tumor mass before the application of immunoconjugates.

MAJOR FINDINGS

A. New Animal Models

Over the past year, an intense effort has been underway to develop preclinical model systems to evaluate the therapeutic efficacy of immunoconjugates of monoclonal antibodies. A human melanoma, FeMX, xenografted in nude mice and rats has been demonstrated to produce tumors after either sc and iv administration. Pretreatment of 4-6 week old nude mice with 200 mg/kg of Cytoxan followed by iv administration of $3X10^6$ FeMX ascites cells 24 hours later resulted in 100% incidence of pulmonary amelanotic foci by 30 to 35 days post inoculation, a time when all animals died from melanoma. At necropsy, metastatic involvement was observed in the liver, kidney, spleen and along the spinal cord. The sc injection of $3X10^6$ FeMX ascites cells, into nude mice pretreated 24 hours prior with 400 r resulted in a progressively growing tumor with all animals dying within 80 to 85 days. No gross metastatic lesions were observed at necropsy. Nude rats exposed to 750 r followed 24 hrs later by the inoculation of FeMX ascites ($4X10^6$) cells, either iv or sc, developed pulmonary foci as well as progressively growing sc tumors. When animals were necropsied 6 weeks post inoculation, the average number of pulmonary foci ranged between 10 and 25, and the average size of the sc tumors were approximately 20 to 25 mm. Likewise, 100% of nude mice treated with 400 r followed by iv administration of $3X10^6$ Ht-29 ascites cells (human colon carcinoma) developed pulmonary foci. All animals died within 40 days, with no gross metastatic involvement of other organs. Similarily, sc inoculation of $3X10^6$ Ht-29 ascites cells resulted in a progressively growing tumor with animals dying between 100 and 120 days, without gross metastasis found at necropsy. Nude rats inoculated either sc or iv with $4X10^6$ Ht-29 ascites cells 24 hours after exposure to 750 rads exhibited both pulmonary foci and progressively growing tumors when the study was terminated at 6 weeks.

The number of macroscopic pulmonary foci found at 2 weeks ranged between 2 and 5 with the number of visible foci increasing by 7 to 10 in 4 weeks or 12 to 16 foci at 6 weeks. Tumor size at 6 weeks ranged between 12 to 15 mm's in diameter. No gross metastatic involvement was observed in other organs regardless of route of injections during this period. Recently, we have been able to demonstrate the sc growth of a human T cell lymphoma xenografted in nude mice.

Following the sc inoculation of approximately $4x10^6$ 8402 tumor cells, 80% of the animals develop a progressively growing tumor. All animals die within 70 to 80 days. This new model can be utilized to study the efficacy of T-101, a MoAb that has undergone phase I clinical trials in patients with cutaneous T cell lymphoma (CTCL), following conjugation to a specific drug and/or toxin.

B. Therapy Studies

During the past year, therapy studies with simple polypeptide A chain-like toxins, gelonin and pokeweed antiviral protein (PAP) covalently conjugated to antimelanoma antibody 9.2.27 were performed. Utilizing 50 µg of a gelonin or PAP conjugate of 9.2.27 administered iv as multiple treatments to nude mice bearing prepalpable FeMX tumors resulted in a 3 to 4 day delay in tumor emergence as compared to untreated animals. However, there was no apparent difference in subsequent tumor growth between the two groups. In addition, there was no significant difference in median survival time when the study was terminated at 60 days.

C. Localization Studies

Due to the lack of a good therapeutic response utilizing 9.2.27 - immunoconjugates in vivo, an intense effort has been underway, utilizing trace-labeled 9.2.27 or conjugates, to determine the amount of localization of the antibody at the tumor site versus uptake by elements of the RES. Preliminary studies with ^{125}I-labeled 9.2.27 have indicated that at 48 hours after injection, approximately 1 to 2% of the MoAb localizes in a small palpable (7-8 mm) FeMX tumor xenografted in nude mice. Approximately, 2 to 5% of the labeled MoAb was found in the liver, kidney and spleen. In contrast, only .01% or less of a gelonin and/or PAP conjugate of ^{125}I-labeled 9.2.27 (50 µg) reached the tumor site of similar size FeMX-induced tumors. Two to 5% of the conjugates were taken up by the liver, kidney and spleen. In an attempt to block the RES and hopefully increase the uptake of 9.2.27 by the FeMX tumor, a study was recently undertaken with RPC-5, a myeloma protein of the same subclass ($IgG2_a$). Tumor-bearing mice received 1 mg of cold RPC-5 iv at 72, 48 or 24 hours before systemic delivery of ^{125}I-labeled 9.2.27 (100 µg). Results indicated that, regardless of time of delivery of RPC-5, pretreatment with nonspecific immunoglobulin failed to enhance the uptake of ^{125}I-labeled 9.2.27 by the tumor (3%) and/or decrease the uptake by elements of the RES: liver (3%), kidney (4.5%) and spleen (5%).

Recently a collaborative effort was established with Dr. Tom Bumol, Eli Lilly, to evaluate a 9.2.27-DAVLB (descetylvinblastine) drug conjugate prepared by this investigator. Initial in vitro studies have demonstrated good immunoreactivity on our FeMX melanoma cells as assessed by flow cytometry (FACS) and ^{125}I binding assays. Studies are now underway to determine localization at the FeMX tumor site versus uptake by elements of the RES utilizing ^{125}I-labeled conjugates. If good localization is observed at the tumor site, therapy studies are to be initiated against the FeMX tumor in nude mice. Likewise, a P97-mitomycin C conjugate has been obtained from Dr. Hara, Teijin Institute for Biomedical Research, Tokyo, Japan. This conjugate is undergoing similar in vitro and in vivo studies as described for the Eli Lilly drug conjugate.

A collaborative effort has been established with Drs. Joe Nemec (PRI) and Arnold Welch (NCI). The purpose is two-fold: (1) to develop a new method of labeling MoAb's with an α emitter, astatine-211, which is a halogen chemically similar to ^{125}I. Initial studies will concentrate on using Iodine instead of astatine since the chemistry for both is identical and Iodine is more readily applicable for both diagnostic and therapeutic purposes. Our initial efforts will focus on the conjugation area followed by the in vivo evaluation of such preparations for their therapeutic and diagnostic potential utilizing various human xenografts in nude mice and rats available within the MoAb section

SIGNIFICANCE

Studies in the nude mouse or rat xenografted with human tumors are needed for understanding potential toxicity and immunomodulatory effects, and for developing optimal therapeutic protocols utilizing immunoconjugates against primary and metastatic human tumors.

I. Proposed Course

A new drug conjugate, Actinomycin D-poly-L-lysine-9.2.27 (see Dr. Alarif's report), shows good promise for eventual therapy against the FeMX melanoma tumor xenografted in either nude mice or rats. This conjugate has demonstrated good specific ^{125}I-binding assays and killing in cytotoxicity assays. The purity of conjugate is being assessed via HPLC chromatography and SDS-PAGE gel electrophoresis. Localization studies will be performed in the near future. If successful, this conjugate will be evaluated for prelinical therapeutic efficacy, first against a prepalpable tumor, then against an established FeMX tumor, and finally against experimentally induced pulmonary foci. Procedures found to be successful in this system will be extended to other drugs such as methotrexate, adriamycin and 5FU conjugated to MoAb's 9.2.27 (human melanoma), 2945 (human colon) or D3 (reactive with guinea pig L-10 hepatocarcinoma).

II. Localization Studies

Efforts will continue to find ways to increase the uptake of specific MoAb's or drug conjugates to established tumors while decreasing the uptake of such preparations to elements of the RES. Pretreatment of tumor-bearing animals with either nonspecific MoAb's of the same subclass or heat aggregated immunoglobulins administered at different time intervals or doses will be done before iv delivery of a specific labeled MoAb. PEG (polyethylene glycol monomethyl ether) which has been shown to decrease antibody formation to foreign proteins and enzymes as well as decreasing Fc receptor binding both in vitro and in vivo is presently under investigation, to determine if it will enhance uptake into tumors and decrease nonspecific uptake by the RES.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09228-05 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Further Characterization of Natural Killer (NK) Cells in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Nama, title, laboretory, end institute affiliation)*

PI:	C. W. Reynolds	Senior Staff Fellow	BTB, NCI
Others:	D. Reichardt	Biologist	BTB, NCI
	H. Fukui	Guest Researcher	BTB, NCI

COOPERATING UNITS *(if eny)*

LCC, NCI-FCRF (J. Ward); IB, DCBD, NCI (P. Henkart); University of California, San Diego (S. Hedrick)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	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 both normal bone marrow and tumor targets are all included in the large granular lymphocyte (LGL) subpopulations. Studies with transplantable LGL leukemias in F344 rats have demonstrated close similarity with normal LGL. Similarities were also noted between these LGL tumors and previously reported cases of human Tγ-CLL. Biochemical analysis of these rat LGL leukemias has resulted in the purification of cytoplasmic granules containing a highly cytolytically active protein of approximately 60 Kd. Rabbit antibodies against these granules block both rat and human NK, antibody-dependent cellular cytotoxicity (ADCC), and inhibit the growth of the fungus, Cryptococci neoformans. Molecular biologic studies have also shown the lack of β-chain rearrangement or mRNA expression, suggesting that the T cell receptor is not involved in the recognition of target cells by LGL. The present studies demonstrate that a cytoplasmic granule component is necessary for the lytic activity of LGL in both NK and ADCC, and provide the first direct evidence that a secretory event involving these granules is part of the lytic process for both tumor cells and fungi.

PROJECT DESCRIPTION

PERSONNEL

Craig Reynolds	Senior Staff Fellow	NIS	BTB	NCI
Della Reichardt	Biologist	NIS	BTB	NCI
Hiro Fukui	Guest Researcher	NIS	BTB	NCI

OBJECTIVES

The objectives of this project are: (1) to investigate details regarding the morphological, functional, and antigenic characteristics of rat NK cells, (2) to further characterize the transplantable spontaneous LGL leukemias in F344 rats, and (3) to analyze specific membrane receptors and intracellular molecules responsible for target cell lysis.

MAJOR FINDINGS

I. Characteristics of LGL

Detailed studies on isolated rat LGL have demonstrated these cells to be a distinct population of cells, about 75 μm^2 in size, positive for acid phosphatase and β-glucuronidase; and negative for alkaline phosphatase, esterase, peroxidase and lysozyme. In collaboration with Dr. Jan Rozing (Netherlands), we were able to demonstrate, by the use of newly produced monoclonal antibodies and flow cytometry, that rat LGL were an antigenically distinct population of cells that share an number of cell surface antigens with monocytes, suppressor/cytotoxic T cells and polymorphonuclear leukocytes (PMN). LGL are not typical T cells, B cells, monocytes or PMN but rather share some of the characteristics with these other cell types. In addition, the presence of various cell surface antigens suggested that in vivo or in vitro use of the appropriate antibodies might provide a basis for identifying, isolating or eliminating NK cells.

II. Comparison of Spontaneous Rat LGL Leukemias With Normal Rat LGL

Our recent discovery of spontaneously occurring LGL tumors in aged Fischer (F344) rats has provided a very useful system for obtaining a large number of LGL for detailed biochemical and genetic studies. Normal LGL and the rat NK (RNK)-leukemias are morphologically identificable as large lymphocytes with azurophilic granules in the cytoplasm. Both the normal LGL and LGL tumor lines express receptors for the Fc portion of IgG (FcγR), the NK-associated asialoGM_1 antigen, and the T cell-associated antigens recognized by the BC-84, W3/13, and OX-8 monoclonal antibodies. The expression of the Thy 1.1 antigen (previously shown to be a marker on early lymphocyte precursors in rat bone marrow) on most LGL leukemias but not normal LGL probably reflects the relatively immature state of the transformed LGL. Ultrastructurally, RNK-leukemia cells have cytoplasmic granules which appear as lysosomes in association with Golgi vesicles. The RNK lines efficiency kill the NK-sensitive targets YAC-1 and G_1-TC, but have little or no activity against the NK-resistant targets (C58NT)D and P815.

Most lines also demonstrate appreciable levels of ADCC against antibody-coated P815. This pattern of specificity is very similar to that seen with normal rat LGL. The fact that most RNK-tumor lines have both NK and ADCC activities supports the possibility that the same LGL can kill both NK-sensitive and antibody-coated targets.

Many of the clinical and laboratory findings for these rat LGL tumors are very similar to the small number (<35) of reported cases of human Tγ-lymphoproliferative disease (Tγ-LPD). These include splenomegaly, hepatomegaly, icterus; elevated bilirubin, aminotransferase and serum alkaline phosphatase levels; reduced serum albumin levels, and anemia. As in human Tγ-LPD, this anemia seems to be due to a combination of factors including erythrophagocytosis by the RNK-tumor cells and an infiltration of the bone marrow by leukemia cells. Thus, the RNK-tumor lines may serve as an excellent experimental model for further examining the ontogeny, function, and response to treatment of human LGL Tγ-LPD.

III. Analysis of Target Cell Receptors and Cytolytic Molecule(s) from the Cytoplasmic Granules of LGL Tumor Lines

The LGL leukemias are also an excellent source of cells for projects which involve the isolation and characterization of subcellular or secretory products which may be present in very small amounts. For example, the LGL leukemias from the rat have been a very useful source of cells for the isolation, purification, and characteriztion of LGL cytoplasmic granules. The use of LGL tumors for these studies was critical since the amount of granule material required to do these detailed experiments clearly could not have been obtained from normal LGL. In collaboration with Dr. Pierre Henkart (Z01CB05018-15), we have identified at least one molecule from LGL granules which is highly cytolytic. This molecule has not been found in the cytoplasmic granules from other cells; including T cells, PMN's, mast cells and macrophages. Antibodies produced in rabbits against the LGL granules were also found to be highly inhibitory for both rat and human ADCC activity. Further purification of the cytolytic molecule within the granules suggests that it is a protein of approximately 60,000 MW and acts via polymerization and insertion into the target cell membrane (similar to poly C_9 complement pores).

The granules have also been shown to effect the in vitro growth of the fungus Cryptococci neoformans (Dr. June Ann Murphy, Univ. Oklahoma). Pretreatment of Cryptococci with whole granules or soluble granule contents completely inhibited the growth of this microorganism. Addition of antigranule Ab or removal of Ca^{+2}/Mg^{+2} blocked this effect, suggesting that a similar molecule may be affecting the growth of these fungus and causing the lysis of tumor cells.

SIGNIFICANCE

Recent data in experimental animals have indicated that the natural cell-mediated immune system may play a significant role not only in immune surveillance against tumors but also in resistance to microbial infection and rejection of bone marrow grafts. Further studies in experimental animal systems should facilitate our understanding of natural cell-mediated immunity in man. Our present results have clearly demonstrated that LGL are a distinct

population of lymphocytes which do not express the genes for the β-chain of the T cell surface receptor. 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 recognition receptors for target cells, 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 of the overall function, lytic mechanism, and relevance of NK cells in human tumor systems.

PROPOSED COURSE

Future studies will include experiments to analyze a number of cell surface structures on the LGL leukemias including the IFN-binding site, receptor(s) for target cells, and proposed additional structures which might be required for NK cell activation and/or granule release. These studies will include the production of cDNA probes from LGL tumor messenger RNA for detecting the gene(s) which encode for membrane-bound structures, rearranged in LGL but not other tissues, and not expressed on B cells. This approach will hopefully help to characterize the LGL recognition receptor at both the protein and DNA level and may also help to determine the biological relationship of LGL and T cell receptors.

In addition to the further biochemical and functional characterization of the intracellular cytoplasmic granules isolated from rat LGL tumors, we are attempting to determine whether these LGL leukemias can produce high levels of various cytokines, including interleukin-1 (IL-1), interleukin-2 (IL-2), colony stimulating factor (CSF), γ-IFN and B cell growth factor (BCGF), all which have been associated with human LGL. In addition, we are studying LGL granules for the presence and molecular nature of NKCF or other types of secreted cytolysins. This system could provide a very useful model for examining the mechanisms of lysis by NK cells.

Studies are also now underway to examine the biological half-life and turnover rates of LGL. This is an especially important question in BRM-treated animals where there is often an increase or decrease in NK activity. Our intent is to examine what effect these BRMs have on the proliferation of LGL and how these changes may alter the antitumor effectiveness of NK cells following BRM treatment.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09246-17 BTB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. B. Herberman	Chief	BTB, NCI
Others:	J. R. Ortaldo	Head, Natural Immunity Section	BTB, NCI
	L. Mason	Microbiologist	BTB, NCI
	B. J. Mathieson	Senior Investigator	BTB, NCI

COOPERATING UNITS *(if any)*

University of Perugia, Italy (C. Riccardi); University of Rome, Italy (A. Santoni); Preclinical Screening Laboratory, Program Resources, Inc. (J. Talmadge)

LAB/BRANCH
Biological Therapeutics Branch

SECTION
Natural Immunity Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Studies have been performed to determine the mechanism of cytotoxicity by mouse natural cytotoxic (NC) cells and to contrast this information with the mechanism for cytotoxicity by natural killer (NK) cells. The NC-susceptible target cell, WEHI-164, has been found to be highly susceptible to both mouse and human recombinant tumor necrosis factor (TNF) and antibodies to mouse TNF strongly inhibited NC activity. In contrast, the NK-susceptible target cell YAC-1 was resistant to lysis by TNF and anti-TNF did not affect NK activity.

Studies have continued on the regulation of mouse NK activity. The development of NK activity after birth was found to be appreciably accelerated by repeated inoculations of infant mice with interleukin 2 (IL-2). Similarly, repeated inoculations of low doses of IL-2 were able to induce more rapid reconstitution of NK activity by donor bone marrow cells in lethally irradiated mice. These results point to an important role of IL-2 in the in vivo differentiation or expansion of mouse NK cells.

Mouse model systems for induction of hyporesponsiveness to augmentation of NK activity, after multiple inoculations of natural or recombinant interferon, have been developed. This hyporesponsiveness was found to be generalized, not only in the blood or spleen but also in the liver and lungs. Subsequent inoculation with other biological response modifiers, such as IL-2, poly ICLC or MVE-2, resulted in substantial augmentation of NK activity, pointing toward a combination treatment approach to overcoming the NK hyporesponsiveness.

Studies have been performed on the regulation of mouse NK activity by 17β-estradiol. As previously described, prolonged treatment resulted in depressed NK activity which was associated with decreased numbers of large granular lymphocytes (LGL). In contrast, shorter periods of treatment with this estrogenic hormone resulted in augmented NK activity and increased numbers of LGL. These results indicate that hormonal regulation of NK activity is more complex than previously realized and that estrogens can have both positive and negative regulatory effects.

PROJECT DESCRIPTION

PERSONNEL

Ronald B. Herberman	Chief		BTB	NCI
John R. Ortaldo	Head	NIS	BTB	NCI
Llewllyn Mason	Microbiologist	NIS	BTB	NCI
Bonnie Mathieson	Senior Investigator	NIS	BTB	NCI

OBJECTIVES

The objectives of this project are: (1) to determine the characteristics and mechanisms of cytotoxicity of natural effector cells in mice; (2) to determine the factors and mechanisms by which biological response modifiers regulate levels of NK activity in mice; (3) to evaluate the role of natural cell-mediated immunity in resistance against tumor growth.

MAJOR FINDINGS

I. Role of Tumor Necrosis Factor (TNF) in Activity of Natural Cytotoxic (NC) Cells

Natural cell mediated cytotoxic reactivity in mice has been associated with two types of effector cells, the natural killer (NK) cell and the NC cell. In contrast to substantial insights into the characteristics of the NK cell and its mechanism of cytotoxicity, relatively little is known about the NC cell. NC activity is measured primarily against the WEHI-164 tumor cell line, with high levels of cytotoxic reactivity observed after 18 hrs. of incubation with even low effector to target cell ratios. The potent reactivity against this target cell and the requirement for a long incubation period raised the possibility of involvement of a soluble mediator and we hypothesized the involvement of tumor necrosis factor (TNF). Mouse as well as human TNF has recently been cloned and therefore recombinant mouse TNF and antibodies against it as well as against natural mouse TNF were available for study. Incubation of the WEHI-164 cell line with either mouse or human recombinant TNF resulted in high levels of cytotoxicity, which could be completely inhibited by the addition of the relevant antibodies. Antibodies to recombinant or natural mouse TNF were then tested for their ability to inhibit NC activity of normal spleen cells against the WEHI-164 cell line. Both of these antibodies strongly inhibited NC activity, whereas other antisera were without detectable effects. In contrast, antibodies to TNF had no effect on NK activity against YAC-1 target cells. These data demonstrate that TNF plays a critical role in cytotoxicity by mouse NC cells and suggests that this cytokine may be an important effector molecule for host defense against tumors.

II. Regulation of NK Activity in Mice

Our previous studies have demonstrated that infant mice have low or undetectable NK activity, which appears to be attributable to a low number of large granular lymphocytes (LGL) and of NK cell progenitors. Further, incubation of infant spleen cells with interleukin 2 or interferon for 12 to 18 hrs. induced little cytotoxic reactivity. Thus, the low NK activity of infant mice appears to be

due to delayed maturation of this effector population, with spontaneous NK activity developing at 3 to 4 weeks of age. To understand the basis for this development and the factors regulating the appearance of NK activity, attempts were made to alter the time course of development by repeated injection of cytokines into infant mice. Interferon and especially interleukin 2 were found to accelerate the time for development of detectable cytotoxic activity by 10-14 days, with adult levels reached by 3 weeks of age. Taken together, the results suggest that the time course of maturation of the NK activity is largely dependent on the time of spontaneous production of interleukin 2 and/or other cytokines, and that the developmental process can be accelerated by exogenous administration of these factors.

Similar analyses have been performed in mice after lethal irradiation and bone marrow transplantation. NK activity in the chimeric mice usually is first seen around 10 days and returns to pretreatment levels after 14 days. It has been found that repeated administration of low doses of interleukin 2 after bone marrow transplantation accelerates the time for reconstitution of NK activity, with peak levels achieved within 7 to 10 days. This accelerated reconstitution did not seem dependent on residual NK activity in the recipients, since pretreatment of the recipients with antisera to asialo GM_1, just prior to irradiation and transplantation, did not affect the time course or levels of reactivity achieved.

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 clinical therapy. Multiple injections of interferon (human recombinant αA/D or recombinant mouse interferon γ) into young adult mice resulted in hyporesponsiveness to NK augmentation, whereas a similar course of injections into weanling mice resulted in sustained augmentation of NK activity. Further studies were then performed to understand the basis for the hyporesponsiveness in young adult mice. We considered the possibility that the hyporesponsive state was restricted to certain organs or that activated NK cells were redistributed from the spleen to other sites. However, testing of NK activity from the lungs and blood as well as spleen indicated that the hyporesponsiveness was systemic, with no evidence of augmentation of NK activity after a 2 week course of daily interferon injections. In contrast, the hyporesponsiveness to NK augmentation in the spleen which was induced by other biological response modifiers, e.g. MVE-2 or P. acnes, was shown to be compartmentalized, with augmented NK activity retained in the lungs and liver. Studies were then performed with multiple injections of interleukin 2 and the results obtained were very similar to those seen with interferon, with hyporesponsiveness to NK augmentation developing at all sites examined. Mice rendered hyporesponsive by multiple injections of interferon were found to still be quite responsive to augmentation of NK activity by other agents, including IL-2, poly ICLC and MVE-2. These results indicate that sequential administation of different biological response modifiers might be more effective for sustained augmentation of NK activity than repeated administration of the same agent.

Of the agents known to depress or negatively regulate NK activity in mice, estrogenic hormones have been particularly effective. To understand the basis for this hormone-induced depression of NK activity, we have investigated the effects of 17 β-estradiol on mouse NK activity. In addition to cytotoxic function, we evaluated the effects on the number of LGL, the cellular subset which has been shown to be responsible for NK activity. As previously observed, prolonged estrogen administration, for 60 days, decreased NK activity and this was associated with a decrease in the number of LGL and with significant bone marrow aplasia. Unexpectedly, shorter periods of treatment with estrogen resulted in increased NK activity. After 7 days of treatment, the increased cytotoxic activity was associated with high density cells, usually devoid of NK activity. After 30 days of treatment, augmented NK activity was still observed and this was associated with an increase in the number of LGL. The cytotoxicity by both the LGL and by the high density spleen cells was completely abrogated by treatment with antiserum to asialoGM_1 plus complement, whereas treatment with antibody to Thy 1.2 plus complement only partially decreased the reactivity. These results indicate that exposure of mice to estrogens may first stimulate the development of NK cells, with the earliest activity associated with morphologically distinct but antigenically similar cells, with the subsequent expansion of the number of LGL. More prolonged treatment with estrogen results in a marked decrease in the effector cell population, perhaps attributable to the depression of bone marrow function.

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 and NC cells. Particular emphasis will be placed on determining the basis for production of TNF by NC cells. It will be of interest to determine whether the release of this cytokine is truly spontaneous or whether this activity results from stimulation of the effector cells by interaction with NC-susceptible target cells.

Studies will be continued on the basis for spontaneous development of NK activity in mice. In particular, we will evaluate the possible role of endogenous production of interleukin 2 or interferon. Both adult and infant mice will be injected repeatedly with antibodies to the receptor for mouse interleukin 2 or with antibodies to various types of mouse interferon, to determine whether such treatments inhibit the development or maintenance of spontaneous NK activity.

In addition to further studies to understand the basis for the hyporesponsiveness to augmentation of NK activity after repeated frequent injections of interferon, studies will be performed to determine the interval for repeated injection of interferon which might circumvent the development of such hyporesponsiveness. It is possible that less frequent injections of interferon, or brief cycles of interferon, might result in more sustained immunomodulatory activity and concomitant antitumor therapeutic activity.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09247-05 BTB

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October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 charecters 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 professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. R. Ortaldo	Head, Natural Immunity Section	BTB, NCI
Others:	R. B. Herberman	Chief	BTB, NCI
	T. Sayers	Guest Researcher	BTB, NCI
	A. Procopio	Visiting Fellow	BTB, NCI
	A. Gronberg	Visiting Fellow	BTB, NCI
	I. Blanca	Guest Researcher	BTB, NCI
	C. Woodhouse	Visiting Fellow	BTB, NCI
	C. Morgan	Head, Monoclonal Antibody/Hybridoma Section (10/01/85 to 12/21/84)	BTB, NCI

COOPERATING UNITS *(if any)*

NCI-FCRF (G. Scala); MET, NCI (T. Waldmann); IB, DCBD, NCI (P. Henkart); PO, NCI-FCRF (H. Rabin)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	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 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. NK cytotoxic factors (NKCFs) are being examined for their specificity and mechanism of action. Three distinct steps have been defined for NKCF; a) production, b) binding to targets, and c) subsequent target lysis. With procedures able to independently measure these events, a variety of agents which have been reported to inhibit NK cell-mediated killing are being tested to determine their site of action. These NKCFs are produced by LGL and have a general specificity pattern similar to intact killer cells. Comparisons were made between NKCF and recombinant lymphotoxin (LT) and tumor necrosis factor (TNF). The results demonstrated that NKCF is distinct from both these cloned factors. In addition, LGL have been shown to produce a variety of cytokines including IFN-α and β , interleukin 1 and 2, and B-cell growth factor in response to target cells or lectin. The effector cells for mediating antibody-dependent cellular cytotoxicity (ADCC) with various mouse monoclonal antibodies was shown to be associated with LGL. In addition, this ADCC effector cell was regulated in vitro by IFNs and IL-2 in a manner similar to the augmentation of NK activity.

PROJECT DESCRIPTION

PERSONNEL

John Ortaldo	Head	NIS	BTB	NCI
Ronald Herberman	Chief		BTB	NCI
Thomas Sayers	Guest Researcher	NIS	BTB	NCI
Antonio Procopio	Visiting Fellow	NIS	BTB	NCI
Alvar Gronberg	Visiting Fellow	NIS	BTB	NCI
Isaac Blanca	Guest Researcher	NIS	BTB	NCI
Clive Woodhouse	Visiting Fellow	MAHS	BTB	NCI
Charles Morgan	Head	MAHS	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 LGL 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 FINDINGS

I. 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 and have employed these purified cells to continue examination of characteristics of the NK cells.

In studies with monoclonal antibodies, most, if not all, of the active NK cells were shown to be OKT10, OKM1 and 3G8 (Fcγ) positive. The B73.1 and 3G8 antibodies appear to be very useful reagents for enumerating and separating LGL. In contrast, the anti-HNK1 reagent (anti-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 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, 3G8 positive), the predominant peripheral progenitor was only positive for OKT11. Initial results indicate

that the bone marrow progenitors of NK-like activity are negative for all markers on mature NK cells (OKT10-, OKM1-, 3G8-) but are positive for common leukocyte markers.

II. Mechanism of Human NK Activity

Studies have been initiated to study cytotoxic factors produced by NK cells (NKCFs). 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. Using NKCF as a model for cytolysis by LGL, we have analyzed a variety of agents previously demonstrated to inhibit NK activity. These have included: 1) phosphorylated sugars, 2) protease inhibitors, 3) antibodies, 4) the absence of divalent cations (Ca^{++} and Mg^{++}) 5) lipomodulin, 6) nucleotides, 7) prostaglandins, and 8) inhibitors of lysosomal enzymes. The following have been shown to inhibit lysis of NKCF: phosphorylated sugars, and antibodies to rat granules, whereas the other agents or treatments tested have no detectable effect (ATP, cAMP, protease inhibitors, PGE_2). In regard to production of NKCF, preliminary data indicate that 1) the absence of Ca^{++}/Mg^{++}, 2)PGE_2, and 3) ATP inhibit production, whereas phosphorylated sugars do not. All of the inhibitors will be tested for their effects on: 1) production of NKCF after target-effector interaction, 2) binding of NKCF to target cells, and 3) NKCF lysis (after 6 hrs. of adsorption and washing) of targets. Phosphorylated sugars and antibodies to rat LGL granules were found to inhibit the binding of NKCF to targets, whereas the other agents tested had no detectable effect (ATP, cyclic AMP, protease inhibitors, prostaglandin E_2). Monensin inhibited post-NKCF binding, indicating a late effect on target cell lysis. In addition to the above studies, purification studies were performed to begin biochemical characterization of human NKCF. The results indicated that radiolabeled NKCF has an apparent molecular weight between 20,000 and 40,000. This material demonstrated a pattern of binding to target cells which was similar to the pattern of lysis by NKCF. Such approaches with radiolabeled NKCF should be useful for the further study of the biochemical characteristics of human NKCF and of its mechanism of action.

III. ADCC by LGL with Mouse Monoclonal Antibodies

Studies were performed to explore one of the possible factors which may contribute to the therapeutic efficacy of antibodies in patients with melanoma. In view of the ADCC observed with 9.2.27 or MB3.6 and mouse effector cells and the potential antitumor benefits to be achieved from such interactions, the ADCC with human effector cells was measured. For comparison, parallel studies were performed with a rabbit antiserum against human melanoma. MB3.6 is a mouse IgG3 monoclonal antibody directed against the GD_3 ganglioside and 9.2.27 is directed against a 250,000 dalton antigen which has been found in high levels on melanoma cells. Peripheral blood mononuclear leukocytes were tested, and the anti-GD_3 antibody produced substantially more cytotoxicity than 9.2.27. The strongest ADCC was observed with the rabbit antimelanoma antibody. Cell populations enriched for LGL had high levels of ADCC activity with the anti-GD_3 antibody and with the rabbit antiserum but had minimal effects with 9.2.27. In contrast, monocytes and T cells had no detectable ADCC activity. NK and ADCC activities by human LGL have been shown to be augmented substantially by pretreatment of the effector cells with interferon

and IL-2. Similarly the ADCC by monoclonal antibodies against the melanoma target cell could be augmented by pretreatment of LGL with either recombinant IFNα or recombinant IL-2, but recombinant IFNγ induced only a modest degree of augmentation of ADCC.

In our studies, considerably stronger and more consistant ADCC activity was observed with the γ3 antibody to GD_3 than with the γ2a antibody, 9.2.27. This was somewhat surprising since γ2a antibodies have been considered best for mediation of ADCC. Therefore, antibodies of various isotypes were studied for ADCC activity. In a survey with more than 60 monoclonal antibodies against human colon carcinoma, most of the γ3 antibodies were quite active in inducing ADCC, whereas antibodies of the other isotypes gave ADCC with substantially lower frequency. Such results are encouraging since they should help to design therapeutic protocols with monoclonal antibodies with the capability of mediating ADCC, perhaps in combination with interferon α or IL-2 treatment.

IV. Secretory and Other Non-cytotoxic Functions of Human Large Granular Lymphocytes (LGL)

In addition to their central role in mediating NK activity, there are indications that human LGL also have considerable immunoregulatory functions, including the ability to secrete a variety of cytokines. Highly purified populations of LGL, depleted of all detectable T cells and monocytes, could be stimulated to produce substantial levels of: 1) interferons, with the type depending on the stimulus; 2) interleukin 2; 3) interleukin 1; 4) colony stimulating factor; and 5) B cell growth factor; as well as NK cytotoxic factor(s) (see above). Studies are now in progress to determine the ability of IL-2-dependent cultures of LGL, and particularly clones derived from such cultured LGL, to produce these factors, and to determine the association of production of one or more of these factors with phenotypically distinguishable subsets of LGL. The available data indicate that cultures of LGL can produce interferon and interleukins 1 and 2. The production of IL-1 by fresh LGL has been found to be restricted to the Ia+, OKM1+ subset, whereas the LGL responsible for IL-2 and BCGF production were OKM1-, Ia- but 3 . The OKT8+ subset$^+$ of LGL did not produce factors but rather seemed to have a negative regulatory effect on factor production. These findings are of considerable interest, implying both an important immunoregulatory role for LGL and the potential for positive self-regulation of NK activity.

SIGNIFICANCE

Natural cell-mediated immunity may play an important role in host resistance 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. The further characterization of phenotype of NK cells offers the ability to directly enumerate NK cells in various 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 will be performed. A 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(s) 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, by the use of isotopically or fluorescein-labeled cells; and (5) the cytolytic activity of LGL against autologous primary tumor cells and studies on the interactions of LGL with mouse monoclonal antibodies will be continued.

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 solubulized target structures will be performed and reagents prepared (monoclonal antibodies) to purify, identify, and enumerate target cell structures.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 09255-03 BTB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of NK Cells in the Control of Metastatic Spread and Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Gorelik	Guest Researcher	BTB, NCI
Others:	R. B. Herberman	Chief	BTB, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biological Therapeutics Branch

SECTION
Natural Immunity Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

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.)*

Antimetastatic effects of heparin and warfarin was demonstrated against various tumor cell lines B16F10, BL6 melanoma cells, Lewis lung carcinoma (3LL), Madison lung carcinoma M109. The antimetastatic effects of the anticoagulant agents can be enhanced by stimulation of the host NK reactivity by poly I:C treatment. In contrast, depression of NK cell function by cyclophosphamide (Cy) or anti-asialo GM1 serum abrogated the antimetastatic effects of heparin or warfarin. In some experiments, this abrogation was partially due to the effect of the residual NK cells surviving after 1 injection of Cy or anti-asialo GM1 serum. Antimetastatic effects of the anticoagulants completely disappeared after 2 treatments with Cy or antiserum. Since young (3 weeks old) C57BL/6 or beige mice have some NK reactivity, the heparin and warfarin exerted the antimetastatic activity in these mice which disappeared after one injection of anti-asialo GM1 serum. The importance of NK cells in the antimetatatic effects of the anticoagulant drugs supports by the fact that restoration of NK reactivity of Cy treated mice after adoptive transfer normal spleen cells associated with the restoration of the antimetastatic effects of heparin. However, spleen cells of mice with depleted NK function after anti-asialo GM1 treatment failed to reconstitute NK reactivity of the recipient mice as well as the antimetastatic activity of heparin. These results indicate that fibrin coagulation on the tumor cells may be one of the mechanisms responsible for the in vivo tumor cell protection. Anticoagulant drugs make tumor cells more vulnurable to the destruction by NK (or other cytotoxic) cells. These data shed new light on the understanding of the mechanisms of the anti-metastatic effects of the anticoagulant drugs and the in vivo role of natural cell-mediated immunity.

PHS 6040 (Rev. 1/84) GPO 914-916

PROJECT DESCRIPTION

PERSONNEL

Elieser Gorelik	Guest Researcher	NIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI

OBJECTIVE

To investigate the mechanisms in antimetastatic defense. For these purposes we have studied interactions of tumor cells with the factors of the hemostatic system in the blood, and the role of NK cells in the antimetastatic effects of anticoagulant drugs.

METHODS EMPLOYED

The antimetastatic effect of anticoagulant drugs (heparin or warfarin) was tested in normal (9 weeks old) C57BL/6, beige or nude mice. Heparin was administered i.p. 30 minutes to 1 hour before tumor cell inoculation at a dose of 2 units/g body weight, which provided the maximum antimetastatic effect without any toxic or lethal effects. Warfarin was given in the drinking water (8 mg/liter) 2 days before tumor cell inoculation. Blood coagulation and prothrombin times were monitored by standard techniques.

NK activity of C57BL/6 mice was suppressed by i.p. inoculation of 0.25 ml of antiasialoGM1 serum diluted 1:40 or was augmented by i.p. inoculation of poly IC (200 µg).

The number of visible metastatic foci in the lungs was counted 17 days after i.v. inoculation of $5 \times 10^4 - 1 \times 10^5$ of the B16F10 or B16BL6 melanoma sublines. Elimination of tumor cells in mice treated with heparin or warfarin was assessed by i.v. inoculation of (^{125}I)dUrd labeled melanoma cells. The level of radioactivity remaining in the lungs, liver, spleen, and blood was determined at various time periods after tumor cell inoculation.

MAJOR FINDINGS

We continue our investigations of the antimetastatic effects of anticoagulant drugs (heparin and warfarin) in mice with depressed or stimulated NK reactivity. Stimulation of NK cell activity with poly I:C potentiated the antimetastatic effect of heparin or warfarin. This effect was observed with various tumor cell lines: B16F10, BL6 melanoma cells, Lewis lung carcinoma (3LL), Madison lung carcinoma (M109). When mice were pretreated with anti-asialo GM1 serum (1:40, 0.25 ml i.p.) or Cyclophosphamide (Cy) (200 mg/kg) the antimetastatic effect of heparin or warfarin was abrogated. However, in some experiments this abrogation was not complete. Treatment of mice with anti-asialo GM1 serum or Cy caused a substantial increase in the number of experimental metastases in the lungs of mice. Administration of heparin or warfarin in mice treated with anti-asialo GM1 serum or Cy had some antimetastatic effects since the number of the pulmonary metastatic foci in these mice was reduced to the control level. Anticoagulant drugs might affect on the various mechanisms involved in the metastatic process (adherence to the endothelium,

extravasation) or action of the residual NK cells. Although one injection of anti-asialo GM1 serum or Cy had a profound inhibitory effect on NK cell function, but it is possible that some of their activity still remained. In order to test these assumptions we performed the following experiments:

C57BL/6 mice were treated with various dilutions of anti-asialo GM1 serum (1:20 - 1:120), applied once or twice with 2-3 days interval. In mice treated twice with high concentration of antiserum, the antimetastatic effect of heparin and warfarin was completely abolished. Beige mice or young (3 weeks old) C57BL/6 mice have relatively low levels of NK cell activity. Nevertheless it was enough for heparin or warfarin to express their inhibitory effect on metastasis formation. The presence of NK cells in these mice was confirmed by the fact that Poly I:C stimulated the antimetastatic defense in these mice and potentiated by treatment with heparin and warfarin. However, 1 injection of anti-asialo GM1 serum (1:40, 0.25ml) was sufficient to completely abolish the antimetastatic effect of heparin or warfarin.

Thus, these data indicate that fibrin coagulation is not obligatory for attachment of tumor cells to the vessels and extravasation since in mice with suppressed NK reactivity after treatment with heparin and warfarin tumor cells were able to extravasate and develop numerous metastatic foci. What seems important for the antimetastatic effect of anticoagulants is the presence of active NK cells. This conclusion has been supported by experiments with adoptive transfer of splenic NK cells.

Cy treatment (200 mg/kg) suppressed NK cell activity and dramatically increased the number of the BL6 melanoma metastases in the lungs. Heparin inhibited the formation of the metastatic foci in normal but not in Cy-treated mice. I.V. inoculated spleen cells (50×10^6) reconstituted NK reactivity of Cy-treated mice and the antimetastatic effect of heparin. The efficiency of the transplanted cells increased in combination with heparin treatment, since the number of metastatic foci in these mice was significantly lower then in mice transplanted with spleen cells without heparin treatment. When NK activity of the donor spleen cells was depleted by treatment with anti-asialo GM1 serum they failed to reconstitute either NK reactivity of the recipients or the antimetastatic effect of heparin.

SIGNIFICANCE

These data indicate that NK cells are crucial for the antimetastatic effect of anticoagulant drugs. Fibrin coagulation on the tumor cell may be one of the mechanisms responsible for the protection of tumor cells from destruction by NK cells. Anticoagulant drugs make tumor cells more vulnerable to the destruction by NK cells. Although these experiments were designed to evaluate the role of NK cells in this phenomenon it seems possible that fibrin deposition could also prevent tumor cells from destruction by other effector cells (macrophages, immune lymphocytes). Understanding the mechanisms which help tumor cells to escape from immune destruction has practical importance for increasing the efficiency of immunotherapeutic methods.

FUTURE PLANS

This project will be discontinued, since we would like to concentrate our efforts on another project.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09256-03 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 cherecters 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 professionel personnel below the Principel Investigator.) (Neme, title, leboratory, end institute affiliation)*

PI:	J. R. Ortaldo	Head, Natural Immunity Section	BTB, NCI
Others:	R. B. Herberman	Chief	BTB, NCI
	P. Allavena	Visiting Fellow	BTB, NCI
	A. Procopio	Visiting Fellow	BTB, NCI
	S. Yamada	Guest Researcher	BTB, NCI
	T. Sayers	Guest Researcher	BTB, NCI

COOPERATING UNITS *(if any)*

Roche Inst. of Molecular Biology, Nutley, NJ (S. Pestka); Med. Br., NIH (Dr. Ozols)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	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.)*

Human natural killer (NK) cells and K cells mediating antibody-dependent cellular cytotoxicity have been shown to be large granular lymphocytes (LGL). 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; however, it was capable of augmentation of other leukocyte activities and demonstrated antiproliferative and antiviral activities similar to other IFN-α's. This finding has led to studies regarding 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. This IL-2-mediated augmentation appears to parallel production of IFN-γ by LGL, but abrogation of antiviral activity with anti-IFN- γserum did not abolish NK boosting. Cultures and clones of highly purified LGL, grown in the presence of IL-2 have demonstrated morphology an d cytotoxic patterns similar to fresh LGL. In addition to NK activity, cultured and clones of LGL have been shown to produce a variety of lymphokines (IL-1, IFN, CSF, BCGF).

PROJECT DESCRIPTION

PERSONNEL

John Ortaldo	Head	NIS	BTB	NCI
Ronald Herberman	Chief		BTB	NCI
Paola Allavena	Visiting Fellow	NIS	BTB	NCI
Antonio Procopio	Visiting Fellow	NIS	BTB	NCI
Sachiro Yamada	Guest Researcher	NIS	BTB	NCI
Thomas Sayers	Guest Researcher	NIS	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 FINDINGS

I. Regulation of Human NK Activity

To obtain better insight into the nature of the diversity of the biologic effects of interferons (IFNs), various preparations of human natural, recombinant, and hybrid recombinant alpha IFNs 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 cytolysis and cytostasis. However, at low levels of IFN (10-50 units), appreciable differences among the various species were seen. In an attempt to determine a structurefunction relationship, a series of recombinant IFN-α molecules were studied. Recombinant IFN-α J, which has antiviral and antiproliferative activity, was unable to rapidly boost NK activity and interfered with boosting by other IFN-α species. In addition, considerable potency differences were seen with regard to the natural IFN-α species, with several being very active at <1 units of antiviral activity but others requiring >70 units to result in significant augmentation. 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 recombinant IL-2, 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. Of considerable interest has been the ability of IL-2 to augment NK activity in the absence of

growth promotion. In addition, this IL-2-driven boosting occurred with TAC (the receptor for IL-2 growth)-negative LGL and was not abrogated with monoclonal antibodies to TAC. These results indicate an additional immunoregulatory function of this growth factor.

II. Cytotoxicity by Cultured and Cloned Cells

Only about 25% of the LGL clones demonstrated cytotoxicity against the panel of targets tested. The majority of these cytolytic clones demonstrated a broad pattern of killing, similar to fresh LGL populations. However, about 25% of the cytotoxic clones demonstrated selective killing of only some of the panel of NK susceptible targets. None of the T-cell clones demonstrated killing of NK-susceptible targets. The LGL clones were found to vary widely in their expression of various 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.

Since fresh peripheral blood LGL can secrete a variety of cytokines, IL-1, IL-2, INF- αand β and BCGF, it was of interest to determine whether cytotoxic reactivity and cytokine production were clonally distributed. We obtained clones from highly purified preparations of human LGL, and cultured them in IL-2-containing medium for several weeks. All the clones tested spontaneously produced detectable levels of IFN-γ and 35/40 clones (87%) produced higher levels when stimulated with PHA. A smaller proportion (9/54) or (16%) of clones secreted IL-1 after stimulation with LPS, while (17/49) or (34%) of the clones (17/49) produced IL-2 in response to PHA stimulation. Cytokine production was associated with both cytotoxic and non-cytotoxic clones and did show the correlation with their surface phenotype (i.e. espression of OKT3, OKT8, OKM1, or B73.1) as has been observed for fresh LGL. The ability to produce IL-1 or IL-2 was not usually found within the same clone; however, three clones produced either IL-1 and IL-2 when stimulated in different experiments, but not both at the same time. These results indicate that LGL-derived clones have the capability of producing multiple cytokines, suggesting that the LGL population may play an important immunoregulatory role and may also be capable of self-regulation of cytolytic activity.

An in vivo xenograft model for human ovarian carcinoma expresses the ovarian carcinoma associated antigen, CA125, and bears estrogen receptors. We have performed adoptive cell therapy with various human effector cell populations. Human lymphoid and monocytoid effector cells were isolated and activated in vitro with recombinant interleukin 2 or interferon gamma. A significant extension of survival time in this ovarian carcinoma model could be achieved when IL-2 activated LGL were transferred in animals bearing tumors. T cells activated with IL-2 also induced a significant prolongation of survival of animals bearing the ovarian carcinoma. Monocytes with or without activation induced no significant augmentation of survival. In vitro results indirect isotopic release assays to measure the cytotoxic activity of effector cells before and after activation paralleled the level of protection that was demonstrated in vivo. Therefore, this xenogeneic model for human ovarian carcinoma, and our results with the adoptive cell transfer, are quite encouraging for proceeding in these sequestered cell transfer experiments in human patients bearing nontreatable ovarian carcinoma by standard therapies.

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 the 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 (>12 weeks) in vitro.

Cultures and clones of LGL (maintained on IL-2) have been shown to exhibit apparent clonal restriction of specificity. Since cultured 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, these 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 cloned LGL can be altered by maturational agents; (2) to enumerate the frequency of secreting LGL clones and the diversity of the secreted factors; (3) the biochemical mechanism involved in augmentation of NK and ADCC by interferon or IL-2; (4) the efficacy of IL-2 activated lymphoid cells in adoptive cell transfer experiments.

PUBLICATIONS

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Ortaldo, J. R., Allavena, P., Mason, L., and Herberman, R. B.: Culture of NK cells: Specificity, phenotypes, functions and comparison with activiated T cells. In Herberman, R. B. (Eds.): Mechanisms of Cytotoxicity by NK Cells. Ortaldo, Academic Press, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09257-03 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Functional Activity of Large Granular Lymphocytes in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Neme, title, leboratory, and institute affiliation)*

PI:	C. W. Reynolds	Senior Staff Fellow	BTB, NCI
Others:	R. B. Herberman	Chief	BTB, NCI
	T. Barlozzari	Visiting Fellow	BTB, NCI
	H. Fukui	Guest Researcher	BTB, NCI

COOPERATING UNITS *(if eny)*

Chugai Pharmaceutical, Tokyo, Japan (H. Fukui); Stanford University, Palo Alto, CA (Dr. Eugene Butcher)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.2	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.)*

The present series of studies have directly demonstrated an important in vivo antitumor role for large granular lymphocytes (LGL), the population of cells known to mediate natural killer (NK) and antibody-dependent cell mediated cytotoxicity (ADCC). The adoptive transfer of LGL into rats with depressed NK/ADCC 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 antitumor role for LGL and suggest that the adoptive transfer of highly enriched LGL populations should be further considered as one potential immunotherapeutic regimen in cancer patients. Studies with the BRM, OK432, have shown this agent to augment NK activity and increase survival of tumor-bearing rats. In other experiments we have shown a number of differences in the organ, age and strain distribution between the NK and ADCC effector cell (K cell) populations. Additional experiments are now in progress to utilize these differences between NK and K cells to further investigate the in vivo relevance of these two natural immune systems. We are also investigating additional in vivo roles for NK cells in bone marrow transplantation and in the antiviral and antifungal immune responses.

PROJECT DESCRIPTION

PERSONNEL

Craig W. Reynolds	Senior Staff Fellow	NIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Teresa Barlozzari	Visiting Fellow	NIS	BTB	NCI
Hiroyasu Fukui	Guest Researcher	NIS	BTB	NCI

OBJECTIVES

The objectives of this project are: (1) to directly investigate the role of LGL in the in vivo resistance to tumor growth, metastasis and syngeneic bone marrow transplantation and (2) to compare the NK and ADCC activities of LGL, with the eventual goal of determining the in vivo antitumor activity of both NK and K cells.

MAJOR FINDINGS

I. The Role of LGL in In Vivo Antitumor and Bone Marrow Transplantation Immunity

In our present studies, rats treated with anti-asialoGM_1 (asGM_1) antibody showed a parallel decrease in NK activity and frequency of LGL in the spleen and peripheral blood, indicating that the antiserum-induced depression of NK activity was due to an elimination of most effector cells.

To further determine the possible role of LGL in tumor cell rejection in vivo, we studied LGL involvement in the clearance of ^{125}IUdR-labeled tumor cells from the lungs, an assay previously shown to correlate well with in vitro NK activity. Two hours after iv injection of labeled tumor cells, animals treated with anti-asGM_1 antiserum were found to have significantly more ^{125}IUdR-labeled tumor cells in the lungs. These results demonstrate a decrease in the in vivo rate of clearance of tumor cells from the lungs. Animals treated with anti-asGM_1 were also found to have a greater than 10-fold increase in the number of lung metastases which developed following the iv injection of syngeneic MADB-106 mammary tumor cells. Furthermore, the adoptive transfer of 3-10x10^6 highly enriched LGL into NK-depressed animals 2 hours before tumor challenge partially or fully restored: 1) in vitro cytotoxic activity against established cell lines; 2) in vivo ability to eliminate radiolabeled cells from the lungs; and 3) ability to inhibit the development of pulmonary metastases. These results are the first unequivocal evidence that LGL, which are highly enriched in NK activity, play an important role in the rapid elimination of circulating tumor cells, with subsequent inhibition of metastasis.

Similar adoptive transfer studies in anti-asGM_1 treated rats have also shown a significant role for NK cells in the inhibition of syngeneic bone marrow stem cell growth and differentiation. Anti-asGM_1 treatment of lethally irradiated recipients increased the number of colony forming units in the spleen (CFU-S) following syngeneic bone marrow transfer. The adoptive transfer of LGL but not T cells into these irradiated/anti-asGM_1 treated recipients significantly reduced the number of CFU-S seen. In addition, in vitro cytotoxicity assays

using highly purified LGL and bone marrow stem cells showed significant lysis of the bone marrow cells. These results further emphasis the important in vivo role of NK cells in the control of hematopoetic stem cell growth and differentiation.

II. Comparison of NK and ADCC Activity in Normal and BRM-treated Rats

Our previous studies have been related to the in vivo role of LGL. Much of this effort has been related to NK activity; however, LGL function not only as NK cells but also as K cells in antibody-dependent cell-mediated cytotoxicity (ADCC). At present, it is not clear which of these functions is responsible for in vivo resistance to tumor growth in our experimental system. In the present studies, we have compared the distribution of these two activities in rats with regard to: a) organ, b) strain, c) age, and d) Percoll density gradient fractionation. Appreciable NK and ADCC activities were observed in peripheral blood lymphocytes (PBL), splenic lymphocytes (SPL) and peritoneal exudate cells (PEC) but not in cell preparations from the lymph node, bone marrow and thymus. However, the ADCC reactivity in the PBL from F344, athymic nude, nude/+, Lewis, PVG/RTLRL and PVG/OLA rats, was consistantly 3-10 fold higher than NK activity, while the PEC from the same animals showed appreciable NK activity but little ADCC. On a per cell basis, however, there was about the same NK and ADCC activity from the spleens of these rats. In contrast to the results in the above strains, the ADCC activity in WF/N rats was significantly lower in the PBL and SPL as well as PEC. Variations of NK and ADCC activity with age were also assessed in both a high ADCC (F344) strain and low ADCC (WF/N) strain of rats. In PBL or SPL from F344 rats, the level of ADCC but not NK activity increased with age. On the contrary, in WF/N rats, the level of both NK and ADCC activities remained stable up to 35 weeks of age. To further investigate the mechanism for reduced ADCC activity in WF/N rats and to compare the cell populations which exhibit NK and ADCC activities, PBL and SPL from various strains of rats were fractionated by discontinuous Percoll density gradients. In all strains tested, the NK and ADCC activities were associated mainly with cells in the low-density fractions. These fractions also contained a high frequency of LGL and cells with receptors for the Fc portion of IgG ($Fc\gamma R^+$ cell). In PBL, the % LGL/%$Fc\gamma R^+$ cell in the low density Percoll fraction were 65.9/62.4 from nude, 42.6/39.1 from nude/+, and 40.8/38.7 from F344 rats. In contrast, the low density PBL fraction from WF/N rats contained 50.8% LGL/<10% $Fc\gamma R^+$ cells. These results suggest that both $Fc\gamma R^+$-LGL and $Fc\gamma R^-$-LGL exist, and that the reduced ADCC activity in WF/N rats is due to a low frequency of $Fc\gamma R^+$-LGL. Following BRM-treatment there was a significant increase in NK activity in all strains tested but an increase in ADCC activity only in strains with naturally low % $Fc\gamma R^+$ LGL. This increase in ADCC activity correlated with an increase in the % $Fc\gamma R^+$ cells and suggests that the augmentation of ADCC activity was due to an increase in $Fc\gamma R$ expression on LGL.

SIGNIFICANCE

Our present experiments involving the adoptive transfer of LGL has provided the first direct evidence that LGL play an important role in inhibiting the development of metastases and in the regulation of growth and differentiation of

syngeneic bone marrow transplants. Our studies on the comparison of the NK and K cell systems in rats are an important prerequisite to the further development of new treatment modalities which might selectively augment either NK or K cell activity. Since these new treatment modalities will be more focused towards the most relevant in vivo antitumor effector mechanism(s) they could provide new therapeutic strategies for the treatment of patients with tumors which are selectively sensitive to either NK or K cell activity.

PROPOSED COURSE

The ability to selectively deplete NK activity and to specifically reconstitute this activity with highly purified LGL has provided a unique and extremely useful method for further examining the in vivo function(s) of these cells. The same type of selective anti-asGM$_1$ depletion and specific LGL reconstitution experiments are also now being used to examine the role of LGL in carcinogenesis. For these experiments, rats have been treated with antisera either 3 days before or up to 50 days following N-methyl-N-nitrosourea (NMU) or methyl (acetoxymethyl) nitrosomine (DNM-OAc) administration. One to two hundred days following carcinogen administration, animals will be sacrificed and scored for: 1) total number of tumors, 2) latent period or growth rate of tumors, 3) histological and anatomical sites of tumors, and 4) development of microscopic and macroscopic metastatic foci. If an effect on any of these three parameters is seen, anti-asGM$_1$-treated animals will be reconstituted with LGL, T cells and monocytes to definitively identify which cell was involved. These anti-asGM$_1$ depletion and LGL reconstitution studies will also include kinetic experiments to independently determine the time points at which NK cells may be involved in inhibiting the development of primary tumors or metastases. These experiments should provide useful and novel data regarding the role of LGL in the development and metastatic spread of carcinogen-induced primary tumors in rats.

Since it is not yet clear whether it is the NK or ADCC system which is the most important in vivo, further experiments will be conducted in order independently evaluate these functions in tumor-bearing rats. Specifically, rats with low NK but not ADCC (or low ADCC but not NK) will be tested for the growth of susceptible tumor cell lines. A further confirmation of the role of ADCC or NK cells will come from reconstitution experiments utilizing highly enriched NK or ADCC effector cells. Alternatively, NK-susceptible or Ab-coated target cells can be injected in vivo, prior to tumor cell inoculation, as a form of in vivo NK or ADCC cold-target inhibition.

Future experiments will also continue to examine the adoptive transfer of both fresh and in vitro cultured LGL into tumor-bearing animals. With our previous data in mind, we will examine the kinetic and dose requirements for inhibiting the growth of peritoneal tumors (ip LGL transfer) and pulmonary metastases (iv LGL transfer). Specifically, tumor-bearing animals will be treated with cytoreductive therapy (cyclophosphamide or surgery) followed by the adoptive transfer of normal or activated LGL. In addition, BRMs known to augment LGL function will also be given in an attempt to further enhance the in vivo function of these cells.

Studies regarding the localization of adoptively transferred LGL are also continuing. In experiments with Drs. Eugene Butcher (Stanford University) and Robert Wiltrout (Z01 CM 09262-03 LMI), we are attempting to study the mechanisms of LGL homing by examining whether rat and human LGL have surface receptors for tissue specific high endothelial venules (HEV). These studies should lead to a better understanding of LGL migration into selected normal and neoplastic tissues and may eventually help in the development of new therapeutic strategies for inducing increased and selective migration of LGL into tumors or sites of infection.

PUBLICATIONS

Barlozzari, T., Reynolds, C. W., Wiltrout, R., and Herberman, R. B.: Direct evidence for the role of LGL with high NK activity in the inhibition of tumor metastases. J. Immunol. 134: 2783-2789, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09259-03 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. J. Mathieson	Biologist	BTB, NCI
Others:	J. R. Ortaldo	Head, Natural Immunity Section	BTB, NCI
	Y. Yoda	Guest Researcher	BTB, NCI
	L. Mason	Microbiologist	BTB, NCI
	R. H. Wiltrout	Senior Staff Fellow	BTB, NCI
	E. Schlick	Visiting Associate	BTB, NCI
	R. Salup	Guest Researcher	BTB, NCI
	R. B. Herberman	Chief	BTB, NCI

COOPERATING UNITS *(if any)*

Memorial Sloan-Kettering Cancer Center, New York (F.W. Shen); Program Resources, Inc. (R. Overton).

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	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.)*

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, bone marrow and liver have been characterized. Effector cell activity has been monitored against appropriate targets for both natural killer (NK) activity and natural cytotoxic (NC) activity. Phenotype has been determined by complement (C)-mediated, antibody-dependent cytotoxicity, by flow cytometry analysis (FCA) of immunofluorescence (IF) and by visual morphological assessment using Staphylococcus aureus protein A-dependent (SpA) binding to monoclonal antibody (MoAb). Large granular lymphocytes (LGL) from nylon wool nonadherent cells subjected to density separation techniques have been characterized with a series of MoAb to T-cell differentiation antigens, to myelomonocytic antigens and to other hematopoietic subsets. Initially we examined liver-derived LGL from animals whose NK activity has been augmented by biological response modifiers (BRM). In addition, LGL obtained from livers of BRM-treated mice have been compared to splenic subpopulations enriched for LGL. Purification procedures for splenic LGL from normal and BRM-activated animals required modification and additional steps to eliminate low density T cells ($Lyt2^+$ or $L3T4^+$) and B cells. Pronounced differences in phenotype between the liver derived and spleen-derived LGL populations has been observed. The phenotypic differences do not appear to be related to the BRM used for NK augmentation but to the organ used as a source of LGLs. We have currently begun experiments to select lymphocyte subsets before culture in lymphokine to identify the lymphokine activated killer (LAK) cells and to compare these cells with NK cells and T cells. Characterization of bone marrow progenitors of NK activity has been initiated to determine whether the precursors share any of the markers found on mature, functional NK cells.

PROJECT DESCRIPTION

PERSONNEL

Bonnie Mathieson	Biologist	NIS	BTB	NCI
John Ortaldo	Head	NIS	BTB	NCI
Yasuhiro Yoda	Guest Researcher	NIS	BTB	NCI
Llewllyn Mason	Microbiologist	NIS	BTB	NCI
Robert Wiltrout	Senior Staff Fellow	NIS	BTB	NCI
Erich Schlick	Visiting Associate	LS	LMI	NCI
Raoul Salup	Guest Researcher	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 mouse and human cells that have natural killer (NK) or natural cytotoxic (NC) activity and lymphokine activated killer (LAK) cells, and 2) to study the origin, differentiation and development of these cells in vivo and in vitro.

Our specific aims are to: (a) acquire and develop a panel of monoclonal antibodies and other reagents with selective or differential reactivity against mouse and human NK and mouse NC cells, (b) develop enrichment and selection techniques to isolate precursors of these cells from bone marrow, (c) characterize the isolated precursors of NK cells from bone marrow by phenotype, growth requirements in vitro and pattern of cytotoxicity on different tumor cell lines and normal lymphoid targets, and (d) compare such precursors with prothymocytes, early thymocytes and myelomonocytic cells.

METHODS EMPLOYED

Donor animals were obtained from the Animal Production Area of the FCRF. C3H/HeN or C57BL/6 (B6) and B6-Ly 5.2 congenic mice were used routinely for these studies.

To characterize NK cells and LAK cells in the mouse, we have used a panel of monoclonal antibodies (MoAb) that detect: 1) the T cell specific antigens Lyt2 and L3T4 as well as Ly1 and Thy-1 which are expressed on T cells but also some other cells; 2) Ly9 (Lgp 100) which is expressed on all lymphocytes; 3) Ly5, which is expressed on all leucocytes including NK cells; 4) Qa5, an MHC associated, class 1 antigen which is easily detected on NK cells; 5) Gma-1, a myelocytic antigen that also appears to be expressed on some LGL enriched populations; and 6) asialo GM_1 and Fc receptor, markers that have been associated with NK cells in mice and other species. MoAb serological reagents have been obtained from commercial sources or through collaborative agreements, titered and tested for appropriate reactivity.

Subset enrichment methods. Nylon wool nonadherent (NWNA) cells from various tissues were subjected to Percoll density gradient fractionation to enrich LGL in the low density region of the gradient. The enriched fraction contains about 15-30% LGL in mice in contrast to the high level of purification seen for

human or rat cells. Anti-Ig plate adherence was used to further remove contaminating B cells that interfered with the immunofluorescence analyses. Anti-Lyt2 and anti-L3T4 MoAb were used to further deplete T cells by indirect anti-Ig coated plate separations of mouse cells.

Liver interstitial cells that contain a high proportion of LGL (See Robert Wiltrout, project # Z01CM09262-02 BTB) were obtained by collagenase digestion of minced perfused livers from MVE-2, Poly IC:LC, or C. parvum-treated animals. These cells were further purified by removal of nylon wool adherent cells and Percoll density enrichment.

Various lymphocyte subsets have been purified prior to culture in IL2 for identification and characterization of lymphokine activated killer (LAK) cells. Purification methods rely on anti-Ig plate adherence of selected specific T cell subsets treated with MoAb to T cell subset markers and selective complement dependent elimination (see below).

Cell Surface Phenotype. Phenotype of the isolated cell populations was determined by one of three methods: a. Flow cytometry analyses (FCA) to quantify cell surface antigen expression detected by immunofluorescence (IF) were performed on an Ortho Cytofluorograph. b. Cytotoxic elimination experiments with antibody and complement (C) served two purposes: 1) To directly assess the phenotype of the eliminated cells relative to the level of NK or LAK function, 2) To eliminate unwanted or irrelevant cell subsets in enrichment methods for spleen, blood and bone marrow before their use in IF or functional analyses. c. For visual assessment of antigen expression on morphologically identified cells, protein A dependent methods were employed.

Separation of Human Bone Marrow Progenitors BM was aspirated in the presence of heparin from the posterior iliac crest of normal human volunteers and autologous heparinized peripheral blood was obtained by venipuncture. Mononuclear cells (MNC) were separated from BM using Ficoll Hypaque and were passed through a nylon wool column. Flow cytometric analysis (FCA) of nylon wool non-adherent cells (NW-NA) cells before the culture showed that NW-NA BM cells contained low numbers of $T101^+$ cells and $OKT11^+$, indicating the presence of contaminating T cells. For complete depletion of mature T cells and NK cells it was necessary to remove by density separation cells that formed rosettes with 2,5-aminoethlyisothiouronium (AET)-treated sheep red blood cells (SRBC), (E^+ cells). This was followed by cytolytic pretreatment with a monoclonal antibdoy (MAb) mixture of T101, OKT11, OKM1 and VEP13, plus complement (C).

MAJOR FINDINGS

Cell Surface Analysis by Cytotoxic Elimination. We have confirmed previously reported findings from this and other laboratories, indicating that mouse splenic cells enriched for LGL and/or for cells exhibiting NK activity are also enriched for cells with low or negligible Thy-1 and Lyl expression. This was determined in coordinate cytotoxic elimination experiments and cell surface antigen monitoring by flow cytometry. $Lyt2^+$ cells in these fractions could be eliminated by treatment with anti-Lyt2 MoAb + C. This reduced total cell numbers without markedly depleting the level of NK activity or NC activity. Experiments with monoclonal anti-Qa5 indicate that the NK activity can be eliminated without

affecting the NC activity in spleen, liver, or bone marrow populations. Attempts to eliminate NK or NC activity with the myelomonocyte specific anti-Gma-1 MoAb have indicated that the NK cells from spleen, liver, and bone marrow express this antigen only at low levels. This was also confirmed by FCA of anti-Gma-1.2 on Percoll-enriched LGL from spleen, liver, and bone marrow. Anti-Thy-1 elimination experiments also confirm the Thy-1 expression on a large proportion of the isolated NK cells, independent of the T lymphocyte subset markers Lyt2 and L3T4. Collectively, these data support the concept that spleen or bone marrow LGLs exhibiting NK activity are more related to the T-lymphocyte lineage than to the myelomonocytic subsets of hematopoietic cells.

Enrichment and Depletion Experiments. In a series of experiments with spleen, bone marrow and peripheral blood cells, Percoll enrichment of LGLs also consistently enriched both NK and NC activity in the low density fractions. NK and NC activity are coordinately enriched with the LGLs from spleens of normal control animals or from spleens of animals treated in vivo with such BRMs as poly IC:LC or C. parvum.

Flow cytometry analyses (FCA). Using FCA, we have now examined purified liver LGL or highly enriched splenic LGL with a panel of 25 MoAb against cell surface markers detected by MoAb. There results very clearly indicate that there are major quantitative differences in the surface expression of a number of these antigens between the liver-derived LGL and splenic LGL. In particular liver LGL have higher levels of Thy-1, MAC-1, Asialo GM_1, Fc receptor and Gma-1. We are currently assessing the phenotypic characteristics of LAK cells and comparing these cells with both NK cells and subsets of T cells.

Coordinate analysis of morphology and cell surface markers. Using the property of Staph. protein A (SpA) binding to IgG_2 subclasses of antibody, we have begun to analyze the cell surface expression of LGL versus myelomonocytic cells and other lymphocytes. We have used allelic MoAb that are of IgG_2 isotypes for Lyt2, Gma-1, and Ly5. These reagents allow us to use a second stage binding with either Staph A bacteria or with protein A-coupled sheep erythrocytes as a visual marker to morphologically identify antigen positive cells that bind the antibody. These results indicate that the LGL clearly bind anti-Ly5, but do not bind either the Lyt2 or the Gma-1 reagents. This methodology allows direct assessment of the cell surface antigen expression on morphologically distinct cells where appropriate reagents are available. However, it may be limited by antigen density on the surface so that cells with low antigen density might not be detected, whereas FCA would be able to detect a low but positive level of binding, but cannot identify cell subsets by morphological criteria.

To confirm the presence of NK progenitors in the human BM and to characterize them, it was necessary to culture functionally active NK cells from immature progenitors. We have been successful in obtaining NK cells in cultures of human BM cells in the presence of interleukin 2, after eliminating all mature T cells, mature NK cells and CFU-C. NK cells cultured from progenitors were LGL, very similar to fresh human LGL, which have NK (K562) activity, surface phenotypes of $NKH1^+$, $OKM1^+$, $OKT10^+$, $3G8^+$ and $OKT3^+$. CFU-C colonies did not grow from the pretreated BM cells which yielded NK cells. Thus NK progenitors in the BM are distinguishable from CFU-C and are not simply LGL expanded from mature contaminating T cells or NK cells.

SIGNIFICANCE

Several theories have been proposed to explain the occurrence and biological relevance of naturally occurring cytotoxic cells to tumor targets. Mouse lymphoid cells that can lyse lymphoid tumor targets such as YAC have been defined as natural killer (NK) cells. NK effector cells may represent a primitive, but unique, lymphocyte subset that acts as a first line of defense against malignant cells or microbial agents. Another possibility is that NK may be products of an alternative pathway of differentiation from prothymocytes, incapable of completing a normal T-cell differentiation pathway without the appropriate or sufficient interaction with a thymic environment.

The type of cells that display NK activity have been identified in the human, in the rat, and in the mouse as large granular lymphocytes (LGL). Phenotypic characterization of LGL from both humans and rats has indicated that these cells have some characteristics of cells in both the T-cell series and in the myelomonocytic series. NK/LGL subset heterogeneity may be related to the state of differentiation or activation of these cells, or subsets of cells with natural cytotoxic function may be derived from different lineages. Better separation or purification of either LGL and the cells with the NK activity in the mouse has now permitted further definitive characterization of these cells.

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, it is likely that these cells comprise a unique lymphoid subset. However, they still share some T cell surface markers such as the low level expression of Thy-1 and Ly1 antigens. This may in turn indicate their more immediate derivation, or closest association with, the T cell lineage.

A direct approach to this problem is to analyze bone marrow precursors of the different cell types and to compare their origin and development of functional capacity. The characteristics of the precursors for these cells are virtually unknown and limited to evidence that NK cell function has been reconstituted by bone marrow cell transfers. These studies have not been monitored for the possibility that host-derived cells are responsible for functional repopulation. The current availability of several MoAb to allelically-determined bone marrow antigens in mice now makes such experiments easier to approach. Our studies with human bone marrow clearly indicate that NK progenitors can be identified as a population of cells separate from myelocytic precursors and lacking mature NK and T cell markers.

PROPOSED COURSE

There are two major aspects of this project which we intend to develop during the next year: (1) 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. (2) Continued phenotypic analysis and functional comparison of NK cells, LAK cells and T cells.

Manipulation of lymphoid precursor populations from murine bone marrow can be informative where relationships between both precursors and functional effectors can be assessed with genetic markers. Therefore, we intend to continue the development of methodology to identify, isolate, monitor, and assess cells with natural cytolytic capacity.

Extensive plans have been formulated to use the monoclonal reagents, especially those with allelic differences, to assess by C elimination or positive selection the precursor populations from bone marrow for NK cells and thymocyte precursors. These selected populations will be transferred into irradiated hosts where we can monitor by allelic differences both the donor and host components in the repopulation. This is essential because of the information for thymocyte repopulation that indicates that the initial cells repopulating the thymus are host-derived. By monitoring both donor and host alleles of Ly5 after selected cell transfer, we will be able to determine the phenotypic characteristics of the NK precursors. In addition, by monitoring intrathymic repopulation at the same time as we monitor the functional reappearance of the Qa5+ NK activity, and myelocytic cells, we should be able to determine whether the kinetics of the repopulation of these different subsets is coordinated or independent.

In summary, we intend to: (a) continue to define the phenotype of both freshly isolated "mature" NK cells, the precursors for LAK cells, and their bone marrow precursors, (b) attempt to determine reliable cell surface criteria for separating the cells with NK and NC activity, (c) define characteristics of NK progenitors by growth and development of NK cells and thymocytes in irradiated hosts and in vitro and to monitor the markers and transitions that may exist between these cells, and (d) continue to analyze the growth conditions and factors needed to distinguish NK and LAK cells. With these several goals we hope to determine more specifically whether NK cells represent one, two or multiple lineages of cells, an offshoot of T-cell differentiation or a unique myelomonocytic lineage of cells.

PUBLICATIONS

Wiltrout, R. H., Mathieson, B. J., Talmadge, J. E., Reynolds, C. W., Zhang, S. R., Herberman, R. B., and Ortaldo, J. R.: Augmentation of organ-associated NK activity by biological response modifiers: Isolation and characterization of large granular lymphocytes from the liver. J. Exp. Med. 160: 1431-1449, 1984.

Mathieson, B. J., Mason, L., Overton, W. R., Winkler, R. T. and Wiltrout, R. H.: Distinctive phenotypes of natural killer (NK) cells isolated from different tissue sources. In Streilein, E. W., Ahmad, R., Black, S., Blomberg, B. and Voellmy, R. W. (Eds.): Advances in Gene Technology: Molecular Biology of the Immune System. New York, Cambridge University Press, 1985, pp. 245-246.

Yoda, Y., Mathieson, B. J. and Ortaldo, J. R.: Differentiation and function of natural killer (NK) cells. Acta. Haematol. Jap., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09262-03 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one lina batwean the borders.)*

Antitumor Effects of NK Cells, rIL2-Stimulated Lymphocytes, & Macrophages in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. H. Wiltrout	Senior Staff Fellow	BTB, NCI
Others:	R. R. Salup	Guest Researcher	LMI, NCI
	J. R. Ortaldo	Head, Natural Immunity Section	BTB, NCI
	C. W. Reynolds	Senior Staff Fellow	BTB, NCI
	B. J. Mathieson	Senior Staff Fellow	BTB, NCI
	P. L. Urias	Chemist	BTB, NCI

COOPERATING UNITS *(if any)*

Queen's University, Kingston, Ontario, Canada (R. S. Kerbel)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	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.)*

Natural killer (NK) cells and activated macrophages (Mø) have been shown to inhibit formation of metastases. NK cells have effects during the bloodborne phase of metastasis, in both normal or biological response modifier (BRM)-treated mice. We have found that highly lytic NK cells can also be induced in the tissues of both the lungs and liver by the pyran co-polymer, MVE-2, and that these organ-associated NK cells are efficient in inhibiting the formation of metastases in the lungs and liver. Further, we have characterized the cells mediating this tissue resistance to metastasis as large granular lymphocytes (LGL), the cells previously associated with NK activity in rats and humans. Additional BRMs which augment liver-associated NK activity by inducing an influx of LGL into the liver include poly ICLC, OK-432, P. acnes, and human recombinant interleukin 2 (rIL-2). All of these BRMs induce a greater augmentation of NK activity in the liver than in blood and spleen. Further, while repeated administration of most BRMs induced a hyporesponsiveness to augmentation of NK activity in the blood and spleen, liver-associated NK activity was further augmented with additional increases in the total number of LGL. Liver-associated macrophages were also activated for tumoricidal activity by various BRMs. The application of these organ-associated natural effector cells to treatment of established liver metastases is currently under study. Similarly, we have studied the ability of rIL 2-stimulated cytotoxic lymphocytes and rIL 2 to enhance the antitumor effectiveness of the chemotherapeutic drug, doxorubicin hydrochloride (DOX). Chemo-immunotherapy of stage I murine renal carcinoma (Renca) cured 67% of Renca-bearing mice, while adoptive immunotherapy or chemotherapy cured less than 20% of the mice. Further, effectiveness of chemoimmunotherapy alone was maximized against stage II or stage III Renca by a bicompartmental approach in which administration of treatment iv and ip cured >75% of Renca-bearing mice, whereas iv or ip treatment produced no cures. These results demonstrate that adoptive immunotherapy can enhance the effectiveness of chemotherapeutic drugs against tumors.

PROJECT DESCRIPTION

PERSONNEL

Robert H. Wiltrout	Senior Staff Fellow	NIS	BTB	NCI
Raoul R. Salup	Guest Researcher	IB	LMI	NCI
Craig W. Reynolds	Senior Staff Fellow	NIS	BTB	NCI
John R. Ortaldo	Head	NIS	BTB	NCI
Bonnie J. Mathieson	Senior Staff Fellow	NIS	BTB	NCI
Patricia L. Urias	Chemist	NIS	BTB	NCI

OBJECTIVES

To ascertain the roles of NK cells, macrophages (Mø), and cytokines in augmenting resistance to metastasis formation, and to study the ability of adoptive immunotherapy by rIL 2-stimulated lymphocytes and rIL 2 to enhance the effectiveness of chemotherapeutic drugs against established murine renal carcinoma (Renca).

(1) To study the ability of various BRMs to augment organ-associated natural immunity.

(2) To determine the potential of BRM-augmented organ-associated natural immunity (NK cells and macrophages) for the prevention and treatment of liver metastases.

(3) To study the mechanism(s) by which BRMs induce an increase in the total number of LGL in the liver.

(4) To study the role of LGL in acute inflammation.

(5) To determine the extent to which adoptive immunotherapy with rIL 2-stimulated lymphocytes and rIL 2 can enhance the effectiveness of chemotherapeutic drugs against established murine renal carcinoma (Renca), and other experimental tumors.

I. Role of Natural Effector Cells in BRM-Mediated Antimetastatic Effects:

A. Modulation of Metastases and NK Activity by BRMs.

Treatment of normal mice with anti-asGM$_1$ serum ablated NK activity, and increased formation of experimentally induced lung and liver metastases, following iv administration of B16 melanoma cells. This suggested that NK cell activity provided the main defense against metastasizing tumors. We have shown that tumoricidal activity mediated by MVE-2 or P. acnes-activated Mø was refractory to treatment with anti-asGM$_1$, confirming that anti-asGM$_1$ also selectively depletes NK activity in BRM-treated mice. Interestingly, MVE-2 was able to induce antimetastatic effects in anti-asGM$_1$-treated mice. This antimetastatic activity was observed even when spleen and blood NK levels, and the rate of lung clearance of radiolabeled tumor cells, which reflects early NK-mediated events in the metastatic process, remained impaired, indicating that the anti-metastatic effects of MVE-2 was not

dependent on intact NK activity in the circulation. These observations suggested that MVE-2 had induced anti-tumor effects at the sites of metastasis formation (lungs and liver), during the post-blood borne phase of metastasis. Therefore, we examined the lungs and livers of mice treated with MVE-2 and anti-asGM$_1$ for natural immune effector activity. Surprisingly, there was not only good Mø-mediated activity, but also high levels of tissue NK activity. Therefore, we focused our attention on the activity of these natural effector cells in these organs.

B. Isolation and Characterization of Natural Anti-Tumor Effector Cells in the Liver.

Our studies concentrated on effector cells in the liver, since it is often seeded by B16 melanoma following anti-asGM$_1$ treatment. Hepatic effector cells were isolated from normal and BRM-treated mice. The nonparenchymal cells consisted of 25-35% Kupffer cells, which could be enriched to 82-90% by adherence. Normal mice yielded 0.5 to 1 x 10^6 Kupffer cells per mouse, while MVE-2-treated mice yielded 1.5 to 4 x 10^6 Mø, P. acnes-treated mice yielded 5.0 to 11 x 10^6. Once activated, these cells were able to lyse the macrophage-sensitive targets L5178Y and P815, while having little effect against the NK-sensitive YAC-1 target.

Examination of the nonadherent cells revealed considerable numbers of large granular lymphocytes (LGL) and large agranular lymphocytes (LAL), the cell types closely associated with NK activity. Livers of normal mice yielded up to 1 x 10^5 LGL + LAL, while up to 2 x 10^6 were obtained from BRM-treated mice, with 20-40% of the isolated cells being LGL + LAL. Depletion of adherent cells on nylon wool increased the percentages of LGL + LALs to 40-70% and fractionation on Percoll density gradients increased the LGL + LAL to 90%. These percentages of LGL in the livers of BRM-treated mice are in striking contrast to their much lower proportions in the blood or spleen. Cells from the livers of normal mice exhibited little NK cell activity, while mice treated with MVE-2, P. acnes, poly ICLC, OK-432, or rIL 2 exhibited much higher levels of NK activity.

Previous studies had demonstrated that multiple administration of various BRMs, including IFN, MVE-2, and poly ICLC, induced a hyporesponsiveness to augmentation of NK activity in the blood and spleen. It was not clear whether this was due to an actual refractoriness of NK cells or to redistribution of the effector cells. Therefore, we studied levels of organ-associated NK activity following multiple treatment with BRMs. Interestingly, total NK activity and LGL number were increased in the liver following multiple treatment with MVE-2, poly ICLC, or OK-432 as compared to a single administration. This result contrasted with the induction of NK hyporesponsiveness and decreased LGL numbers in the blood and spleen. These results imply that repeated treatments with BRMs induce a redistribution of NK-active LGL from the blood and spleen to the liver, or alternatively a preferential recruitment and/or proliferation of LGL into the liver.

C. Contribution of Interstitial NK Activity to Anti-Metastatic Defenses in MVE-2-Treated Mice.

We used anti-asGM$_1$ serum to treat MVE-2- or P. acnes-stimulated mice, and found it did not decrease Mϕ priming or cytolytic activity. Similarly lung and liver NK activity was also not eliminated by the antiserum treatment. The antimetastatic effects induced by MVE-2 in the lungs and livers of anti-asGM$_1$-treated mice correlated with the persistance of this anti-asGM$_1$-resistant NK activity since multiple high doses of the antiserum abrogated both the local NK activity and anti-metastatic protection. This finding provides the basis for a model of anti-metastatic defense in which NK cells not only participate in the early blood-borne phase of metastasis, but in which NK activity also may be induced to play a vital role during the post blood-borne phase of the metastatic cascade, in interstitial tissues or organ sites such as the liver.

D. Role of LGL in Acute Inflammatory Responses.

LGL accumulate quite early (by day 1) in the liver following BRM treatment, before the appearance of other lymphocytes, macrophages, and PMNs. We therefore postulated that LGL may have some role in the development of acute inflammation. Our studies demonstrated that selective elimination of LGL and consequent depression of NK activity by pre-treatment with anti-asGM$_1$ serum, inhibited the development of hepatic inflammation following BRM treatment.

Similarly, the development of hepatic inflammation was also inhibited in beige mice, which have a relatively selective genetic defect in NK activity, following treatment with BRMs. These results support the hypothesis that LGL may provide amplification or chemotactic signals important in the generation of an acute inflammatory response.

II. Chemoimmunotherapy of Established Murine Tumors: Enhancement of Effectiveness of Chemotherapeutic Drugs by BRMs or Adoptive Immunotherapy and Human rIL 2:

A. Application of adoptively transferred rIL 2-stimulated lymphocytes and rIL 2 to chemoimmunotherapy.

We have characterized the tumor growth and progression of a murine renal carcinoma (Renca), which when implanted under the kidney capsule, closely mimics the pattern of invasion and metastasis seen with adult human renal cell carcinoma. Tumor growth has been classified into clinical stages I-IV and early results demonstrated that NK cells were important for the control of spontaneous metastases in Renca-bearing mice, since selective depression of NK activity resulted in increased formation of metastases to lymphnode, liver, and lungs and decreased survival time. Optimum chemoimmunotherapy protocols were then devised for each stage of tumor growth. Initial studies of stage I Renca demonstrated that iv chemotherapy with doxorubicin hydrochloride (DOX) was able to cure only 17% of the tumor-bearing mice, while iv adoptive immunotherapy by rIL 2-stimulated lymphocytes and rIL 2 was unable to cure any mice. In contrast iv administration of combination chemoimmunotherapy cured 67% of the Renca-bearing mice. Similar results were obtained against

1 day old Renca implanted ip. Based on this apparent synergy between chemotherapy and immunotherapy, we designed treatment protocols for stage II Renca. These studies demonstrated that a bicompartmental approach, in which chemoimmunotherapy was administered both iv and ip, was required for maximum effect. More than 75% of the tumor-bearing mice were cured by this regimen, while iv or ip chemoimmunotherapy alone was totally ineffective. In addition, removal of the tumor-bearing kidney was required for optimal therapeutic effect. Further studies of stage III Renca demonstrated that the cyclic bicompartmental administration of chemoimmunotherapy was able to cure >70% of the Renca-bearing mice. Stage IV Renca proved more difficult to treat since the peritoneal cavity was extensively involved with tumor and cytoreductive surgery was impossible. However, surprisingly, initial studies have demonstrated that the survival time of mice with stage IV Renca was doubled by bicompartmental chemoimmunotherapy, raising the possibility that chemoimmunotherapeutic approaches may be useful against advanced disseminated tumors, even when the primary tumor is unresectable.

B. Chemoimmunotherapy with BRMs.

Since BRMs potently augment the activity of NK cells and/or macrophages, we postulated that they might also enhance the effectiveness of chemotherapy against tumors. Preliminary studies have demonstrated that DOX alone cured 40% of mice bearing Renca for 1 day in the peritoneal cavity. The administration of a single dose of MVE-2 cured no mice. However, the combination of DOX + MVE-2 cured 90% of the mice bearing this early stage Renca. Studies are currently underway to determine the efficacy, for more advanced tumors, of chemoimmunotherapy with BRMs.

SIGNIFICANCE

Defining the mechanisms by which BRMs mediate antimetastatic effects in experimental models may help to guide the formulation and interpretation of BRM therapy regimens in clinical trials. Similarly, optimization of adoptive immunotherapeutic protocols which employ rIL 2-stimulated cytotoxic lymphocytes should also prove useful for application to clinical trials. Most importantly, the observations that both BRMs or adoptive immunotherapy with rIL 2-stimulated lymphocytes and rIL 2 enhances the effectiveness of chemotherapeutic agents for treatment of established experimental cancer, provides a conceptual framework for formulation of analogous chemoimmunotherapeutic approaches in clinical trials.

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 Mø, and to understand the respective potential of NK cells and Mø as antitumor effectors in immunoprophylaxis and immunotherapy. These studies will continue by examining antimetastatic effects of BRMs in local organ sites. Further we will study the in vivo dynamics of the LGL population in mice following treatment with BRMs and the possible immunoregulatory roles of LGL in the development of acute inflammation (collaboration with Dr. Craig Reynolds Z01 CM 09257 03). We will also expand our studies on chemoimmunotherapeutic approaches

to treatment of established Renca by investigating the effectiveness of this treatment in several additional tumor models, including human ovarian carcinoma in nude mice (collaboration with Dr. John Ortaldo Z01 CM 09256 02) and the MDAY-D2 metastatic tumor of DBA/2 mice, among others. We will also collaborate with Dr. Bonnie Mathieson (Z01 CM 09259 02) to proceed to characterize the cell(s) which responds to rIL 2 in the chemoimmunotherapy studies, as well as characterize the actual effector cells. We will also study the contribution of the host's immune system during chemoimmunotherapy, as well as test several additional chemotherapeutic drugs for use in chemoimmunotherapeutic studies.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09275-02 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Effect of Mutagen Treatment on the Immunogenic Properties of Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Gorelik	Guest Researcher	BTB, NCI
Others:	R. B. Herberman	Chief	BTB, NCI
	S. Peppoloni	Visiting Fellow	BTB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	

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.)* The immunogenic and metastatic properties of the BL6 and 3LL tumor cells treated wtih MNNG or UV light were further investigated. Tum^- clones from UV-treated 3LL cells have complete cross reactivity with nonhomologous tum^-, tum^+ and parental 3LL tumor cells. Parental 3LL tumor cells were sensitive targets for specific cytotoxic T cells, and they were equally efficient as immunogenic tum^- cells in the cold target inhibition assay, but they were poorly immunogenic in in vivo and in vitro tests. These results indicate that parental 3LL cells possess tumor associated antigenic determinants but express them in nonimmunogenic form. The primary antitumor response was highly sensitive to the immunosuppressive action of x-irradiation (550R) or cyclophosphamide (Cy) (200 mg/kg) treatment. However, antitumor resistance of the preimmunized mice remained fully expressed after these treatments. Cy treatment decreased the total number of spleen cells and the proportion of B-lymphocytes, but substantially increased the proportion of Ly 2.2^+ and L3T4 lymphocytes. Ly 1.1^+ lymphocytes were mostly responsible for the adoptive transfer of anti-3LL resistance. MNNG treatment of BL6 melanoma cells increased the expression of class I MHC antigens and immunogenicity of the treated cells. Mice which rejected a tum^- clone from the BL6T2 subline were resistant to the nonhomologous tum^- melanoma clones, and to tum^+ clones which expressed high level of $H\text{-}2^b$ antigens, whereas tum^+ clones with low level expression of the $H\text{-}2^b$ complex or parental BL6 melanoma with little or no detectable MHC antigens, were able to grow in immune mice. Interferon treatment increased the expression of class I MHC antigens; however it did not influence the ability of BL6 melanoma cells to grow in vivo, perhaps due to transient effect of interferon on the $H\text{-}2^b$ antigen expression. The metastatic properties of MNNG-treated BL6 melanoma cells was substantially reduced, probably due to the increase their immunogenicity. Further understanding the mechanisms responsible for the conversion of the nonimmunogenic tumor cells into the highly immunogenic variants could open the way for the utilization of specific immune mechanisms for the immunotherapy of cancer patients.

PROJECT DESCRIPTION

PERSONNEL

Elieser Gorelik	Guest Researcher	NIS	BTB	NCI
Ronald B. Herberman	Chief		BTB	NCI
Samuele Peppoloni	Visiting Fellow	NIS	BTB	NCI

OBJECTIVES

The main objectives of this project are:
1) To study the effect of treatments by ultraviolet (UV) light or N-methyl-N-nitro-nitrosoguanidine (MNNG) on the immunogenic and metastatic properties of murine tumors.
   a) To select clones or noncloned populations of tumor cells with high levels of immunogenicity.
   b) To use highly immunogenic tumor cells and biological response modifiers for additive or synergistic antitumor and antimetastatic effects.

METHODS OF PROCEDURES

Mutagen treatment of tumor cells was performed by using ultraviolet (UV) light or N-methyl-N-nitro-nitrosoguanidine (MNNG). After exposure of 3LL tumor cells to 72mW/cm^2 of UV light, the 2% surviving tumor cells were expanded and cloned by limiting dilution. Eighty individual cell clones were selected and their ability to grow in vivo was tested by inoculation of $2.5x10^5$ cells into the footpad (i.f.p.) of syngeneic C57BL/6 mice. Nontreated 3LL tumor cells were also cloned and 20 selected clones were inoculated i.f.p. into mice at the same dose.

The highly invasive B16BL6 clone of melanoma cells was incubated in vitro with of MNNG during 1 hr. at 37° C. Cells were washed twice and expanded. The treatment was repeated again 1 month later. Tumor cells after 1 or 2 mutagen treatments were designated as B16BL6T1 or B16BL6T2, respectively. B16BL6T2 were cloned using limiting dilution and 48 individual clones were inoculated i.f.p. into C57BL/6 mice ($5x10^4$ cells/mouse).

Metastatic properties of tumor cells were investigated by i.v. inoculation or by their ability to metastasize from the local (i.f.p.) site of their growth. Tumorigenic and metastatic properties were assessed in normal C57BL/6 or nude mice as well as in mice treated i.p. with anti-asialo GM1 serum (0.25 ml diluted 1:40), which is efficient in the suppression of NK cell function.

Using monoclonal antibodies against H-2^b antigens and flow cytometry, the expression of H-2K^b and H-2D^b antigens on the surface of tumor cells treated with mutagens was investigated.

MAJOR FINDINGS

Immunogenic variants of 3LL and BL6 tumor cells were obtained after in vitro treatment with UV light or MNNG, respectively. Immunogenicity and antigenic specificity of the individual clones and whole monoclonal populations were

further investigated. Tum$^-$ clones from UV-treated 3LL tumor cells were cross-reactive with tum$^-$, tum$^+$, or parental 3LL tumor cells, as determined by the ability of these tumor cells to grow in preimmunized mice or by in vitro assessment the cytotoxic activity of immune spleen cells. Spleen cells from immune mice resensitized in vitro against tum$^-$ clone were equally cytotoxic against various tum$^-$, tum$^+$ and 3LL tumor cells. Furthermore, parental 3LL tumor cells and tum$^-$ clone 75 were equally efficient for cold target inhibition of the cytotoxic activity of the immune lymphocytes. In contrast, 3LL cells were poor immunostimulators testing in vivo or in vitro. Thus, these data indicate that parental 3LL tumor cells possess tumor associated antigenic determinants, but they exist in nonimmunogenic form.

Simultaneous inoculation of the tum$^-$ clone into the left leg and parental 3LL cells into the opposite leg resulted in the complete rejection of both tumor inocula. However, when 3LL tumor cells were inoculated first followed 7-10 days later by the tum$^-$ clone the growth of 3LL tumor cells was not protected. Furthermore, several consecutive s.c. inoculations of viable tum$^-$ cells did not affect the growth of 3LL, whereas growth of the tum$^-$ cell inoculum was completely inhibited. The mechanisms responsible for the failure to reject the established 3LL tumor by the immunization with tum$^-$ cells are still unknown. It hardly can be explained by the effect of the suppressor cells since the tumor-bearing mice were able to reject the viable tum$^-$ cell used for immunization.

Tum$^-$ cells of 3LL or BL6T2 tumors were able to grow in X-irradiated (550R) mice or mice treated with cyclophosphamide (200 mg/kg). However, when mice became immune after rejection of tum$^-$ cells their specific antitumor resistance was not impaired by irradiation or Cy treatment. Thus, the primary antitumor immune response is sensitive but the secondary immune reactions is relatively resistant to the immunosuppressive action of x-irradiation or Cy treatment. The phenotype of the spleen cells of normal or immune mice treated with Cy (200 mg/kg) was investigated. Cy-treatment decreased the total number of spleen cells, the proportion of B-lymphocytes whereas it increased the percent of Lyt 2.2$^+$ and L3T4$^+$ lymphocytes in both normal and immune spleens. Despite the high proportion of Lyt 2.2$^+$ and L3T4$^+$ lymphocytes) Cy-treated immune spleen cells after in vitro restimulation were poor cytotoxic. The Cy-induced suppression of CTL production was not restored by addition of IL-2 to the Cy-treated immune spleen cultures. Thus, the lower level of in vitro cytotoxicity showed by Cy-treated immune spleen cells would not attributed to the lack of lymphocytes with cytotoxic or helper phenotypes, but rather to an impairment of their physiological functions following drug treatment. In vivo experiment with adoptive transfer of the antitumor resistance indicate that Lyt 1.2 positive, but not Lyt 2.2$^+$ lymphocytes are mostly responsible for the antitumor protection.

Analysis of the cross reactivity of tum$^-$, tum$^+$ clones from the BL6T2 melanoma cells demonstrated complete cross-protection when various tum$^-$ clones were reinoculated into mice which rejected non-homologous tum$^-$ clones. However, protection of tum$^+$ clones depended on the level of the expression of H-2^b antigens by these clones. Immune mice were able to reject tum$^+$ clones which expressed high levels of class I H-2^b antigens. Tum$^+$ clones with low level of MHC antigens were able to overcome the antitumor resistance of immune

mice. In parallel the original BL6 melanoma cells or its clones which were almost devoid of H-2^b antigens also were able to grow in immune mice.

Interferon (α&β) at the dose 1000-4000 u/ml and interferon γ (50-100 u/ml) were extremely efficient in the induction of class I H-2^b antigens expressions on the cell surface of H-2^b-negative BL6 melanoma cells. Although interferon treatment caused an increase in H-2 antigen production, it did not increase the immunogenicity of BL6 melanoma cells and they developed tumors in 100% of inoculated mice. This might be a result of the temporary effect of interferon on H-2 genes activity, since by 7 days after interferon removal from the culture the expression of H-2^b antigen decreased. However, when interferon-treated tumor cells were transplanted into the preimmunized mice their growth was prevented. Thus, augmentation of H-2 antigen expression by in vitro interferon treatment made tumor cells more recognizable by immune lymphocytes.

Spleen cells from immune mice after in vitro resensitization with immunogenic clone 39 or BL6T2 cells became cytotoxic. However, CTL failed to destroy BL6 melanoma cells which lack MHC restricted elements. Furthermore, BL6 melanoma cells were more resistant to the cytotoxic action of NK cells or IL-2 activated spleen lymphocytes then BL6T2 melanoma cells. In contrast, BL6 melanoma were more sensitive then BL6T2 cells to the cytotoxic action of the LGL granules. Tumoricidal macrophages equally destroyed BL6 or BL6T2 melanoma cells. The effect of interferon on the susceptibilty BL6 and BL6T2 melanoma cells to the cytotoxic action various effector cells is under present evaluation.

FUTURE PLANS

The immunogenic and metastatic properties of the tumor cells treated with the mutagenic agents will be further investigated. The relative resistance of the secondary immune response to the immunosuppressive action of Cy could be exploited for combined chemoimmunotherapy. Therefore, mice bearing 3LL tumors will be immunized and then treated with Cy. In addition, interferon treatment of mice bearing BL6 melanoma cells will be used to increase in vivo the H-2^b antigen expression by the tumor cells. In combination with the specific immunization, it could increase the efficiency of the specific and nonspecific immune mechanisms. Tumor necrosis factor (TNF) could induce necrosis of the tumor and results in regression. It was found (North, et al. 1982) that TNF induced regression only of the immunogenic tumors. In order to induce necrosis and regression, we intend to immunize tumor-bearing mice with immunogenic tumor cell variants and then treat with recombinant TNF. The in vivo antitumor or antimetastatic activity of normal and specific immune lymphocytes expanded in vitro with IL-2 will be compared. Although nonspecific effector cells could be highly tumoricidal in vitro, they may be unable to specifically concentrate at the tumor size. It is expected that IL-2-expanded lymhocytes from the mice which were immunized with immunogenic variants could have higher antitumor or antimetastatic effects than lymphocytes from normal mice.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09282-01 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Potential Differentiation Capacity of Thymocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. J. Mathieson	Biologist	BTB, NCI
Other:	L. Mason	Microbiologist	BTB, NCI
	J. Wine	Bio. Lab. Tech	BTB, NCI
	F. Ruscetti	Head, Lymphokines Section	LMI, NCI
	K. Matsushima	Visiting Fellow	LMI, NCI
	W. Farrar	Senior Staff Fellow	LMI, NCI
	L. Takacs	Guest Researcher	LMI, NCI

COOPERATING UNITS *(if any)*

Laboratory of Microbial Immunity, NIAID, NIH (B.J. Fowlkes, T.M. Chused, L. Edison), Program Resources, Inc. (R. Overton) and Stanford University Medical Center, Division of Immunology (J. Parnes).

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Natural Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	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 differentiation capacity of isolated subsets from normal mouse thymus has been examined in vivo and in vitro. In experimental cell transfer studies using congenic mice we have determined that the most immature intrathymic subset of adult mouse thymocytes is a dull Lyl+ (dLyl) subset which is lacking both Lyt2 and L3T4 cell surface expression. This subset is seen early in thymus graft analyses. The dLyl cells isolated from adult thymus have been shown to be capable of thymus repopulation in irradiated animals, but these cells are not capable of repopulating other hematopoietic compartments. Thus the dLyl cells are committed thymocyte progenitors with limited capacity for repopulation. Because some of the dLyl cells can also be shown to differentiate into Lyt2+, L3T4+ cells in short term in vitro culture, we have examined the freshly isolated dLyl cells for expression of Lyt2 mRNA. Lyt2 mRNA was not detected in the freshly isolated dLyl cells, although appropriate transcripts could be seen in total thymocyte populations and selected Lyt2+ populations. A minor population of immature dLyl cells can be maintained in vitro for several months without further cell surface differentiation. We are currently examining the growth requirements and other characteristics of these cells using interleukin and mitogen stimulation and addressing questions of expression and induction of cell surface receptors for interleukins known to affect thymocyte proliferation.

GPO 914-918

PROJECT DESCRIPTION

PERSONNEL

Bonnie J. Mathieson	Biologist	NIS	BTB	NCI
Llewellyn Mason	Microbiologist	NIS	BTB	NCI
John Wine	Bio. Lab. Tech.	NIS	BTB	NCI
Frances W. Ruscetti	Head	LS	LMI	NCI
Kouji Matsushima	Visiting Fellow	IS	LMI	NCI
William Farrar	Senior Staff Fellow	LS	LMI	NCI
Lazlo Takacs	Guest Researcher	IS	LMI	NCI

OBJECTIVES

The major objectives of this project are: 1) To study the normal intrathymic differentiation pathways in mice and to specifically compare the phenotype and functional potential of different thymic subsets to cells which have natural cell-mediated cytotoxic reactivity. 2) To develop enrichment and selection methods to obtain the most immature thymic subsets from adult organs and to determine the appropriate stage of fetal cells for manipulation in vitro with biological response modifiers (BRM). 3) To determine whether the immature thymic cells can be shifted from conventional T-cell differentiation toward differentiation into large granular lymphocytes (LGL) with cytotoxic activity. 4) To attempt to obtain and maintain cloned cell lines of intrathymic generative cells and to determine if these clones have either natural killer (NK) or natural cytotoxic (NC) cell function.

METHODS EMPLOYED

Cell suspensions of immature adult thymocytes isolated by methods recently developed by B.J. Fowlkes have been used as potential sources for NK and NC precursors. To obtain immature adult thymocytes the majority of Lyt2+ cells are killed by anti-Lyt2.2 monoclonal antibody (MoAb) plus complement (C) treatment. This is followed by selective cytotoxic elimination of the remaining Lyt2+ cells and the mature L3T4+,Lyl+Lyt2- subset. These procedures yield about 1-3% of the input thymocytes and must be obtained free of contaminating blood and peripheral lymphoid tissue to avoid contamination in the final population.

Monitoring the purification and in vitro and in vivo differentiation is performed by flow cytometry analysis (FCA) in the BRMP facilities or at the NIAID facilities in Bethesda. MoAb and appropriate fluorescent detection reagents have been obtained and used in these studies. Those reagents required for the 2 color FCA are provided by Dr. B.J. Fowlkes.

Selected immature thymocytes have been cultured in bulk culture or microtiter wells with preparations of IL1, IL2, or IL3 and CSF, as well as the common T-cell mitogens. We have also used Con A induced spleen cell supernatants for this investigation.

Cloning of the cells will be performed by plating the cells at limiting dilution on irradiated splenic filler cells. The cells will be precultured

for 1 to 2 weeks in supernatant alone before this cloning to avoid expansion and cloning of conventional Lyt2+ killer T cells, which can be accomplished with IL2 and spleen filler cells. Clones will be tested for their growth requirements, by determining maximal clonal growth, and for potential production of interleukins when they are sufficiently expanded. However, we will initially determine the morphology, the phenotype and the cytotoxicity of the cells on both NK and NC targets for the cultures as they are expanded and grown in the interleukin preparations.

We have been unable to examine further potential differentiation in vivo of some thymocyte subsets because of limitations in the homing potential. We have begun experiments with direct intrathymic injections to permit the growth and differentiation in the appropriate environment to bypass this limitations.

MAJOR FINDINGS

Isolation of Immature Thymocytes - We have isolated a small subset (1-3%) of thymocytes with a phenotype which suggests that these cells are the equivalent of the thymic subcapsular cells. These cells have the following characteristics: they are dull Lyl+ cells (dLyl) by quantitative immunofluorescence; they express no detectable Lyt2 or L3T4. These cells comprise one of 2 subsets within the Lyt2-, L3T4- subset and may be derived from the dLyl subset. These cells have limited self renewal characteristics and are committed to the T cell lineage as demonstrated by their failure to yield multipotential colony forming units in the spleen when transferred in vivo into irradiated animals.

The dLyl cells can also be identified in repopulating thymic grafts between mice congenic for Lyt antigens (Mathieson and Fowlkes). The early kinetics of the appearance of the dLyl population in the grafts makes it highly likely that this population is an intrathymic generative cell.

However, some dLyl cells can be converted in vitro to Lyt2+ cells within 24 hours. In collaboration with J. Parnes (Stanford) we have determined that the dLyl cells when freshly isolated not only lack Lyt2 antigen on their cell surface but also fail to express the antigen at the mRNA level. The dLytl cells express low levels of T200/Ly5 and are Ly9/Lgp100+, indicating their lymphoid nature. These cells also have high levels of J11dq/M1-69, a heat-stable antigen (HSA) detected on the bulk of thymocytes but present only at reduced levels on mature T-cells. Thus, dLyl cells appear to be similar in phenotype to the early fetal thymocytes that, by definition, are immature. We intend to further characterize the surface phenotype of these cells as more reagents become available, particularly with regard to reagents that might.

Morphologically, the dLyl+ cells have large, kidney-shaped nuclei with no granules and a pale area at the site of the nuclear indentation. These large agranular lymphocytes strongly resemble the LGL which have been associated with NK activity in cell suspensions derived from spleen, liver or peripheral blood but not in the thymus of several species. Furthermore NK activity and LGL are increased in thymic (nude) mice which suggests the possibility of an alternative pathway for thymic precursor. However, the thymic subset of dLyl cells has no detectable NK or NC activity and has only

low levels of proliferative capacity without appropriate stimulation. Repeated experiments indicate that a minority of these cells can be maintained in culture for several months with Con A supernatants (CAS), without feeder cells, and that under such conditions these cells will develop cytoplasmic granules and develop the morphological appearance of LGL. Under these conditions, these populations have remained low for NK or NC activity.

Cell suspensions isolated from normal thymuses or in vitro stimulated thymus subsets have been injected into the thymus of irradiated mice. These experiments allow us to manipulate and to assess the differentation capacity of subpopulations whose homing potential is affected or limited by previous technical manipulations.

SIGNIFICANCE

Recent reports have indicated that cloned cytotoxic T-cell lines or Lyt2+ thymocytes plated at limiting dilution can demonstrate either NK-like killing or "promiscuous" killing of NK targets. These reports have indicated that the specificity of T-cell killing may be modified with lymphokine treatment so that a broader range of target killing may be obtained. We would like to determine whether lymphokine-treated "precursor" T-cells from the thymus can be differentiated on an alternative pathway to that of conventional Lyt2+ cytotoxic T-cell immunity.

Early fetal thymocytes (day 15 or earlier) express Ly5 (T200), low levels of Ly1, no detectable Lyt2 or L3T4, high levels of H-2 and asialo GM-1. These phenotypic characteristics are similar to those previously reported for the phenotype of NK cells. If NK cells are derived from a T-cell associated lineage, then NK precursor cells might be obtained from early fetal thymus cells or a normal adult population with the same phenotype. Thus, we have proposed that under appropriate in vitro stimulation, cytolytic cells with appropriate morphology and function to identify them as NK cells might be derived from immature, undifferentiated thymocytes.

Using a thymic graft analyses, we have identified a population of dull Ly1 (dLy1) cells in the normal adult thymus with characteristics suggesting it is responsible for graft repopulation. We have developed methods for isolation of these cells in relative purity from normal adult tissue and are using this adult thymocyte subset in studies of intrathymic differentiation and analysis of their NK progenitor activity. These studies are based on the assumption that these adult precursor thymic cells may have been programmed for a certain level of cytotoxic differentiation, but that the relative specificity associated with MHC restriction and T cell receptor expression which T cells acquire within the thymus comes at a later differentiation step.

It is still unclear whether all thymic progenitor cells have equivalent differentiation potential or whether the progenitors one heterogenous with separate lineages already committed at an earlier stage of differentiation. Further attempts to separate the dLy1 subset on the basis of IL2 receptor and other potentially useful markers will be required to determine whether there are separate lineages of cells or whether the intrathymic environment to which the precursors are exposed determines the fate of their differentiation.

Using the intrathymic injection procedures we should be able to bypass many limitations normally imposed by requiring cells to home to an appropriate environment for differentiation with i.v. injections.

PROPOSED COURSE

We will continue to isolate these immature cells from the mouse thymus and will attempt to clone the cells that have been maintained in culture for short periods by transfer to limiting dilution conditions with feeder cells. Cloned cells will be a valuable source of cells for monitoring granule development in mouse cells if they can be further characterized as LGL with NK or conventional cytolytic T cell activity. It may also be possible to test these cells for reactivity with rabbit anti-rat LGL granule antibodies, which appear to recognize NK-related granules in LGL of human and rat (see C. Reynolds Project # Z01 CM 09257-03 BTB).

To date, we have only used mouse Con A supernatants (SN) to support the growth of potential NK cells. These SN contain putative IL2 activity, based on their ability to support the growth of a cytolytic T-cell line. However, recent reports indicate that IL2 is not sufficient for maintaining cytolytic function of cultured cells and the dLyl cells will not grow in IL-2 although they have receptors for this lymphokine. We are systematically testing various recombinant and purified interleukins and cell growth factors available in the BRMP, in particular IL2, IL3, CSF, and interferon, because of their known effects on NK cells and T cells. The ability of these cytokines to support both the growth of freshly isolated immature thymocytes and any clones which we are able to obtain will be tested. These in vitro populations will be monitored for cytolytic activity on several targets.

Thymocyte subsets with immature characteristics (the dLyl cells, the total $Lyt2^{-}$,$L3T4^{-}$ cells and Lyt2+L3T4+ blasts, will concurrently be studied in grafted thymuses and in situ thymus repopulation of irradiated animals receiving cells i.v. or intrathymically. Appropriate selection subset and transfer of cells between congenic mouse strains with Lyl and Ly5 differences will enable us to analyze both differentiation and localization of intrathymic cells and the development of NK function in other organs in the same hosts.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06146-08 BTB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 Tumor-bearing Host

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. A. Chirigos	Head, Immunopharmacology Section	BTB, NCI
Others:	P. Sinibaldi	Visiting Fellow	BTB, NCI
	R. Ruffmann	Guest Researcher	BTB, NCI
	W. Budzynski	Guest Researcher	BTB, NCI
	M. E. Gruys	Microbiologist	BTB, NCI
	R. Wiltrout	Senior Staff Fellow	BTB, NCI
	C. Reynolds	Senior Staff Fellow	BTB, NCI
	E. Schlick	Visiting Scientist	LMI, NCI
	L. Varesio	Acting Head, Immunobiology Section	LMI, NCI

COOPERATING UNITS *(if any)*

Preclinical Section, PRI (J. Talmadge); Laboratory of Viral Diseases, NIAID (H. Levy); Armed Forces Radiobiology Research Institute (M. Patchen)

LAB/BRANCH

Biological Therapeutics Branch

SECTION

Immunopharmacology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	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.)*

Immunopharmacokinetic studies of several biological response modifiers (BRMs) in vivo have been performed. The majority of BRMs (MVE-2, Poly ICLC, IFN, glucan, Lentinan, Picibanil, $MnCl_2$, C. parvum, BCG) augment both NK cell and MØ tumoricidal activity. However, Imuthiol appeared to be selective for NK cells and Picolinic acid appeared selective for MØ. Multiple treatments with several BRMs maintained high levels of MØ activity but did not maintain elevated NK activity. Several possible mechanism(s) for this hyporesponsiveness to augmentation of NK cell activity were eliminated, i.e. suppresor lymphocytes or MØ and PGE production. The hyporesponsiveness to NK boosting by multiple treatments with BRMs was consistantly found to be associated with a decrease in splenic large granular lymphocytes (LGLs), which are associated with NK cell activity, indicating a failure to maintain the expansion of LGLs in the spleen. Several BRMs were also examined in vitro and in vivo for their capacity to induce the production and secretion of regulatory factors (colony stimulating factor, CSF; Prostaglandin E_1 and E_2, PGE; Interferon, IFN). Poly ICLC, MVE-2, BM41332 and Picibanil induced the secretion of CSF and PGE. Poly ICLC and Picibanil also stimulated IFN secretion. Serum levels of CSF following injection with Poly ICLC or MVE-2 remained significantly elevated for 7 days compared to the 7 minute half-life of exogenously injected CSF. The increased CSF was found to be accompanied by increased bone marrow (BM) cells and stem cells (GM-CFU-C) developing from BM cells. MVE-2 and Poly ICLC treatment following cytoreductive chemotherapy (cyclophosphamide) resulted in an earlier and elevated recovery of depressed NK activity and of bone marrow function.

Extensive studies with the MBL-2 lymphoma show that combined cyclophosphamide and BRM treatment (MVE-2 or Poly ICLC) leads to longer survival and an increased number of long-term survivors. The additive therapeutic effects of BRMs plus chemotherapy appears to be attributable to the ability of BRMs to reconstitute and/or enhance NK and MØ tumoricidal activity as well as to reconstitute bone marrow cellularity.

PROJECT DESCRIPTION

PERSONNEL

Michael A. Chirigos	Head	IS	BTB	NCI
Paola Sinibaldi	Visiting Fellow	IS	BTB	NCI
Ralf Ruffmann	Guest Researcher	IS	BTB	NCI
Wladyslaw A. Budzynski	Guest Researcher	IS	BTB	NCI
Eileen Gruys	Microbiologist	IS	BTB	NCI
Robert Wiltrout	Senior Staff Fellow	NIS	BTB	NCI
Craig Reynolds	Senior Staff Fellow	NIS	BTB	NCI
Erich Schlick	Visiting Scientist	LS	BTB	NCI
Luigi Varesio	Acting Head	IS	BTB	NCI

OBJECTIVES

This project examines the immunological and pharmacological mechanisms by which biological response modifiers (BRMs) regulate the various cellular components of the immune system and cause the production and release of soluble cellular components (e.g., IFN, CSF, PGE, interleukins 1 and 2). 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.

MAJOR FINDINGS

The immunopharmacokinetic studies of several BRMs [MVE-2, Poly ICLC, glucan, C. parvum, Picibanil, αβ IFN, Azimexon, Imuthiol; BM 41.332, $MnCl_2$] showed that all of these agents augmented NK cell activity in vivo, and that six (MVE-2, Poly ICLC, Picibanil, C. parvum, $MnCl_2$, αβ IFN) were particularly effective. The same BRMs also strongly stimulated Mø tumoricidal activity, which remained elevated for a minimum of 10 days. The five most effective BRMs (MVE-2, Poly ICLC, Picibanil, C. parvum, αβ IFN) were tested to assess whether multiple treatment would result in a higher response of effector cells, or maintain activity over a longer period of time. However, two treatments or more resulted in either reduced augmentation of NK cell activity, or a return to, or even a depression below, pretreatment levels. In contrast, the peritoneal Møs from the same mice maintained a high degree of tumor cytotoxic activity. Studies were then conducted to determine the mechansism(s) for such hyporesponsiveness to NK boosting by multiple treatments. No evidence was obtained for: cumulative BRM toxicity leading to depressed splenic mononuclear cells; presence of suppressor Mø or suppressor T cells; or, increased PGE production which could lead to NK cell depression. Since NK activity is associated with large granular lymphocytes (LGL), a comparison of the LGLs in the spleens of mice receiving single vs multiple treatments with BRMs was performed. The results showed that multiple treatments resulted in much less NK activity but also a striking decrease in the percentage of LGLs, relative to the high levels seen after one dose of the same BRMs. Thus the hyporesponsiveness appears to be related to a failure of a maintained expansion in the effector cell population in the spleen. However, further studies are needed to assess whether such failure to maintain the expanded size of the effector cell population is due to a limited number of available

precursor cells or to the lack of continued production of IL-2. Studies are also in progress to determine whether a similar hyporesponsiveness occurs in the liver and/or lungs.

Another potentially important aspect of the effects of BRMs is their capacity to stimulate the production and secretion of regulatory factors such as colony stimulating factor (CSF), prostaglandin E (PGE), and interferon (IFN). The seven BRMs were tested for their in vitro capacity to stimulate these factors. Three BRMs (Poly ICLC, αβ IFN, BRM 41.332) stimulated the secretion of significant amounts of CSF and PGE. Circulating levels of IFN were stimulated only by poly ICLC. CSF and PGE are involved in the positive and negative feedback of myeloid stem cell proliferation. To evaluate the potential implications of CSF on myeloproliferative responses, in vivo effects of Poly ICLC and MVE-2 on immune responses and on bone marrow were conducted. As expected, both BRMs significantly raised the cytotoxic activity of both NK cells and Mϕs. In addition, and of particular interest, both agents induced an increase in CSF in the serum, an increase in bone marrow cells in the femur and in the number of granulocyte-monocyte colony forming units (GM-CFU-C) developing from bone marrow cells. PGE was not increased in the serum of BRM-treated mice, nor did bone marrow cells secrete PGE at levels above control values when incubated for 24 hours.

Results of studies assessing NK cell activity in tumor-bearing mice and the dose of BRM necessary to maximally augment NK cell activity in the peripheral blood, spleen and bone marrow (femur) of tumor bearing mice indicated that: (1) NK cell activity is down regulated during the progressive growth of B16 melanoma or Lewis lung tumor; (2) the NK cell down-regulation resulting from progressive tumor growth could be reversed by maintaining the tumor-bearing mice on drinking water containing indomethacin (a cyclooxygenase inhibitor), indicating that down-regulation of NK cell activity in tumor-bearing mice could be due to the production and secretion of PGE by tumor cells; and, (3) the dose of BRM (MVE-2, Poly ICLC and Picibanil) which maximally augments NK activity in normal mice had to be increased by 25 to 40% to achieve a similar degree of NK cell augmentation in tumor-bearing mice. These observations suggest the need to establish in a tumor-bearing host the maximum immunomodulating dose of BRM for NK cell activity and that PGE may be acting as a negative feedback inhibitor, by down-regulating NK cell activity.

In an attempt to dissect the antitumor role of NK cells from those of Mϕ, antisera directed against the neutral glycosphingolipid asialo GM-1 (asGM-1), which selectively depletes NK cells activity, was employed. Results in mice inoculated i.v. with B16 melanoma show that MVE-2 treatment results in a significant decrease in lung and liver metastases when administered alone or when combined with the anti-asialo GM-1 antibody. These results suggest a antimetastatic role for non-NK effector cells which are stimulated by MVE-2 which are refractory to the anti-serum. Taking advantage of the observation that multiple treatment with MVE-2 also leads to a depressed NK-cell response, but not Mϕ activity, studies were conducted to assess the correlation of effector cell responses to the establishment of lung metastases. Single or multiple MVE-2 treatment resulted in a significant decrease in the establishment of lung tumors. Consistent with previous observations, multiple treatment resulted in a depressed NK cell response both in peripheral blood and spleen.

In contrast, Mø activity remained elevated. This pattern of results suggests the Mø played a prominent role in, or at least were sufficient for, inhibiting the establishment of lung metastases in mice treated with MVE2, but does not exclude the role of NK cells in the lung. Recent results (R. Wiltrout) indicate that suppression of NK activity in spleen or blood may not always accurately reflect NK activities in the lung or liver. Alternatively, a chemotherapeutic agent was used to nonselectively deplete NK activity. Of several therapeutically active drugs which are employed in cancer chemotherapy, cyclophosphamide (Cy) was selected, based on its reported effectiveness in treating various tumor types, particularly in combination with other drugs as well as its reported effect on immunological control mechanisms. Pretreatment of mice with Cy led to an increase in the development of lung metastases from IV-inoculated B16 melanoma cells. However, treatment with MVE-2 one day after Cy abrogated the depressive effect of Cy and led to a decreased number of lung metastases. MVE-2 pretreatment alone decreased significantly the establishment of lung metastases. Cy was previously found to depress NK cell activity in spleen or peripheral blood below normal values, but to exert little effect on Mø activity. Since Cy depressed NK cells, but has little effect on Mø activity, it seems likely that the effects of MVE-2 treatment in this study were related to reconstitution of NK cell activity and/or stimulation of Mø activity.

From previous studies, MVE-2 and poly ICLC were shown to cause an increase in colony stimulating factor (CSF) and bone marrow cellularity as well as NK cells and Mø activity. MVE-2 and poly ICLC were therefore examined in mice treated with Cy, to assess whether they would cause an earlier reconstitution of bone marrow cellularity and effector cell populations. Indeed, treatment with poly ICLC or MVE-2 caused a dose-dependent increase in bone marrow cells, in mice pretreated with Cy. Thus, whereas spontaneous recovery of the bone marrow cells from the myelosuppressive effects of Cy took about 11 days, additional treatment with BRMs led to not only reconstitution to normal levels by the 6th day, but to a rebound above normal levels.

Peripheral blood NK cell activity was depressed for at least 11 days after Cy treatment. BRM treatment resulted in a substantial increase in peripheral blood NK activity, and in a further increase in Mø cytotoxicity in the Cy-treated mice. Thus the reversal by MVE-2 of the Cy-induced increase in lung metastasis could be due to the restoration of both bone marrow and NK and Mø activity.

Studies were conducted to establish whether combining cytoreductive chemotherapy and BRM would lead to a more effective antitumor therapy. MVE-2 was tested for its effect when combined with Cy. The time for treatment with MVE-2 was found to be critical. A more effective response was achieved when MVE-2 was administered 3 to 6 days following a maximum therapeutic dose of Cy. Delaying MVE-2 treatment for 10 days resulted in a decrease of its efficacy. MVE-2 was not effective when combined with a lower dose of Cy, as indicated both by the lack of an increase in survival period and the lack of long-term survivors.

Further studies were conducted to establish the supportive therapeutic value of MVE-2 when combined with a maximum therapeutic dose of Cy. The therapeutic response to Cy treatment alone was significant whether it was administered

early (day 1) or late (day 5). Combined treatment with MVE-2 resulted in extended survival periods and a substantial proportion of long-term survivors when Cy was administered early. One treatment with MVE-2 appeared to be as effective as multiple treatments. In contrast, MVE-2 was not as effective when Cy treatment was delayed till day 5. Late treatment with MVE-2 alone did not elicit any therapeutic response. The importance of timing of administering Cy and a BRM was further supported from studies with a second BRM, Poly ICLC, employed in the same treatment protocol.

Timing of both the cytoreductive treatment with Cy, and with the BRM, appears to be critical. Delaying Cy treatment allows a progressive increase in the number of MBL2 cells which would reduce the efficacy of Cy treatment. In addition, delaying treatment with BRM following Cy treatment was less effective due to the progressive increase in the number of residual MBL2 cells occurring during the period between Cy treatment and administering the BRM. Regarding the latter possibility, we have established that the efficacy of BRM treatment alone is greatest when administered to mice with a low tumor burden. It is evident from the current studies that the more effective the cytoreductive treatment was, the more effective was the response to BRM treatment.

SIGNIFICANCE

The Immunopharmacology Section investigates and develops potential therapeutic agents which may alter biological responses which are 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 are being conducted with chemical and biological agents with respect to their: immunoadjuvant effects; most efficacious treatment regimens; and, usefulness in combined treatment modalities, and these studies have 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.

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. Tumor-bearing animals will receive intensive cytoreductive chemotherapy. BRMs which are capable of protecting and/or stimulating bone marrow and augmenting effector cells will also be administered concomitantly or intermitently with chemotherpy. Bone marrow cellularity and effector cell responses will be monitored to assess whether bone marrow and effector cells are maintained or augmented by the combined treatment regimen. This information will lead to the development of combined treatment protocols leading to maximum antitumor effects.

Several BRMs augment both Mø and NK cell activity. Studies will be conducted to assess whether the augmentation is mediated through the induction and secretion of cytokines (IL-1 and IL-2). Increased IL-1 production has been shown to be a functional property of activated Møs. BRMs which activate Mø tumoricidal activity in vitro and/or in vivo will be tested for their ability to induce IL-1. IL-2 expands the lymphoid cell population and directly augments NK cell activity. BRMs that augment NK cell activity in vitro and/or in vivo will be tested for their capacities to induce IL-2. This information will lead to a better understanding of the mechanism(s) by which BRMs increase Mø and NK 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 Mø and NK cells. Mø activation by several BRMs leads to the secretion of two cell regulating factors, PGE and CSF. We have demonstrated that PGE and CSF production and secretion by BRM-activated Møs occur through independent mechanisms. Since PGE has been shown to inhibit effector cell responses through a negative feedback inhibition mechanism, it may be possible to further enhance effector cellular responses by inhibiting PGE synthesis with cyclooxygenase inhibitors. Indomethacin will be administered at various time intervals in relation to BRMs and Mø and NK cell activity will be examined. Treatment protocols leading to enhanced effector cell responses will be examined in tumor-bearing mice to assess whether a better therapeutic response can be achieved.

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SUMMARY REPORT

LABORATORY OF MOLECULAR IMMUNOREGULATION

October 1, 1984 to September 30, 1985

INTRODUCTION

The Laboratory of Molecular Immunoregulation became an official entity on February 2, 1983. Headed by Dr. Joost J. Oppenheim, formerly of NIDR, the LMI 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; studies the lymphokine/cytokine modulation of cellular functions that participate in host defense; devises new diagnostic tests to better define immune status and pursue more critical evaluation of BRMs; evaluates the effects of BRMs on immunoregulatory pathways and host defense mechanisms and generates new BRMs that modify host defense mechanism. The LMI consists of three sections: Biochemistry, Lymphokines, and Immunobiology. The main research activities of the staff during the past fiscal year are summarized below for each section.

BIOCHEMISTRY SECTION

The Biochemistry Section is programmed to conduct research on the isolation, purification, and characterization of antigens, soluble mediators of the immune response, cellular growth factors and their receptors; investigates the use of these substances as therapeutic agents, as stimulants in the production of monoclonal antibodies, and as reagents in the development of clinically useful diagnostic immunoassays; purifies proteins to assay for amino acid sequence analysis, a first step in determining the nucleotide sequence and to subsequently obtain the cDNA code for the protein.

The Biochemistry Section has recently undergone another major personnel change with the departure of Dr. Edward Kimball and discontinuance of his studies of human urinary excretion of transforming growth factors (TGF) and interleukin 1 (IL 1). The section now consists of Dr. Joost J. Oppenheim who functions as the Head of the section and the research activities provided in support of the section by Dr. Connie R. Faltynek of the Program Resources, Inc. The search for outstanding investigators with molecular biology and biochemistry training and some experience in immunology is continuing in an effort to strengthen these investigative approaches in the LMI, BRMP.

Dr. Faltynek has initiated studies on the expression and regulation of receptors for human interferons (IFNs) on normal and malignant peripheral blood leukocytes. For these studies she has radioiodinated recombinant IFN-α A and IFN- γ by techniques which preserved the biological activity of the molecules. She has observed that highly purified resting T lymphocytes and large granular lymphocytes from normal donors constitutively express receptors for IFN- α and IFN-γ . Dr. Faltynek has assayed the number of IFN receptors per cell and the affinities of the IFN-receptor interactions. The level of IFN receptor expression has varied with the activation state of the normal T lymphocytes. Proliferating normal lymphocytes express 3 to 5 fold more receptors for IFN α than

resting lymphocytes. In contrast, the number of receptors for IFN γ was transiently decreased by 10 fold by 18 hrs after activation of lymphocytes by lectins.

Dr. Faltynek has also observed that the cells from IFN-αA responsive hairy cell leukemia patients expressed more IFN-αA receptors than the cells from the nonresponsive chronic lymphocytic leukemia patients. However, due to differences in cell size, the receptor density on these two types of leukemic cells was similar. Taken together, her data suggest that although it may not be sufficient as a predictive parameter for clinical responsiveness to IFN, the absolute number of receptors per cell may be an important criterion in the antiproliferative response to IFN.

LYMPHOKINE SECTION

The Lymphokine Section investigates the mechanism of action and therapeutic usefulness of lymphokines and other lymphocyte-derived growth and differentiation factors in the treatment of cancer; produces lymphokines, purifies lymphokines, develops and standardizes assays for lymphokines, and monitors the effects of treatment with lymphokines on immune responses.

Dr. Francis W. Ruscetti, the Head of the Lymphokine Section, investigates the growth regulation of immune and hematopoietic cells by studying the interaction of cytokines with their respective cell surface receptors. Recombinant, purified IL 2 was used to identify biochemical parameters associated with stimulation of these resting T-cells into cell cycle progression. Calcium levels, measured spectrophotometrically using Quin-2 dye, were markedly increased within one minute of IL 2 stimulation. No increase was observed with a non-homologous ligand. IL 2 also stimulated rapid turnover in the phosphoinositol pathway followed by hydrolysis of phosphoinositides. Translocation of protein kinase C from cytosol to cell membrane also correlated with the capacity of T cells to express receptors for IL 2.

Two types of IL 2 receptors with high and low affinity for IL 2 have been identified. Binding of the high, but not the low affinity receptor, induces lymphocyte proliferation. IL 2 itself promotes the expression of the low affinity receptor. Polyclonal lymphocyte stimulants such as phorbol esters induce expression of both types of receptors.

Dr. Ruscetti has evaluated IL 2 receptor expression on different types of transformed T cell lines. He has observed that some HTLV-I transformed lines express IL 2 receptors constitutively. These cells do not produce their own IL 2 but can demonstrate increased growth in response to IL 2. Consequently, endogenous IL 2 can potentially increase the tumor cell growth of some T cell tumors.

Dr. William L. Farrar, a Senior Staff Fellow in the Lymphokine Section, has been investigating the intracellular events that are induced by interleukins 2 and 3. He has observed the following early intracellular events initiated by each ligand on their respective cloned interleukin-dependent cell lines: i) the rapid mobilization of calcium, ii) stimulation of phosphatidylinositol turnover, iii) subcellular redistribution of protein kinase C (PK-C),and iv)

the rapid phosphorylation of a number of proteins which are substrates of PK-C. Since protein phosphorylation has been long regarded as a requisite mechanism of regulating protein/enzyme functions, the identity of the substrates as well as the phosphotransferase network involved becomes critical for understanding the biochemical basis of growth regulation and gene expression. IL 2 and IL 3 both stimulated the phosphorylation of a 68 kd protein within the cytosol of their target cells, suggesting that both ligands may share identical transmembrance signalling apparatus involving the 68 kd PK-C substrate. Among the membrane-associated proteins phosphorylated by PK-C is the IL 2 receptor. PK-C activation is also associated with IL 2 stimulation of IFN γ and IL 2 receptor mRNA synthesis.

Dr. Erich A. Schlick, a Visiting Scientist in the Lymphokine Section, has completed his investigations of the role of hematopoietic growth factors and autocrine tumor cell factors. He has investigated the ability of selected biological response modifiers (BRMs) to modulate growth and differentiation of GM-CFU-C and nucleated bone marrow cells (BMC) in normal and cyclophosphamide (CY)-pretreated mice. In vivo treatment of normal mice with either MVE-2 or poly ICLC induced an increase in secretion of colony stimulating factor (CSF) by BMC and macrophages, which was followed by an increased proliferation rate of GM-CFU-C and BMC. Both BRMs were also able to ameliorate the bone marrow depressing effects of CY pretreatment and to induce significantly enhanced Mϕ activities when given about 3 days after CY.

He has also established that MVE-2 can induce CSF-mediated anti-tumor effects on certain leukemic tumor cells. Treatment of mice bearing the Wehi-3B differentiation positive myelomonocytic tumor (which differentiates in response to CSF) first with CY and then 3 days later with the potent CSF-inducer MVE-2 increased survival time significantly and rendered 20-50% of the tumor-bearing mice disease free. No effects were seen with the differentiation negative tumors. These results suggest that BRMs which induce CSF may reconstitute hematopoiesis and macrophage functions or may block leukemogenesis by inducing terminal differentiation of susceptible target cells.

Preliminary data obtained by Dr. Schlick suggests that autocrine regulation of tumor growth exists. Two murine tumors (a Moloney virus-transformed T lymphoma and a spontaneous lung carcinoma M109) secrete factors that stimulate their own growth in a clonogenic assay and in suspension culture. These findings could provide the basis for manipulating tumor growth, e.g., by inhibiting the autocrine factor(s) or their production. Dr. Schlick will return to Germany by June of 1985, to pursue his immunopharmacology studies further.

IMMUNOBIOLOGY SECTION

The Immunobiology Section conducts studies on cell-mediated immunity to tumors and on the immunobiology of the antitumor effector mechanisms; investigates changes in intracellular biochemical events as well as the signals and mechanisms involved in cellular activation; conducts studies on the changes in cell surface markers during the process of cell differentiation and activation and the use of these markers in identifying, defining, and isolating subpopulations of various cell types; and investigates the role of cytokines such as interferon, colony stimulating factor and interleukin 1 as well as serum factors in cell growth and differentiation.

The Immunobiology Section has been relatively stable except for the transfer at mid-year of Dr. Robert Wiltrout to the Natural Immunity Section of the Biological Therapeutics Branch (BTB). His annual report will therefore be submitted with that of BTB. In addition, Dr. Kikuo Onozaki, an Expert returned to Japan in April of 1985, but his findings will be discussed.

Dr. Luigi Varesio, the Acting Head of the Immunobiology Section, has continued his molecular studies of the activation and modulation of macrophages with BRMs. Since mature macrophages do not proliferate in vitro the specific cellular response to BRMs can be easily distinguished from side effects due to changes in cell cycle. Dr. Varesio has shown that different biochemical pathways are involved in the activation of macrophages by IFN γ or IFN α and IFN β. However, despite distinct pathways of activation, all three types of IFN induced a major decrease in RNA synthesis in macrophages induced to express cytotoxic activity upon either in vitro or in vivo activation. In addition, activated macrophages show a specific alteration of rRNA metabolism causing a selective inhibition of accumulation of 28S rRNA. These results show that ribosomal RNA is important in regulating macrophage activation and that BRMs can act at the level of metabolism of ribosomal RNA. Within the context of macrophage activation he has defined for the first time biochemical events which are characteristic for cytotoxic cells. Understanding the molecular basis of the association between altered ribosomal RNA processing and macrophage activation may lead to the discovery of new regulatory functions of ribosomal RNA, and studies to test this possibility are in progress.

Dr. Elisabetta Blasi, a Visiting Fellow under the supervision of Dr. Varesio, has developed a new approach to study the differentiation of bone marrow (BM) cells into mature leukocytes. Recombinant retroviruses carrying activated v-onc were utilized to infect fresh murine BM cells. She has developed conditions for immortalization of murine macrophage precursors by infecting BM cells with v-myc and V-raf. The immortalized cells will provide a new model for studying the role of oncogenes in hematopoiesis. Parallel experiments are in progress to immortalize human BM cells as well as T and B cell precursors.

Dr. Howard A. Young, an expert molecular biologist with the Immunobiology Section, has been investigating the genetic control of interferon γ gene expression. In order to determine if DNA sequences near the IFN-γ coding region influence IFN-γ gene expression, Dr. Young has inserted cloned human IFN-γ genomic DNA into mouse fibroblasts and T cells. Initial results indicate that this gene was not expressed in fibroblasts under any conditions of gene induction. In contrast, human IFN-γ gene expression can be obtained in murine T cells under the appropriate conditions of induction as with PMA or IL 2.

The capacity of the intracellular human IFN-γ to affect murine cell functions has also been studied. Since the mouse cells do not express receptors for human IFN-γ, any changes observed in the cells should be a result of the internal expression of this lymphokine. Dr. Young has observed that mouse cells expressing human IFN-γ do not exhibit enhanced resistance to viral infection but do show increased expression of MHC class I antigens and oligo 2'5'A synthetase activity. This model system is the first example of IL 2 induction of a transfected gene and will permit dissection of the DNA surrounding the IFN-γ coding sequences in order to determine which sequences are involved in the control of IFN-γ expression.

Dr. Young is cloning cytolysin, a cytolytic factor derived from the cytoplasmic granules of a tumor cell line with natural killer (NK) activity (in collaboration with Dr. Craig Reynolds and other BTB investigators). Using a cDNA expression library and antiserum to the purified 60 kd cytolytic component of the granules (termed "cytolysin"), Dr. Young has obtained a cDNA clone which strongly reacts with the 60 kd antiserum. This cDNA clone also reacts weakly with whole granule antiserum. Preliminary results indicate that the immunoreactive cDNA clone is approximately 700 nucleotides in length and represents a multicopy (5-10) gene.

Dr. Elizabeth Kovacs, a Guest Researcher under the supervision of Dr. Young, has shown that production of IL 1 by a human monocytic tumor cell line, THP-1, can be increased 50% by treatment of the cells with 5-azacytidine and that another human monocytic tumor cell line, U937, can be induced to produce IL 1 following LPS stimulation after a similar treatment with 5-azacytidine. Although these effects are temporary and last for only 6-8 weeks, these results suggest that demethylation can promote gene expression for cytokine production by some tumor cell lines.

Dr. Kovacs has also shown that IL 1 treatment of a sensitive mouse thymocyte cell line, D10G4.1, results in increased expression of the c-myc proto-oncogene as well as both IL 2 mRNA and IL 2 receptor mRNA. Additional studies are currently underway to determine if the expression of other genes such as IFN-γ and the fos oncogene, are also altered by IL 1 treatment of these target cells.

Dr. Scott K. Durum, a Senior Staff Fellow in the Immunobiology Section has been studying the mechanism of production and antitumor effects of IL 1 in the mouse. Macrophages (Mø) release potent levels of IL 1 in response to a wide variety of immune and inflammatory stimuli. However, in the presence of anti-Ia antibodies, although IL 1 is still synthesized by murine Mø, IL 1 fails to be released from the cell. Biochemical studies suggest that Ia participates in the processing of the IL 1 precursor accompanying the release process. Hence, a new concept is developing - Ia control of processing and export of IL 1.

Another new concept has recently evolved concerning IL 1 production by murine B cells. Unlike Mø, which produce IL 1 in response to a wide variety of stimuli, B cells required contact with T cells to produce IL 1. Also unlike Mø, which release IL 1 into the surrounding milieu, murine B cell IL 1 remained cell associated. These observations provide new insights into the complex bi-directional mechanisms of T-B interactions.

Dr. Boris Tartakovsky, a Visiting Fellow under the supervision of Dr. Durum, is embarking on studies aimed at exploiting the ability of IL 1 to enhance immune responses against tumors. IL 1 has been shown to promote weak immune responses. Since tumors may succeed in vivo because of an ineffective immune response, it is, therefore, compelling to explore applications of IL 1 as an antitumor agent.

Dr. Kouji Matsushima, a Visiting Fellow under the supervision of Dr. Joost J. Oppenheim, has been studying the cell sources, production, properties and some of the effects of human interleukin 1 (IL 1). Dr. Matsushima has shown that normal human B cells as well as EBV transformed B cell lines with

accessory cell activities, produce IL 1-like factors. Normal B cell derived IL 1 is biochemically and antigenically similar to monocyte derived IL 1. In contrast, IL 1 produced by the transformed B cell line shows some unique biochemical properties and is not inhibited by rabbit anti-human monocyte derived IL 1. This IL 1-like activity may, therefore, be a product of a unique or altered gene.

Studies of the rate of production of IL 1 by monocytes reveal it to appear intracellularly within 30' after stimulation, and extracellularly by 60'. The m.w. of the intracellular IL 1 is 23 kd with a minor peak at 30 kd. The intracellular IL 1 is largely (>95%) associated with the cytosol fraction, and only 1-2% is present in the membrane and particulate fractions of the cells. These data suggest that IL 1 is rapidly processed from a largely biologically inactive 30 kd precursor to an active 23 kd cell associated and 17 kd soluble forms, perhaps by enzymatic cleavage. We have purified the soluble (pI 6.9, 17 kd) form of human IL 1 and obtained a partial amino acid sequence of the amino terminal end of the molecule. This sequence agrees with the reported cDNA sequence for human IL 1. We have radiolabelled this IL 1 with ^{125}I and demonstrated inhibitible binding sites on some human B cell lines.

In collaboration with Drs. M. Benczur and J. Ortaldo of the BTB, Dr. Matsushima also demonstrated that IL 1 augments the NK activity of human large granular lymphocytes. Dr. Kikuo Onozaki has demonstrated IL 1 augments the in vivo tumoricidal activities of human monocytes and that IL 1 also is directly cytostatic and cytocidal for a limited number of tumor cell lines. These in vitro observations reinforce the hypothesis that IL 1 may be a BRM which promotes host antitumor defense mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09251-03 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies of Hematopoietic and Tumor Cell Growth Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. A. Schlick	Visiting Scientist	LMI, NCI
Others:	F. W. Ruscetti	Senior Investigator	LMI, NCI
	F. Bettens	Guest Researcher	LMI, NCI
	J. J. Oppenheim	Chief	LMI, NCI

COOPERATING UNITS *(if any)*

Immunopharmacology Section, Biological Therapeutics Branch, NCI; Clinical Section, Biological Therapeutics Branch, NCI.

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokines Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	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.)*

Antineoplastic treatment regimens consisting of chemotherapy often result in a dysfunction of hematopoietic precursor cells of the granulocyte-macrophage (GM-CFU-C) lineage. We were, therefore, interested in testing the ability of selected biological response modifiers (BRMs) to modulate growth and differentiation of GM-CFU-C and nucleated bone marrow cells (BMC) in normal and cyclophosphamide (CY)-pretreated mice. In vivo treatment of normal mice with either MVE-2 or poly ICLC induced an increase in secretion of colony stimulating factor (CSF) by BMC and macrophages, which was followed by an increased proliferation rate of GM-CFU-C and BMC. Both BRMs were also able to ameliorate the bone marrow depressing effects of CY pretreatment and to induce significantly enhanced Mø activities when given about 3 days after CY. Second, it has been established that MVE-2 can CSF-mediated anti-tumor on certain leukemic tumor cells. In vivo experiments showed that treatment of mice bearing the Wehi-3B differentiation positive myelomonocytic tumor first with CY and then 3 days later with the potent CSF-inducer MVE-2 increased survival time significantly, rendered 20-50 % of the tumor-bearing mice disease-free. No effects were seen with the differentiation negative tumors. The present results support the concept that selected BRMs might be of value in reconstituting granulocyte and macrophage functions, and may further prevent leukemogensis by induction of terminal differentiation. Attempts have also been made to identify whether immortalized human T cells secrete growth factors are essential for long-term growth of human pluripotent hematapoietic stem cells in vitro. Identification of such factors (e.g. multi CSF) provides a model for studying physiologic regulation of marrow cell growth and differentiation and could also allow sustain proliferating stem cells in vitro as a source of BMC after treatment with chemotherapy. Preliminary evidence suggest that an autocrine regulation of tumor growth exists. Two murine tumors (a Moloney virus-transformed T lymphoma and a spontaneous lung carcinoma) secrete factors that stimulate their own growth in a clonogenic assay and in suspension culture. These findings could provide the basis for trying to interfere with the respective tumor growth, e.g. by inhibiting the factor(s) or their production.

PROJECT DESCRIPTION

PERSONNEL

Erich A. Schlick	Visiting Scientist	LS	LMI	NCI
Francis W. Ruscetti	Senior Investigator	LS	LMI	NCI
Florence Bettens	Guest Researcher	BS	LMI	NCI
Joost J. Oppenheim	Chief		LMI	NCI

OBJECTIVES

The overall objective is to investigate the role of hematopoietic and tumor cell growth factors and their modulation by biological response modifiers (BRMs). Specifically, three areas are addressed: 1) To assess the capacity of selected BRMs to induce colony stimulating factor (CSF) in vitro and in vivo and to evaluate their role in growth and differentiation of normal and malignant hematopoietic progenitor cells; 2) To evaluate and characterize hematopoietic growth factors, determine the intracellular signal by which they stimulate differentiation and the genes that must be activated during the differentiative process, and 3) To evaluate the production of self-stimulatory growth factor(s) by tumor cells and to characterize their role in neoplastic cell growth.

METHODS EMPLOYED

Resident murine Mø were obtained by peritoneal lavage and purified by plastic adherence. Sterile single cell suspensions of bone marrow were prepared from murine femurs and from the iliac crests of healthy human volunteers.

Assays for bone marrow progenitor cells as well as assays for functions of mature cell types: Nonadherent bone marrow cells were incubated in soft agar in the presence of serial dilutions of the sera/conditioned media to be tested for CSF. Myeloid and erythroid progenitor cells were determined using standardized soft agar assays. Mø activity was determined using a MBL-2 tumor growth inhibition assay as previously described.

Autocrine cytokines: Cell-free conditioned media from different tumor lines were tested for their capacity to support their own growth by a tumor clonigenic assay and by ^{3}H-thymidine uptake. Purification of these cytokine is being attempted using traditional and high pressure liquid chromatography.

Molecular techniques for measuring changes in genetic structure and gene activation such as Northern and Southern hybridizations are being utilized.

MAJOR FINDINGS

I. In vivo modulation of CSF,PGE and myeloid progenitor cells

Significantly increased concentrations of CSF could be detected in the serum of mice treated with either MVE-2, poly ICLC or the 2-cyanaziridines BM 41.332 and azimexone. Serum CSF levels were elevated as early as one hour post injection and remained increased for up to five days. Peritoneal Mø and bone marrow cells from these in vivo pretreated mice showed a parallel increased

secretion of CSF, when kept in culture for 48 hrs. The increased secretion of CSF was followed by an increase in granulocyte-Mø committed stem cells and nucleated bone marrow cells.

BRMs stimulated only a small increase in PGE secretion by peritoneal Mø, and had no effect on PGE secretion by bone marrow cells or serum PGE levels. Furthermore, pretreatment of the mice with indomethacin, three hours before treatment with BRMs, almost completely suppressed PGE secretion by peritoneal Mø, but left the CSF secretion as well as the bone marrow cellularity elevated, indicating that PGE played only a minor role in vivo on regulation of myelopoiesis.

II. Combined effects of cyclophosphamide and BRMs on myelopoiesis and Mø activity

Although we knew from these in vivo studies that some BRMs are promoting CSF-mediated growth and differentiation of myelomonocytic progenitors, we had to ascertain whether these BRMs would be able to restore myelomonocytic dysfunctions caused by antineoplastic chemotherapeutic treatment. Cytoreductive chemotherapy with cyclophosphamide induced a time-dependent strong suppression of bone marrow cellularity (CFU-C, nucleated bone marrow cells) and of the number and activity of Mø. Administration of either MVE-2 or poly ICLC to cyclophosphamide-pretreated mice was able to stimulate CSF secretion and to reverse the bone marrow depressing effect and to induce significantly enhanced Mø activity. The beneficial effects of both BRMs, however, could only be obtained by observing a critical time interval of about 3 days between the treatments.

III. Prevention of leukemogenesis by induction of terminal differentiation of leukemic cells by CSF-inducing biological response modifiers

It has been established that MVE-2 can induce CSF-mediated anti-tumor effects on certain leukemic tumor cells. Serum from mice pretreated with in vivo with MVE-2 induced terminal differentiation of cloned myelomonocytic tumor cells but had no effect on clones of the tumor cells that are unresponsive to CSF. In vivo experiments showed that treatment of mice bearing the Wehi-3B differentiation positive myelomonocytic tumor first with CY and then 3 days later with the potent CSF-inducer MVE-2 increased survival time significantly, rendered 20-50 % of the tumor-bearing mice disease-free. No effects were seen with the differentiation negative tumors. The present results support the concept that selected BRMs might be of value in reconstituting granulocyte and macrophage functions, and may further prevent leukemogensis by induction of terminal differentiation.

IV. Autocrine regulation of murine tumor cell growth

Preliminary data suggest that a Moloney virus-transformed T lymphoma and a spontaneous lung carcinoma secrete a factor(s), which can stimulate their own growth in a clonigenic assay and in suspension culture. Preliminary gel filtration chromatography of the acidified material from the lung carcinoma revealed elution of activity at an estimated molecular weight range of 8-10,000. Preliminary results further suggest that the(se) factor(s) are distinct from IL 1,

IL 2, IL 3 and CSF, and are selectively active on those tumor cells that also produce them.

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 secretion) are thus a prerequisite for designing combined therapeutic modalities (e.g., chemotherapeutic agents and BRMs) in tumor-bearing hosts.

In addition, it has been established that MVE-2 can induce CSF-mediated antitumor effects on certain leukemic tumor cells. Serum from mice pretreated in vivo with MVE-2 induced terminal differentiation of cloned myelomonocytic tumor cells, but had no effect on clones of the tumor cells that are unresponsive to CSF. Our results support the concept that selected BRMs might be of value in reconstituting granulocyte and macrophage functions, and may further prevent leukemogensis by induction of terminal differentiation. Thus, CSF inducing BRM's may be therapeutically useful by restoring hematopoesis or based on direct antitumor effects.

Since T-cells have been shown to secrete materials that stimulate hematopoietic stem cells, we investigated whether immortalized T-cells secrete factors capable of supporting long-term growth of both myeloid and erythroid progenitors in Dexter-type human bone marrow suspension cultures. Identification of such a factor(s) might provide a useful model for understanding the regulation of bone marrow cell growth and differentiation. By providing the means to sustain actively proliferating hematopoietic stem cells in vitro, such cultures could also be used as a source of autologous bone marrow cells after bone marrow suppression by chemotherapeutic treatment.

Growth stimulating factors have been demonstrated in several human tumor lines. Most of these transforming growth factors (TGF), however, only stimulate reversible phenotypic but not genotypic transformation of normal cells in soft agar cultures, while lacking growth stimulating effects on other malignant cells or on themselves. Evidence for such autocrine stimulation of tumors would provide a basis for trying to interfere with tumor growth, e.g. by inhibiting the factor or its production.

PROPOSED COURSE

The present results indicate that BRMs promote CSF-mediated recovery of bone marrow cells and Mø from chemotherapy, which could provide the opportunity for more extensive chemotherapy. This possibility will be tested by using cyclic treatment with chemotherapeutic drugs combined with intermittent BRM injections. BRMs thus could either allow a shortening of the interval between standardized

chemotherapeutic doses or an increase in the doses of chemotherapeutic drugs given at a standard interval. Furthermore, CSF-inducing BRMs might provide a tool for specific treatment of certain leukemias, by inducing terminal differentiation. This possibility will be tested in vitro and in vivo by comparing the response to BRMs of the WEHI-3B myelomonocytic leukemia D^+ (differentiation positive) subline, which responds to CSF-2 and CSF-3 with terminal differentiation to the response of the D^- subline, which is unresponsive to CSF induced differentiation.

Since the binding of hematopoietic growth factor to specific receptors thereby stimulating terminal differentiation, it is important to determine whether intra-cellular signals for differentiation are the same for normal and leukemic cell differentiation. Pattern of gene expression such as cellular proto-onc gene expression after factor stimulation will be determined is leukemic and normal cells. We will further characterize the biological and physical properties of the growth factor(s) derived from the HTLV-transformed T-cells. CM of these cells will be processed using traditional and HPLC mnethods for separation.

The studies on self-stimulating growth factor production by tumor cells will be continued to determine the biological and biochemical characteristics of these tumor growth factors. The concentrated culture supernatants of both tumor lines will be subjected to gel filtration and reverse phase HPLC and the active material(s) characterized, to determine whether the factor(s) stimulating tumor growth in both assay systems are identical. It will then be necessary to extend the role of these purified factor(s) to fresh and other cultured cells of the same disease.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09254-03 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Intracellular Signals Mediating the Growth and Differentiation of Cells Modulated by Lymphoid-Derived Growth Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. L. Farrar, Jr.	Senior Staff Fellow	LMI, NCI
Others:	S. W. Evans	Visiting Fellow	LMI, NCI
	M. Taguchi	Visiting Fellow	LMI, NCI

COOPERATING UNITS *(if any)*

Lymphokine Section, NCI (F. Ruscetti, M. Sparks); Immunobiology Section, NCI (H. A. Young, E. Bonvini); Program Resourses, Inc. (J. Rossio).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokine Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	0.75	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 spece provided.)*

The interleukins comprise a family of polypeptides derived from cells of haematopoietic origins that regulate not only the growth and differentiation of haematopoiesis but also the clonal expansion and maturation of the immune system. Although many of these regulatory proteins have been molecularly cloned, relatively little is known about their intracellular mechanism(s) of action in regulating S phase progression or gene expression. We have examined several parameters of cellular signal transduction intitiated by ligand-receptor interactions of interleukin 2 (IL 2) and interleukin 3 (IL 3). Among the early intracellular events initiated by each ligand on their respective cloned interleukin-dependent cell lines are i) the rapid mobilization of calcium, ii) stimulation of phosphatidylinositol turnover, iii) subcellular redistribution of protein kinase C and, iv) the rapid phosphorylation of a number of proteins which are substrates of protein kinase C. Since protein phosphorylation has been long regarded as a requisite mechanism of regulating protein/enzyme functions, the identity of the substrates as well as the phosphotransferase network involved becomes critical for understanding the biochemical basis of growth regulation and gene expression. IL 2 and IL 3 both stimulated the phosphorylation of a 68 kd protein within the cytosol of their target cells, suggesting that both ligands may share identical transmembrance signalling apparatus involving the 68 kd protein kinase C substrate. Among the membrane associated proteins prosphorylated by PK-C is the IL 2 receptor. PK-C activation is also associated by IL-2 stimulation of mRNA synthesis of gamma interferon and Tac epitope mRNA.

PROJECT DESCRIPTION

PERSONNEL

William L. Farrar, Jr.	Senior Staff Fellow	LS LMI NCI
Stuart W. Evans	Visiting Fellow	LS LMI NCI
Massaki Taguchi	Visiting Fellow	LS LMI NCI

OBJECTIVES

To examine and identify the intracellular events initiated by recombinant cytokine ligands which stimulate growth and differentiation of lymphoid cells and haematopoietic stem cells. The physiochemical activities stimulated by growth promoting ligands in normal cells will also be studied in malignant cells of corresponding tissue/lineage specificities.

1. Early Biochemical Events Associated with Interleukin 2 (IL 2) and Interleukin 3 (IL 3) Interaction with Cloned Target Cells

Two recombinant growth factors have been studied, IL 2 and IL 3 which have no nucleotide structural homologies or common target cell specificities. Both interleukins share a number of physiological similarities in that they stimulate G_1 to S phase progression and interact with high affinity sterospecific receptors. Although the nucleotide sequence of both ligands is known, little information is available concerning the biochemical mechanism(s) by which either ligand regulates growth or gene expression. We have examined a number of early metabolic events including i) phosphatidylinositol turnover, ii) calcium mobilization, iii) activation of subcellular distribution of protein kinase C, iv) stimulation of Ca^{2+} dependent phosphatase activity and v) ligand-dependent modulation of adenylate cyclase activity.

2. Regulation of Gamma Interferon Gene Expression

We have examined the ability of IL 2 to induce mRNA transcription and translation of an important immunoregulatory molecule, gamma interferon (IFNγ). In addition, studies are in progress (Sparks and Ruscetti) to examine the specific sequence of oncogene expression induced by IL 2 and IL 3 in their respective cell lines. Evidence indicates that at least three unique oncogenes are expressed as a result of IL 2 or IL 3 interaction with high affinity receptors. The same specific sequence is seen with either ligand in cells of distinct lineage. The data also suggests a specific metabolic phosphotransferase may mediate the nuclear expression of these oncogenes.

METHODS EMPLOYED

1) The studies have used a variety of biochemical methods including O'Farrel 2-dimensional gel analysis, protein kinase assays, HPLC-chromatography, immunoprecipitation, and intact cell protein radiolabelling. 2) Subcellular separations are accomplished by differential centrifugation, where enzymes are purified and assayed from their relative compartments. 3) Ligand initiated calcium studies are are performed using QUIN II fluorescence as a measure of intracellular calcium levels. Phospholipid turnover studies are examined using

radiolabel inositol precursors. Separation of phospholipids is accomplished using single-dimension TLC.

MAJOR FINDINGS

1. Intracellular Events in Lymphocyte Activation

We have examined several parameters of cellular signal transduction intitiated by ligand-receptor interactions of two distinct polypeptide growth lymphokines, interleukin 2 and interleukin 3. Among the early intracellular events initiated by each ligand on their respective cloned interleukin-dependent cell lines are i) the rapid mobilization of calcium, ii) transient stimulation of phosphatidylsitol turnover, iii) subcellular redistribution of protein kinase C from cytosol to particulate membrane, iv) the rapid phosphorylation of a number of proteins which are substrates of protein kinase C and, v) that IL 2 uncouples adenylate cyclase response to adrenergic agonist. Since protein phosphorylation has been long regarded as a requisite mechanism for regulating protein/enzyme functions, the identity of the substrates as well as the phosphotransferase network involved becomes critical for understanding the biochemical basis of growth regulation and gene expression. IL 2 and IL 3 both stimulated the phosphorylation of a 68 kd protein within the cytosol of distinct lineage cell lines, suggesting that both ligands may share identical transmembrane signalling apparatus involving the 68 kd protein kinase C substrate. Among the membrane associated proteins phosphorylated by PK-C is the IL 2 receptor. Interleukins also stimulate via PK-C activation and calcium mobilization synthesis of the mRNA for gamma interferon and the Tac epitope mRNA.

SIGNIFICANCE

In order to understand the mechanism of action of growth factors which induce S phase progression it is necessary to identify the intracellular signals capable of initiating or modulating the proliferative response. It has been hypothesized that phorbol esters modulate mitogenesis through pathways that converge with those of other hormones and that the protein kinase C phosphotransferase system may represent a point of biochemical identity between the mitogenic actions of hormones and phorbol esters. Our studies suggest that IL 2 and IL 3 activate a protein phosphorylation system, PK-C, that is also associated with the biological activity of tumor promoters and certain oncogenes. The finding that IL 2 and IL 3 each initiates PK-C activation defines an essential biochemical event necessary for the growth of lymphoid cells and haematopoietic stem cells suggest a possible mechanism of action for, as of yet undefined, oncogenic elements that may be involved in leukemogenesis.

A number of receptors with tyrosine kinase associated activity may be phosphorylated in vivo by the addition of phorbol esters to intact cells incubated in the presence of orthophosphate. Our data demonstrate that phorbol esters phosphorylate the Tac antigen in intact normal and neoplastic T lymphocytes. Purified PK-C was shown to directly phosphorylate isolated Tac immunoprecipitates. These observations and those of previous studies involving other growth hormones suggest an attractive hypothesis that the phosphorylation status of the IL 2 receptor may reflect a physiochemical relationship to receptor affinity which may

constitute a regulatory physiological process for both normal and neoplastic growth. Clearly, both high and low affinity IL 2 receptors have been described on T lymphocytes and the modulation of their relative expression is apparently controlled by the specific ligand, IL 2. The mechanism, by which receptor affinity is regulated by IL 2 or antigen remains as important issue for the understanding of those elements which control or rate limit lymphocyte growth. The demonstration that IL 2, IL 3 and diacylglycerol stimulate the increased phosphorylation of an identical 68 kd protein provides conclusive biochemical evidence that PK-C activation represents a component of the intracellular signalling process initiated by both IL 2 and IL 3. The observations suggest that a number of lymphocyte derived growth factors which regulate growth distinct lineages of cell types may also share a common pathway which involves the activation of PK-C and that these growth factors may represent a unique class of mitogenic polypeptides whose mechanisms of growth regulation may be similar.

Phorbol esters mimicry of IL 2 biological activity is most obvious for the induction of gene products which are also under IL 2 regulatory control. The observations that phorbol ester stimulates gamma interferon production and Tac expression are two notable examples of phorbol ester substitution of IL 2 for the biological expressions of lymphocyte gene products. These obervations suggest a functional role of PK-C activation by IL 2 may be related to the regulation of genetic expression of secretory genes (i.e. gamma interferon) as well as activation of gene structures involved in the maintenance of cell growth and survival (i.e. Tac).

PROPOSED COURSE

Within the immediate future we will focus on comparative studies of growth factor induced metabolic pathways with homologous transformed factor-independent cell lineages. Such studies will focus on phsophoprotein analysis, de novo protein synthesis 2-D PAGE analysis, oncogene expression and specific DNA binding proteins. We will examine cellular systems for translocating phospho-proteins with DNA affinity or affinities for phorbol-induced genome.

Studies will be initiated to examine the biochemical effects of a unique sterol compound which induces gene expression in lymphoid cells and differentiation in cells of haematopoietic lineage. Those studies include the identification of cytosolic and nuclear receptors.

We will continue to examine other aspects of ligand induced initiation of gene expression including 1) ligand interaction with specific dephosphorylation enzymes, 2) evidence to support a role for GTP hydrolysis and the relevant gene structures required for IL 2 induction of gamma interferon.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 09264-03 LMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Normal and Neoplastic T-Lymphocyte and Hematopoietic Cell Proliferation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. W. Ruscetti	Senior Investigator	LMI, NCI
Others:	J. A. Mikovits	Chemist	LMI, NCI
	M. B. Sparks	Guest Worker	LMI, NCI
	W. L. Farrar	Senior Staff Fellow	LMI, NCI
	E. A. Schlick	Visiting Scientist	LMI, NCI

COOPERATING UNITS *(if any)*
Biological Therapeutic Branch, NCI (J. Ortaldo); Dartmouth Medical School (K. A. Smith) Upstate Medical Center (B. J. Poiesz); Program Resources, Inc., NCI-FCRF (J. L. Rossio); Immunobiology Section, LMI, NCI (E. Bonvini).

LAB/BRANCH
Laboratory of Molecular Immunoregulation

SECTION
Lymphokine Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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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.)*

Binding of the regulatory peptide, interleukin-2 (IL-2), to its specific receptor is a prerequisite for activated T lymphocytes to grow. Deprivation of IL-2 from T-cells results in all the cells coming to rest in G_0. Recombinant, purified IL-2 was used to identify biochemical parameters associated with stimulation of these resting T-cells into cell cycle progression. Calcium levels, measured spectrophotometrically using Quin-2 dye, were markedly increased within one minute. No increase was observed with a non-homologous ligand. Also, it was shown that IL-2 stimulates rapid turnover in the phosphoinositol pathway followed by hydrolysis of phosphoinositides. It has been recently demonstrated that IL-2 binding produces a rapid and transient redistribution of protein kinase C (PK-C) from cytosol to cell membrane. This PK-C activity was correlated with the ability of T-cells to express T-cell receptors on their cell surface. Using radio-labeled IL-2 binding and cytofluorometric assays for anti-Tac, a monoclonal antibody to the IL-2 receptor, IL-2 was shown to stimulate increased IL-2 receptor expression on T-cells within 24 hrs. An increased level of IL-2 receptor became detectible by 4 hrs. Analysis of IL-2 receptor numbers after IL-2 stimulation showed major differences between IL-2 (6-9,000 per cell) and anti-Tac (35-45,000 per cell) binding. Resting, previously activated cells stimulated with phorbol esters have nearly equal numbers of binding sites with both ligands. Scatchard analysis of data reveals that the number of binding sites with high affinity ($>10^{-10}$M) correlated with IL-2 binding numbers and not with anti-Tac binding. Thus, IL-2 stimulation of T-cell growth results in an increased number of Tac antigen binding sites with lower affinity on the cell surface. Herpes-viruses, such as Herpesvirus saimiri (HVS), and RNA tumor viruses, such as Human T-cell leukemia virus (HTLV), can transform T-cells. Studies on metabolic events and IL-2 receptor regulation have shown major differences of growth control between normal and such transformed T-cells.

PROJECT DESCRIPTION

PERSONNEL

Francis W. Ruscetti	Expert	LS LMI NCI
Judy A. Mikovits	Chemist	LS LMI NCI
Maria B. Sparks	Guest Researcher	LS LMI NCI

OBJECTIVES

1. To determine the rate-limiting intracellular steps involved in stimulation by IL-2 of T-cell entry into S phase with promotion of T-cell proliferation.

2. To identify and characterize immunomodulators which promote or inhibit activation of the IL-2 and IL-2 receptor genes.

3. To compare the mechanism of T-cell transformation in vitro by RNA and DNA viruses, to gain a better understanding of the steps involved in the development of the transformed state.

4. To determine intracellular steps involved in stimulation of hematopoietic cell differentiation by IL-3 and GM-CSF to gain a better understanding of the differences between by normal and neoplastic myeloid differentiation.

METHODS EMPLOYED

1. Use of immunoaffinity and high performance liquid chromatography to purify growth and differentiation factors like IL-2, IL-3, GM-CSF, BCGF.

2. Use of monoclonal antibodies and specific binding to identify and immunoprecipitate cell surface receptors like the IL-2 receptor.

3. Development of in vitro assays to study normal T-cell growth, malignant T- cell transformation, and normal and neoplastic hematopoietic cell differentiation.

4. Assays for various lymphokines - IL-2 , colony-stimulating activity (CSA), burst promoting activity (BPA), and B-cell growth factor (BCGF) are used.

5. Molecular techniques for measuring changes in genetic structure and gene activation are utilized.

MAJOR FINDINGS

Binding of the regulatory peptide, interleukin-2 (IL-2), to its specific receptor is a prerequisite for activated T lymphocytes to grow. Deprivation of IL-2 from T-cells results in all the cells coming to rest in G_0. Recombinant, purified IL-2 (provided by Biogen, Inc.) was used to identify biochemical parameters associated with stimulation of these resting T-cells into cell cycle progression. Calcium levels, measured spectrophotometrically using Quin-2 dye, were markedly increased within one minute. No increase was observed with a non-homologous ligand. IL-2 stimulates rapid turnover in the

phosphoinositol pathway followed by hydrolysis of phosphoinositides.

In addition, it has been recently demonstrated by one of us (Farrar et. al., Nature, in press) that IL-2 binding also produces a rapid and transient redistribution of protein kinase C (PK-C) from cytosol to cell membrane. These data suggest that calcium ion and protein kinase C-linked events regulate the IL-2 mediated growth response and that phosphoinositol-derived metabolites play a role as second messengers in this response. In support of this, PK-C activity have been correlated with the ability of T-cells to express T-cell receptors on their cell surface. No IL-2 receptor expression was observed without a concomitant translocation of PK-C activity.

Using radio-labeled IL-2 binding and cytofluorometric assays for anti-Tac, a monoclonal antibody to the IL-2 receptor, IL-2 was shown to stimulate increased IL-2 receptor expression on T-cells within 24 hrs. An increased level of IL-2 receptor mRNA present in cells exposed to IL-2 was observed. Analysis of IL-2 receptor numbers after IL-2 stimulation showed that there are major differences between IL-2 (6-9,000 per cell) and anti-Tac (35-45,000 per cell) binding. Resting, previously activated cells stimulated with phorbol esters have nearly equal numbers of binding sites with both. Scatchard analysis of data reveals that the number of binding sites with high affinity ($>10^{-10}$M) correlated with IL-2 binding numbers and not with Tac binding. Thus, IL-2 stimulation of T-cell growth results in an increased number of Tac antigen binding sites with lower affinity on the cell surface.

Recent studies by Cantrell and Smith (J. Exp. Med. 158: 1332, 1983) have shown that the continuous presence of saturating amounts of IL 2 at all times does not prevent the IL-2 receptors from disappearing (with a concomitant loss of growth potential) from the cell surface. Studies were performed by us to look for possible modulation of the IL-2 receptor by non IL-2 agents. Since natural killer cells (NK) as well as T cells can express funtional IL-2 receptors, the two cell types were separated on Percoll gradients. In collaboration with Dr. Poiesz of Upstate Medical Center, it was recently shown that dexamethasone not only inhibits IL-2 production but also inhibits development of IL-2 receptors. Surprisingly, addition of purified IL 2 overcame the dexamethasone-mediated inhibition of receptor expression with a concomittant rise in the levels of IL-2 receptor mRNA. Although the majority of these IL-2 receptor are of low affinity, it seems that positive feedback with IL-2 is needed for optimal development of cell surface IL-2 receptors.

IL-2 by itself is not able to stimulate acquisition of its specific receptor on resting T-cells. Priming with antigen or lectin is required for this to occur. However, IL-2 by itself has been to have marked effects on NK cells such as Tac acquisition and interferon secretion within 24 hrs as well as long-term growth. In colloboration with John Ortaldo, the effect of IL-2 on NK cells is being examined. By measurement of anti-Tac binding and a radio-labelled IL-2 binding assay, it was confirmed that purified NK cells have no detectable IL-2 receptors and that within 24 hrs of IL 2 stimulation, they possess IL-2 receptors of both high and low affinity on their cell surface.

In contrast to cultures of normal T-cells, CTLL and CT6, two long term IL-2 dependent cell lines routinely used to perform biological assays for IL-2, did

not modulate their IL-2 receptors but constitutively expressed them. This provides an explaination, at least in part, for why there are so few cell lines that can be used to assay IL-2 and indicates that these cell lines are not normal.

Attempts to grow mature neoplastic T-cells in vitro have resulted in the development of many IL-2-independent T-cell lines, some of which were originally dependent on exogenous IL-2 for growth but subsequently rarely become independent of IL-2 and some that were factor-independent immediately upon culturing. Some of these cell lines can constitutively release IL-2 and were capable of responding to the same IL-2 with an increased rate of proliferation. To explore possible autocrine stimulation of leukemic cell growth, several cell lines were studied. In collaboration with Kendall Smith, dexamethasone-sensitive and resistant clones of MLA-144, a gibbon ape lymphoma cell line which constitutively produces IL-2 were studied. In those clones in which the production of IL 2 was completely inhibited, cell proliferation declined to 10-20% by day 4 and in two clones complete cell death occurred. Addition of either purified human or MLA-144-produced IL-2 overcame the dexamethasone-mediated growth inhibition. Incubation of these cell clones with purified IgG of anti-IL-2 antibody inhibited cell growth. Thus, this is the first direct evidence that some lymphoid tumors can grow by an autocrine-stimulated growth mechanism.

However, studies on other transformed T-cell lines have indicated that IL 2-mediated growth regulation is a rare event in neoplastic growth. Herpesviruses, like Herpesvirus saimiri (HVS), and RNA tumor viruses, like Human T-cell leukemia virus (HTLV), can transform T-cells in vitro. Transformed cell lines were developed by infecting peripheral blood cells of individual marmosets and owl monkeys by HVS or HTLV. In contrast to normal T-cells, they do not require added IL-2 for growth. An IL-2-specific cDNA probe, which hybridizes to RNA from PHA-treated primate lymphocytes, failed to hybridize to RNA from either HVS or HTLV-transformed marmoset or owl monkey T-cells. The addition of purified IL 2 to HVS-transformed cells stimulated a 2-to 5-fold increase in cell growth while it had no effect on the growth of HTLV-transformed cells. Results on IL-2 receptor studies indicated that: 1) IL-2 receptors on both HVS and HTLV transformed cells do not cycle on and off the plasma membrane as in normal T-cell growth and 2) HVS-transformed T-cells have normal levels of IL-2 receptors while the receptor density on HTLV-transformed T-cells is 8-to 10-fold higher. The addition of purified natural or recombinant IL-2 increased the level of receptors 2-to 3-fold after 24 hrs on the HVS-transformed cells, but had no effect on receptor density on normal or HTLV-transformed cells.

SIGNIFICANCE

Gaining an understanding in the similarities 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 IL-2 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

1. The role of IL-2 in regulating the initiation of IL-2 receptor expression will be investigated as a model for how IL-2 signals activation of gene expression. An attempt will be made to determine what intracellular signals IL-2 stimulates. These studies will be done in collaboration with Drs Farrar, Rossio and Bonvini and will involve studying metabolic products of lipooxygenase and phoshoinsitol pathways. Immunomodulators which can affect T-cell growth growth such as cyclosporin A, interferon, and steroids will be reevaluated for their effects on receptor expression. The characteristics of up-regulation of IL-2 receptors in long-term culture will be determined.

An attempt will be made to determine if the IL 2 receptor with low affinity has any biological function after IL-2 binding

2. Since the binding of IL-2 to its specific receptor stimulates the entry of T-cells into S phase only after a long lag period, attempts will be made to determine the intra-cellular signals which may be rate-limiting for cell cycle progression. Pattern of gene expression such as cellular proto-onc gene expression after IL-2 stimulation will be determined.

3. It remains to be determined whether or not the IL-2 receptor and its physiology play an important role in maintaining the proliferation of transformed cells. Attempts will be made to produce biological variants of cell lines in which the receptor number can be markedly down-regulated, to determine whether down-regulation significantly reduces the growth rate of these cell lines. Other questions to be approached include: would such cell lines, in in the presence of a molecule down-regulating the IL-2 receptor, be subject to normal regulatory influences? Are such cell lines producing variant growth factors that are able to bind to or in other ways stimulate receptor internalization? Do most T-cell tumor lines release activities which up-regulate IL-2 receptors on normal cells? Can this help explain the difference between normal and neoplastic IL-2 receptor physiology? Experiments utilizing synchronized tumor cell lines and studying cell cycle progression and receptor turnover in the presence and absence of IL-2 may help provide such information.

4. Attempts will be made to further characterize important lymphokines which are essential for the functional differentiation of hematopoietic cells. It has been found in collaboration with Dr. Erich Schlick that some of these cell lines are producing factor(s) which can stimulate the growth of human myeloid cells in suspension culture. Further characterization of such factor(s) will be carried out. In addition, the intracellular pathways utilized by differentiating factors will be ascertained. Particular attention will be paid to differences in differentiation and proliferative pathways.

PUBLICATIONS

Eastment, C. E. and Ruscetti, F.W.: Regulation of erythropoiesis in long-term hamster marrow cultures: Role of bone marrow adherent cells. Blood 65: 736-743, 1985

Brown, R. L., Griffith, R. L., Ruscetti, F. W., and Rabin, H.: Modulation of IL-2 release from a primate lymphoid cell line in serum-free and serum-containing media. Cell. Immunol. 92: 14-21, 1985.

Reeves, W. G., Zamkoff, K. W., Poiesz, B. J., Paolozzi, F. P., Tomar, R. H., Moore, J. L., and Ruscetti, F. W.: T-cell growth required for optimal induction of T-cell factor receptor expression in PHA-stimulated T-cells. J. Biol. Res. Mod. 4: 83-95, 1985.

Eastment, C.E. and Ruscetti, F. W.: The development of factor-producing and factor-dependent clones from hamster long-term marrow suspension cultures. Exp. Hematol., in press.

Smith, K. A. and Ruscetti, F. W.: Effect of glucocorticoids on IL-2 dependent T-cell leukemia cell growth. J. Immunol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09216-05 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Varesio	Visiting Scientist	LMI, NCI
Others:	E. Blasi	Visiting Fellow	LMI, NCI
	M. Clayton	Microbiologist	LMI, NCI

COOPERATING UNITS *(if any)*

National Cancer Institute (R. Crouch).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	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.)*

Understanding the intracellular and extracellular regulatory mechanisms controlling the activation of macrophages is necessary to maximize their anti-tumor functions. Moreover, macrophages provide a distinct advantage for the study of the molecular biology of the cellular response to biological response modifiers (BRMs) since mature macrophages do not proliferate in vitro. Thus the specific cellular response to BRMs can be easily distinguished from side effects due to changes in cell cycle. We have shown that different biochemical pathways are involved in the activation of macrophages by IFNγ or IFNα and IFNβ. However, despite distinct pathways of activation, all three types of IFN induced a major decrease in RNA synthesis in macrophages expressing cytotoxic activity upon in vivo or in vivo activation. In addition, activated macrophages show a specific alteration of rRNA metabolism causing a selective inhibition of accumulation of 28S rRNA. These results show that ribosomal RNA is important in regulating macrophage activation and that BRMs can act at the level of metabolism of ribosomal RNA. Within the context of macrophage activation we have defined for the first time biochemical events which are characteristic for cytotoxic cells. Understanding the molecular basis of the association between altered ribosomal RNA processing and macrophage activation, may lead to the discovery of new regulatory functions of ribosomal RNA and studies to test this possibility are in progress.

PHS 6040 (Rev. 1/84)

GPO 914-918

PROJECT DESCRIPTION

PERSONNEL

Luigi Varesio	Visiting Scientist	IS	LMI	NCI
Elisabetta Blasi	Visiting Fellow	IS	LMI	NCI
Michael A. Clayton	Microbiologist	IS	LMI	NCI

OBJECTIVES

To understand the mechanism by which biological response modifiers control the levels of activation of tumoricidal activity in macrophages.

MAJOR FINDINGS

1. Induction of tumoricidal macrophages by interferon. The ability of recombinant interferon-γ (IFN-γ) to activate mouse macrophages was investigated. The use of recombinant IFN-γ has the advantage of being devoid of contaminating lymphokines. Two preparations of IFN-γ were utilized, one which was not glycosylated and which was highly purified from Escherichia coli and another which was glycosylated and which expressed the transfected COS-7 monkey cells. Both preparations of recombinant IFN-γ activated murine macrophages to kill lymphoma and melanoma tumor targets, suggesting that glycosylation of the protein or the presence of other mammalian proteins is not essential for activation. Significant levels of cytolytic activity were induced by IFN-γ (1 to 10 units/ml). This activity was undiminished by treatment of the IFN-γ preparations with polymixin B at doses which neutralized endotoxin (50 ug/ml). Similarly, IFN-γ, at low concentrations, inhibited migration by macrophages. Based on antiviral activity, IFN-γ was shown to be 100 to 1000 times more potent than was IFN-β as a macrophage-activating agent. Taken together, these results demonstrate that murine IFN-γ is a macrophage-activating factor which is effective at biologically active concentrations.

2. Protein synthesis dependent and independent pathways for the activation of cytotoxic macrophages. The role of protein synthesis during the activation of macrophages (Mø) by lymphokines (LK) was studied. Peritoneal murine macrophages elicited by proteose-peptone (pMø) were activated with LK (supernatants from normal mouse spleen cells pulsed with concanavalin A) and tested for cytotoxicity in an 18 hr assay against ^{111}In-labeled L5178Y lymphoma target cells. Reversible (cycloheximide and puromycin) or poorly reversible (emetine and pactamycin) inhibitors of protein synthesis were added during activation, and their effects on pMø-mediated cytotoxicity and pMø protein synthesis were measured. Minimal concentrations of inhibitors, that reduced the rate of protein synthesis by more than 90% without toxic effects on macrophages, were used. Exposure of pMø to LK for 2 to 18 hr in the presence of reversible inhibitors of protein synthesis did not affect the induction of cytolytic activity, indicating that protein synthesis was not required during the activation period. In contrast, activation of macrophages for 2 hr in the presence of poorly reversible inhibitors of protein synthesis resulted in a considerable reduction of cytolytic activity. The impairment of cytotoxic activity was also evident when pMø were treated with a irreversible drug during the first 2 hr of an 18 hr exposure to LK or when LK-activated macrophages were tested

for 2 hr with the drugs before the addition of the target cells. These results demonstrate that active protein synthesis is not required during the activation of pMø by LK, but that new proteins have to be synthesized to allow the expression of the cytotoxic activity in LK-activated pMø.

Since we have shown that IFN-γ , a common component of crude LK preparation can induce cytotoxic Mø we investigated whether active protein synthesis was needed for activation of macrophages by IFN-γ . Moreover, IFN-α and IFN-β were also studied, since they differ from LK in their mechanism of macrophage activation. We found that inhibition of protein synthesis during treatment of pMø with IFN-α or IFN-β prevented the development of cytotoxic activity. In contrast, IFN-γ was fully capable of inducing cytotoxic pMø in the presence of Cy. Moreover, pMø treated with mixtures of IFN in the presence of Cy were activated for cytotoxicity only by IFN-γ together with IFN-α or IFN-β , but not by IFN-α plus IFN-β . These results indicate that the activation of pMø by IFN-γ is independent of new protein synthesis, whereas the activation of pMø by IFN-α and/or IFN-β requires active protein synthesis, suggesting that the mechanism of induction of cytotoxic pMø by IFN-γ differs from that by the other types of IFN.

3. RNA metabolism during macrophage activation. We have recently observed that crude lymphokine-containing supernatants that induce cytotoxic Mø in vitro, contain a factor that inhibits Mø RNA synthesis (RIF). RIF activity is similar to that of macrophage activating factor (MAF), inasmuch as both share an endotoxin requirement and a similar strain distribution of susceptible Mø. In contrast to the depression of RNA labeling by RIF, normal levels of RNA synthesis were seen under conditions in which lymphokines induced in vitro suppressors Mø but not cytotoxic Mø.

The association between down regulation of RNA synthesis and the expression of cytotoxic activity observed in response to lymphokines in vitro raises the possibility that this metabolic alteration is a general marker of in vitro-induced cytotoxic Mø and may also be shared by those Mø activated in vivo.

To determine the association of the decrease in RNA synthesis with tumoricidal activity of Mø, the properties of in vivo-activated Mø were analyzed. In vivo stimulation with various agents resulted in cytotoxic and/or suppressor Mø. However, only Mø with cytolytic activity showed a down regulation of RNA synthesis, whereas there was no correlation with the expression of suppressor activity.

In attempting to clarify the differences in RNA metabolism between noncytotoxic and tumoricidal macrophages activated in vivo and in vitro, we have studied the relative accumulation of various species of RNA in macrophages with the use of agarose gel electrophoresis. Macrophages activated in vitro to a cytotoxic stage with LK and traces of lipopolysacharide (LPS) have an impaired accumulation of mature ribosomal RNA (rRNA), with a decreased accumulation of 28S rRNA compared to 18S rRNA. In contrast, macrophages primed in vitro with LK free of detectable endotoxins, that exhibit suppressive rather than tumoricidal activity, do not manifest a decreased 28S:18S rRNA ratio. The conclusion that the decreased 28S:18S rRNA ratio was associated with the activation of macrophages to a cytolytic stage was supported by the finding that cytotoxic macrophages activated in vivo by i.p. injection of Propionibacterium acnes (formerly

designated C. parvum) also demonstrated a decreased accumulation of 28S comparable with that observed in in vitro-activated macrophages. Moreover, activated macrophages that lost their cytolytic activity upon prolonged in vitro culture had an augmented accumulation of 28S rRNA. These results provide the first direct evidence that the expression of cytolytic activity is associated with modulation of a specific class of RNA. The unbalanced accumulation of rRNA appears to be a late molecular event in the activation process occurring during the transition from primed to cytotoxic macrophages, because inflammatory and primed macrophages had normal rRNA accumulation.

SIGNIFICANCE

Little is known concerning the intracellular events that regulate the response of macrophages to activating agents. The existence of an intricate program of intracellular reactions is suggested by the rigorous sequence of signals needed to activate cytotoxic macrophages. Information on regulatory events in macrophage activation is a prerequisite to identify drugs that, by mimicking the metabolic changes induced by the macrophage-activating factors, could amplify the macrophage-mediated anti-tumor activities.

Peritoneal murine macrophages are an advantageous cell type to use for the above studies, since their activation is not associated with cell proliferation. This fact is quite important since rRNA metabolism, methylation reactions and gene expression are cell cycle dependent and agents such as IFN can inhibit cell proliferation. Therefore, the macrophage model may help to differentiate the primary metabolic effects induced by IFN from those derived indirectly from the alteration of the proliferative status of the target cells. Thus, studies on the effects of IFN on macrophages may provide novel information not only on the cytolytic activity of macrophages, but also on the biology of IFN. Of interest is the observation that the nonglycosylated E. coli-derived IFN-γ is active and therefore may be of value for therapeutic studies, since it can be easily produced in large amounts.

PROPOSED COURSE

Attempts will be made to understand the role of rRNA metabolism in cytotoxic macrophages. DNA probes, specific for mature ribosomal RNA and nucleolar precursors of ribosomal RNA will be cloned, and utilized to study the pathways of ribosomal RNA maturation.

Drugs affecting ribosomal RNA metabolism will be tested for their effects on macrophage-mediated cytotoxicity in order to establish cause-effect relationships between down-regulation of RNA synthesis and activation.

Transcriptional changes in messenger RNA during macrophage activation will be studied utilizing available probes specific for secretory products of macrophages, and for oncogenes that are actively transcribed in the myeloid lineage such as c-fos, c-myc, and c-myb. However, further analysis of the molecular events associated with the activation of macrophages and in particular studies on gene transcription, are limited by the relatively low numbers of macrophages that can be harvested from the peritoneal cavity of mice. Continuous cell lines of immortalized macrophages would be highly advantageous for these projects.

The reports of immortalization of murine and rat fibroblasts and human monocytes with cloned genes coding for viral proteins and oncogenes indicate that a similar approach might be used to immortalize macrophages or macrophage precursors. Preliminary transfection experiments are in progress in order to establish the conditions for transferring antibiotic-resistance gene macrophages.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 09260-03 LMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Interleukin 1 in Immunity and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. J. Oppenheim	Chief, LMI	LMI, NCI
Others:	K. Matsushima	Visiting Fellow	LMI, NCI
	K. Onozaki	Expert	LMI, NCI

COOPERATING UNITS *(if any)*
BTB, NCI (M. Benzcur, J. Ortaldo, R. Herberman); Laboratory of Molecular Virology and Carcinogenesis, Litton Bionetics, Inc. Basic Research Program, NCI-FCRF (L. Henderson, S. Oroszlan, T. Copeland); Dupont Chemical Co., Glenarden DE (M. Kelly); Genentech, So. San Francisco, CA (B. Aggarwal).

LAB/BRANCH
Laboratory of Molecular Immunoregulation

SECTION
Immunobiology Section

INSTITUTE AND LOCATION
FCRF-NCI, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	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 have shown that normal human B cells as well as EBV transformed B cell lines with accessory cell activities, produce IL 1-like factors. The normal B cell derived IL 1 is biochemically and antigenically similar to monocyte derived IL 1, but the IL 1 produced by the transformed B cell line shows some unique biochemical properties and is not inhibited by rabbit anti human monocyte derived IL 1.

Studies of the rate of production of IL 1 by monocytes reveal it to appear intracellularly within 30' after stimulation, and extracellularly within 60'. The m.w. of the predominant intracellular IL 1 activity is 23 kD with a minor peak at 30 kD, whereas the intracellular IL 1 is largely (>95%) associated with the cytosol fraction and only 1-2% is present in the membrane and particulate fractions of the cells. These data suggest IL 1 is rapidly processed from largely biologically inactive 30 kD precursor to an active 23 kD cell associated and 17 kd soluble forms. We have purified the soluble (pI 6.9, 17 kD) form of human IL 1 and obtained a partial amino acid sequence of the amino terminal end of the molecule. We have radiolabelled this IL 1 with ^{125}I and demonstrated inhibitable binding sites on some human B cell lines.

We have also demonstrated that IL 1 augments the in vitro tumoricidal activities both of human natural killer cells and monocytes and that IL 1 also is directly cytostatic and cytocidal for a limited number of tumor cell lines. These in vitro observations reinforce the hypothesis that IL 1 may be a BRM which promotes host antitumor defense mechanisms.

PHS 6040 (Rev. 1/84)

GPO 914-918

PROJECT DESCRIPTION

PERSONNEL

Joost J. Oppenheim	Chief, LMI	IS LMI NCI
Kouji Matsushima	Visiting Fellow	IS LMI NCI
Kikuo Onozaki	Expert	IS LMI NCI

OBJECTIVES

It is the goal of this project to study the pathophysiological role and properties of interleukin 1 (IL 1), an immunoregulatory cytokine, which is released by a wide variety of cell types, and augments immune and inflammatory reactions. The cell sources, effects and biochemical properties of IL 1 are being identified. The possible means by which IL 1 may potentiate host antitumor reactions are also being studied.

METHODS EMPLOYED

We are investigating the cell sources, characteristics and activities of human IL 1 using a variety of cells including human peripheral blood leukocytes, monomyelocytic and B lymphocyte lines and normal B cells. 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 and/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 ranging from 1-3 days and culture supernatants concentrated and chromatographed to obtain more purified cytokines. Interleukin 1 bioassays are performed by assessing the C3H/HeJ thymocytes and/or primary cultures of human foreskin fibroblasts. An IL 1 dependent murine cell line (D10G4.1) provides a more sensitive bioassay for human IL 1 activity. The antiviral assay using WISH cells was used to detect interferon activity. To differentiate between IL 1 and IL 2, bioassays are performed using IL 2-dependent lymphocyte lines that can react only to IL 2. To study the post-translational processing of IL 1, cells were fractionated into cytosol, particulate, and membrane rich fractions and the IL 1 activity was extracted using CHAPS detergent.

MAJOR FINDINGS

We have continued our investigation of the observation that IL 1-like factors that augment murine thymocyte proliferation are produced by many nucleated cell types. We have documented that 10 of 10 EBV transformed human B cell lines, that exhibit the accessory cell function of activating purified T cells, also spontaneously release low levels of extracellular IL 1-like activities. Extraction of these B cells with CHAPS detergent yielded comparable low levels of cell-associated IL 1 activity. In the case of 3 of the cell lines too little IL 1 was produced to be detectable by the thymocyte comitogenic assay, but was detected by assays using the more sensitive D10G4.1 IL 1 dependent cell line.

In order to further test the hypothesis that IL 1 is required for T cell activation, the capacity of purified normal B cells to produce IL 1 was evaluated. Although resting peripheral blood B cells produce no IL 1, normal B cells stimulated by anti μ antiserum or endotoxin (LPS) did release significant IL 1 activity. The ability of B cell-derived IL 1 to augment thymocyte proliferation was inhibited by rabbit anti-human monocyte-derived IL 1 (kindly provided by Dr. M. Kelly, Dupont Company). The biochemical properties of normal B cell-derived IL 1 resembled those of monocyte IL 1. However, the EBV B cell line-derived IL 1 exhibited a higher MW (25 kd) and was a more acidic (pI 5.5) than monocyte IL 1 (17 kD, pI 6.9) and was only parially inhibited by the anti IL 1 and may therefore represent a distinct IL 1 molecule.

Since the predicted IL 1 sequence is reported to code for a biologically inactive 30 kD precursor form of IL 1 which lacks a typical signal peptide, the means by which it comes out of producer cells requires clarification. We have therefore been fractionating cell pellets prior to extraction of IL 1 activity with CHAPS detergent. Study of the kinetics of IL 1 production reveals that no IL 1 is present in monocytes prior to stimulation, but IL 1 can be detected in monocytes by 30' and in soluble form by 60' after stimulation with LPS and or silica particles. The predominant m.w. of the intracellular IL 1 activity was 23 kD. Most of the intracellular IL 1 (>95%) was located in the cytosol fraction, whereas only 1-2% was associated with the particulate and membrane fractions of the cell. The m.w. of the membrane IL 1 was also 23 kD whereas the extracellular IL 1 was 17 kD. The progression from the 30 kd IL 1 gene product, to 23 kD cell-associated and 17 kD soluble forms of IL 1 suggests sequential posttranslational processing by enzymes may be involved in the release of active fragments of IL 1 from cells.

During the past year, we have confirmed that our purification scheme provided good yields of pure normal human monocyte or monocyte cell line (THP1) derived IL 1. We can consistently obtain single silver stained bands on SDS-PAGE at 18-19 kD under non-reducing conditions. In addition, we can elute biologically active IL 1 from non-SDS PAGE. Finally in collaboration with Drs. L. Henderson, S. Oroszlan, and T. Copeland of Litton Bionetics, NCI-FCRF we obtained a partial amino acid sequence of the amino terminal end of the (pI 7.0) human IL 1. This amino acid sequence was in agreement with part of the predicted amino acid sequence based on the published nucleotide sequence that codes for a 30 kD intracellular IL 1 precursor moiety. Our data identifies the site at which the amino terminal end of the soluble extracellular IL 1 molecule is initiated. Using this purified IL 1 we have been able to study the antitumor activities of IL 1 as well as some of its cell-binding properties.

We have documented that pure human IL 1 can promote the in vitro tumorilytic activity of human peripheral blood large granular lymphocytes (LGL) with NK activity (in collaboration with Drs. M. Benczur, J. Ortaldo and R. Herberman). This effect of IL 1 was obtained even in the presence of polymixin B which presumably blocks low levels of LPS contamination. The NK augmenting effect of IL 1 was observed only in fetal calf serum containing medium therefore unidentified serum cofactors may contribute to this effect of IL 1.

We have also observed that IL 1 promotes the tumoricidal effect of peripheral human monocytes. These results were obtained using >97% pure monocytes pulsed

for 24 hrs with IL 1 and then tested for their ability to release ^{125}IUdR or ^{3}HTdR from tumor target cells such as A375 human melanoma cells in 72 hrs. This effect of IL 1 was blocked by indomethacin (1 μg/ml) and emulated by PGE_2, PGE_1 and dibutyryl cAMP, but not by $PGF_{2\alpha}$ or dbcGMP, suggesting that this effect of IL 1 was mediated by cAMP. Since IL 1 is active only on fresh or one day old monocytes, but unlike LPS or a crude lymphokine mixture does not induce 2-4 day old monocytes to become cytocidal, IL 1 is thought to act as a second signal which retains a prior in vivo cytocidal state rather than to activate monocytes de novo.

Control studies revealed that IL 1 was also directly cytostatic and cytocidal for A375 melanoma cells and some L929 mouse fibroblast lines. In collaboration with Dr. B. Aggarwal (Genentech Corp., San Francisco, CA) IL 1 was demonstrated to be biochemically and antigenically distinct from lymphotoxin (LT) and tumor necrosis factor (TNF). In addition, clones of A375 and L929 cells were identified that were susceptible to the cytocidal effects of LT and TNF but resistant to IL 1. IL 1 is cytocidal for only a limited number of cell types. The mechanism of this cytocidal effect remains to be clarified.

The purified human IL 1 can be successfully labelled with ^{125}I using the Bolton-Hunter method. We have evaluated the binding of ^{125}I - IL 1 to EBV B cell lines. The maximal binding of about 200 IL 1 molecules/cell was observed for one of the cell lines. The binding of ^{125}I - IL 1 could be competitively inhibited by unlabelled IL 1 yielding a dose-dependent straight line curve on a Scatchard plot. This suggests that a relatively low number of receptors for IL 1 may be present on B cell line cells that also produce IL 1.

SIGNIFICANCE

Studies of the post translational processing and structures of IL 1 may reveal means of modifying or regulating its production or effects. The hypothesis that IL 1 may have an autoregulatory role, and/or may be involved in perpetuating the growth of transformed cell lines is supported by the observation that cell types and lines that produce IL 1 can also respond to it and in the case of B cell lines can be shown to express receptors for IL 1. On the other hand our in vitro evidence that IL 1 can promote the tumoricidal activities of NK cells and monocytes and also has direct cytocidal effects for some tumor cell lines suggest IL 1 may potentiate tumor rejection.

PROPOSED COURSE

We are interested in the possible role of IL 1 as a growth promoting factor for neoplastic cells. This can be studied by investigating whether inhibitors of IL 1 such as antibodies and "contra-IL 1" can interfere with the autonomous growth of neoplastic cells. The role of the IL 1 receptor, its properties and regulation on normal and abnormal cell types merits investigation. This will require the use of high affinity specific monoclonal anti IL 1. We will make our purified human IL 1 available to produce such anti IL 1 antibodies. Similarly regulation of production and processing of IL 1 will be more clearly delineated and the enzymes involved in this process should be identified to provide a means of regulating IL 1 production and release. The characteristics of variants of IL 1 should be identified and this will be approached in collabor-

ation with Dr. H. Young by using monocyte-derived IL 1 specific gene probes to ascertain whether non-monocytic cell types that produce IL 1-like factors express the same or distinct IL 1 genes. Finally, in collaboration with Dr. W. Farrar we will investigate the mechanism of cell activation by IL 1 to ascertain whether protein kinase C, ion channels, tyrosine kinase and/or phosphoinositol turnover mediate effects of IL 1 on cells.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09283-01 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Analysis of the Genetic Control and Effects of Interferon-γ Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. A. Young	Expert	LMI, NCI
Others:	J. F. Dray	Microbiologist	LMI, NCI

COOPERATING UNITS *(if any)*

Lymphokines Section, LMI (W. L. Farrar, F. W. Ruscetti); Biochemistry Section, LMI (C. R. Faltynek); Immunobiology Section, LMI (S. K. Durum); Biotechnology Group, Meloy Laboratories, Springfield, VA.

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	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 project is designed to determine 1) if intracellular expression of IFN-γ is sufficient to induce specific biochemical changes in the cell expressing this lymphokine in the absence of specific cell surface receptors and 2) if nucleic acid sequences regulate the expression of interferonγ (IFN-γ). We have found that agents which activate protein kinase C induce mRNA expression of IFN-γ in a mouse cytotoxic T cell line. In order to determine if DNA sequences near the IFN-γ coding region influence this expression, we have cloned the human IFN-γ genomic DNA and introduced this gene into mouse fibroblasts and T cells. Initial results indicate that expression of this gene in fibroblasts is not observed under any conditions of gene induction. Our results with murine T cells indicate that under the appropriate conditions of induction, human IFN-γ gene expression can be obtained.

We have analyzed MHC class I antigen expression, oligo 2'5'A synthetase activity and viral resistance in mouse C127 cells expressing human IFN-γ . Since the mouse cells do not bind IFN-γ, any changes observed in the cells should be a result of the internal expression of this lymphokine. We have observed that mouse cells expressing human IFN-γ do not exhibit enhanced resistance to viral infection but do show increased expression of MHC class 1 antigens and oligo 2'5'A synthetase activity, consequences of IFN-γ treatment may be a result of internal IFN-γ expression. The insertion of the human IFN γ genomic DNA into mouse T cells will permit dissection of the DNA surrounding the coding sequences to determine what regions are involved in the IFN-γ gene induction.

PROJECT DESCRIPTION

PERSONNEL

Howard A. Young	Expert	IS LMI NCI
James F. Dray	Microbiologist	IS LMI NCI

OBJECTIVES

The objectives of this study are two-fold: 1) To determine if DNA sequences 5' or 3' to the human interferon-γ (IFN-γ) genomic DNA are involved in the induction of IFN-γ gene expression by interleukin-2, phorbolmyristic acetate, concanavalin A, calcium ionophore and agents which affect protein kinase C activity. 2) To determine if IFN-γ expression in the absence of specific cell surface binding is sufficient to induce specific biochemical responses within a cell.

METHODS EMPLOYED

The human IFN-γ genomic clone was obtained by screening a λ genomic library with a cDNA IFN-γ clone. The 8.6 Kb Bam H1 fragment was ligated to the pSV2 neo vector and introduced into mouse fibroblasts and T cells by calcium phosphate precipitation. Transfectants were selected by growth in Geneticin (Gibco) and IFN-γ mRNA synthesis was measured by "dot blot" analysis using ^{32}P labeled IFN-γ cDNA. IFN-γ binding was measured utilizing I^{125} labeled recombinant human IFN-γ in the presence or absence of unlabeled IFN-γ . MHC class I antigens were measured by fluorescent cell sorting using an antiserum directed against class I proteins and fluorescene conjugated second antibodies. Viral resistance was measured by infecting target cells with either vesicular stomatitis virus or encephalomyocarditis virus.

MAJOR FINDINGS

We have observed that murine IFN-γ gene expression can be induced in mouse T cells by IL-2, PMA, calcium ionophore and OAG, a synthetic diacylglycerol, which is involved in the protein kinase C pathway. To determine if genomic DNA sequences near the IFN-γ coding regions influence the induction of gene expression by these agents, we transfected the human genomic IFN-γ DNA into mouse fibroblasts and T cells.

We have found that the human IFN-γ genomic DNA is expressed in mouse T cells under conditions which induce the endogenous mouse IFN-γ gene (e.g. PMA, IL 2 treatment) but is not expressed in mouse fibroblasts under any conditions of induction. In the stable transformants we have obtained, the number of copies of the human IFN-γ gene appears to be less than 5 in both the fibroblasts and T cells. With regard to the effects of expression of IFN-γ on the properties of the cell producing the protein, we have utilized a bovine papilloma virus expression system producing human IFN-γ and transfected into mouse C127 cells (plasmid construction and cells provided by the Biotechnology Division of Meloy Laboratories). Since human IFN-γ does not cause any changes in mouse cells when added in vitro, we measured a number of parameters to determine if intracellular expression of this lymphokine affected any parameters in the host

mouse cell. We have found the human IFN-γ exhibits no specific binding to transfected murine cells and that the cells do not exhibit any increased resistance to VSV or EMC challenge nor do they produce any endogenous mouse interferons. In addition, the mouse cells do not exhibit any increased MHC class II antigens on the cell surface. However, preliminary data indicates that when compared to mouse cells that contain the IFN-γ gene but do not express it, cells producing human IFN-γ exhibit higher levels of MHC class I antigens on their cell surface as well as slightly higher levels of intracellular oligo 2'-5'A synthetase. This data suggests that some but not all effects of IFN-γ treatment can be exhibited by cells which produce this molecule but do not have specific cell surface receptors.

SIGNIFICANCE

This project will permit a detailed analysis of the genetic regulation of IFN-γ production. Since the expression of this protein is limited to T cells and large granular lymphocytes, these studies will help to elucidate the specificity of gene expression in these cells. This work can lead to the development of chimeric genes, which when introduced into an organism, will only be expressed in T cells or LGL's, thus providing a tissue specific regulation of gene expression. Based on these studies, it may be possible to design unique compounds which will be able to specifically enhance IFN-γ gene expression in vivo, thus augmenting the body's own immunoregulatory system. Furthermore, by understanding the consequences of IFN-γ gene expression, it may also be possible to design altered IFN-γ genes which induce highly specific biochemical changes in the cell. These studies will also demonstrate that lymphokines may not require the presence of a specific cell surface receptor to be active and if the lymphokine can be introduced into a cell by novel mechanisms (e.g. liposomes, antibody conjugates) defined changes (e.g. increased MHC class I antigen expression) in the appropriate target cells may occur without affecting other cells in the body.

PROPOSED COURSE

Upon defining the agents which induce human IFN-γ mRNA synthesis in the mouse T cells, we will biochemically dissect the DNA 5' and/or 3' to the coding region to determine what sequences are involved in IFN-γ gene induction. We will link various non-coding DNA regions of the IFN-γ to a CAT gene and determine, by transient expression experiments, the response of these constructs to the presence of various inducers. Upon defining specific regions of the DNA, we will sequence the DNA to determine its nucleotide characteristics. We will also link the appropriate sequences to a foreign gene, (e.g. human β globin) and introduce this construct into mice to determine if tissue specific regulation of this foreign gene occurs.

In conjunction with Dr. William Farrar, we will be attempting in vitro transcription experiments with the cloned IFN-γ genomic DNA to determine if there are factors in the T cell, but not in fibroblasts, which can specifically enhance the transcription of the IFN-γ gene.

Finally, in conjunction with scientists at Meloy Laboratories, we will construct a human IFN-γ cDNA which lacks the leader peptide sequence. This construct

would result in a IFN-γ molecule which is not excreted from the cell and will enable us to precisely determine if accumulation of intracellular IFN-γ results in the same biochemical changes in mouse cells as that observed with excreted IFN-γ.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09284-01 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Molecular Cloning of Rat Cytolysin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. A. Young	Expert	LMI, NCI
Others:	J. F. Dray	Microbiologist	LMI, NCI
	D. E. Mizel	Chemist	LMI, NCI

COOPERATING UNITS *(if any)*

Natural Immunity Section, Biological Therapeutics Branch, NCI (C. Reynolds); NCI (P. Henkart).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

This project is devoted to the molecular cloning of the gene or genes responsible for the cytotoxicity of granules purified from rat NK-like tumor cells. Using a cDNA expression library and antiserum to the purified 60 Kd cytolytic component of the granules (termed "cytolysin"), we have obtained a cDNA clone which strongly reacts with the 60 Kd antiserum. This cDNA clone also reacts weakly with whole granule antiserum. Preliminary results indicate that the immunoreactive cDNA clone is approximately 700 nucleotides in length.

GPO 814-916

PROJECT DESCRIPTION

PERSONNEL

Howard A. Young	Expert	IS LMI NCI
James F. Dray	Microbiologist	IS LMI NCI
Diane E. Mizel	Chemist	IS LMI NCI

OBJECTIVES

This project has the objective of cloning, by molecular techniques, the gene coding for the cytolytic protein found in granules purified from a rat NK tumor cell line.

METHODS EMPLOYED

A cDNA library has been constructed in the expression vector lambda gtll, using poly A+ RNA purified from the rat NK tumor cell lines. This library was screened with antiserum prepared against a purified 60 Kd cytolytic protein found in the granules present in the NK tumor cells. Immunoreactive clones were screened by utilizing a horseradish peroxidase conjugated goat anti-rabbit second antibody. A strongly immunoreactive clone was plaque purified and the DNA extracted. The cDNA insert was purified by agarose gel electrophoresis and ligated to the plasmid vector SP64.

MAJOR FINDINGS

We have isolated a cDNA clone which is strongly immunoreactive with antiserum directed against a purified cytotoxic 60 Kd granule protein. This clone reacts with a 1:500 dilution of antiserum in 2-3 hours and does not react with normal rabbit serum or with horseradish conjugated second antibody. This clone also reacts weakly with antiserum prepared against whole granules and does not cross react with two cDNA clones isolated utilizing the whole granule antiserum. Purification of the recombinant viral DNA and subsequent digestion with EcoRI indicates that the cDNA clone is approximately 700 nucleotides in length and thus probably represents the C terminal region of the 60 Kd protein.

SIGNIFICANCE

The methods by which NK cells kill target cells is currently incompletely understood. One method clearly involves cell to cell contact and this cytotoxic effect can be mimicked by utilizing granules isolated from the NK cells. By cloning the protein responsible for this cytotoxicity, we can elucidate one mechanism of NK killing. In addition, we will be able to use the cloned DNA as a probe for studying the transcriptional regulation of the relevant gene and for analyzing the relationship between the genes involved in cytotoxic T cell killing and NK killing. Furthermore, we should be able to genetically alter specific portions of the molecule in order to determine how changes in protein structure can affect activity and specificity.

PROPOSED COURSE

We will utilize the recombinant protein to adsorb the 60 Kd antiserum in order to determine if this immunoreactive portion of the protein can remove the antibodies which block the cytotoxicity of both the purified 60 Kd protein and the whole granules.

We will utilize the partial cDNA clone to determine the genomic organization by Southern transfer experiments and mRNA size by Northern transfer hybridizations. Upon determining the size of the mRNA, we will rescreen the cDNA library in order to obtain a full length cDNA. If necessary, we will construct an additional cDNA library to obtain the desired full length clone. Upon obtaining the full length cDNA, we will construct expression vectors for both mammalian and bacterial systems in order to determine the relative activities of the recombinant products. Finally, we will also screen both rat and human genomic libraries in order to obtain the appropriate genomic DNA clones. This will permit a detailed genetic analysis of the genomic structure of the cytolysin gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09285-01 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies on the Regulation of IL-1 Gene Expression and the Molecular Events Occurring Upon IL-1 Treatment of Response Thymocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. J. Kovacs	Guest Researcher	LMI, NCI
Others:	H. A. Young	Expert	LMI, NCI

COOPERATING UNITS *(if any)*

Immunobiology Section, NCI (J. J. Oppenheim); Upjohn Company, Kalamazoo, MI (D. Carter).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

It is the aim of this project to investigate two aspects of IL-1 physiology; 1) whether treatment of cells with agents which alter DNA methylation results in the activation of IL-1 gene expression and 2) what molecular events occur upon IL-1 treatment of an IL-1 sensitive mouse thymocyte cell line. We have found that production of IL-1 by a human monocytic tumor cell line, THP-1, can be increased 50% by treatment of the cells with 5-azacytidine and that another human monocytic tumor cell line, U937, can be induced to produce IL-1 following LPS stimulation after a similar treatment with 5-azacytidine. Treatment of either cell line with another agent which affects DNA structure, bromodeoxyuridine, did not result in activation of IL-1 production.

We have also found that IL-1 treatment of a sensitive mouse thymocyte cell line, D10G4.1, results in increased expression of the c-myc proto-oncogene as well as both IL-2 mRNA and IL-2 receptor mRNA. Additional studies are currently underway to determine if the expression of other genes such as IFN-γ and the fos oncogene, are also altered by IL-1 treatment of these target cells.

PROJECT DESCRIPTION

PERSONNEL

Elizabeth J. Kovacs	Guest Researcher	IS LMI NCI
Howard A. Young	Expert	IS LMI NCI

OBJECTIVES

This project has two main objectives; 1) to determine if IL-1 production by human tumor cells can be influenced by agents which affect DNA structure and 2) to analyze the molecular events which occur upon IL-1 treatment of an IL-1 responsive mouse thymocyte cell line.

METHODS EMPLOYED

We have utilized 5-azacytidine and bromodeoxyuridine to treat two human tumor cell lines. After 48 hours drug treatment and 72 hours of additional cell growth, supernatant IL-1 activity was measured by ^{3}H-thymidine incorporation of mouse thymocytes or the mouse D10G4.1 cell line following treatment with LPS, LPS in combination with silica and calcium ionophore or medium alone.

For analysis of the molecular response of cells to IL-1, the mouse thymocyte cell line, D10G4.1, was treated for various periods of time with IL-1 and RNA extracted according to the procedure of White and Bancroft. The cell lystates were applied to nitrocellulose filters and the filters were hybridized with the appropriate ^{32}P-labeled DNA probes.

MAJOR FINDINGS

We have observed that 5-azacytidine treatment of the human tumor cell line THP-1 results in a 50% increase in the level of supernatant IL-1 activity present after treatment of cells with LPS, calcium ionophore and silica. This increased IL-1 production is transient and decreases to levels observed prior to treatment after 6-8 weeks in culture. We have also been able to induce IL-1 activity from the human tumor cell line U937 following treatment with 5-azacytidine and subsequent induction with LPS. In the absence of azacytidine treatment, no intracellular or extracellular IL-1 could be detected after induction by LPS or any other agents. The ability to induce IL-1 activity in this cell line was also lost after 6-8 weeks in culture presumably based upon hypomethylation of the DNA.

Analysis of the events which occur following IL-1 tratment of the mouse thymocyte cell line D10G4.1, has indicated that increases in myc mRNA are observed several hours following treatment. Additional increases in both IL-2 and IL-2 receptor mRNA's occur a few hours following the increases in myc RNA. Only slight changes have been observed in fos or IFN-γ mRNA levels however. In order to detect these changes, 48 hours of serum starvation was required to place the cells in a sufficiently quiescent state prior to IL-1 treatment. Similar studies performed with fibroblasts did not result in any significant alterations in specific mRNA populations following IL-1 treatment, thus indicating specificity in the target cell for IL-1 effects.

SIGNIFICANCE

These studies represent the first clear demonstration that production of IL-1 activity can be up regulated by methylation of genomic DNA in a manner which permits induction but not constitutive synthesis of the gene product. In addition, we have been able to develop a model system which dissociates the effect of IL-1 on a cell population from the proliferation of the same population. Our results are the first demonstration that expression of specific oncogene mRNA may occur early in the cell cycle, prior to the onset of proliferation and that the function of the oncogene product may be involved in "priming" the cell for entry into the cell cycle.

PROPOSED COURSE

In order to analyze whether or not azacytidine treatment of cells results in hypomethylation of the IL-1 gene, we will require an IL-1 cDNA probe. We are currently screening a cDNA library prepared from the THP-1 cell line utilizing an oligonucleotide probe provided by Dr. D. Carter of the Upjohn Company. In addition, we will be obtaining a partial IL-1 cDNA probe from Dr. P. Auron, MIT, Boston, MA. This cDNA will permit an analysis of the methylation status of both the THP and U937 cells prior to and following azacytidine treatment. In addition, we should be able to verify, by RNA analysis, the presence of increased IL-1 mRNA following drug treatment.

With regards to the effect of IL-1 on the D10G4.1 cell line, we will expand our analysis to include other oncogenes whose expression apears to be correlated with proliferation (e.g., ras, raf) to determine if IL-1 can selectively stimulate the expression of these mRNA populations. In addition, we will assay the protein products of the genes under study to prove that increased mRNA levels can be correlated with increased protein levels. We will also establish whether IL 1 also induces oncogene expression in normal lymphocytes. Finally, we will attempt to introduce specific oncogenes (e.g., myc) into the D10G4.1 cell line in order to analyze whether expression of this oncogene can abrogate the requirement of IL-1 for cell proliferation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09286-01 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Receptors for Human Interferon-α and Interferon-γ on Peripheral Blood Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. J. Oppenheim	Chief, LMI	LMI, NCI
Others:	G. L. Princler	Chemist	LMI, NCI

COOPERATING UNITS *(if any)* PRI, NCI-FCRF (C. Faltynek); Natural Immunity Section, BTB, NCI (J. Ortaldo); Clinical Immunology Services, PRI (A. E. Maluish); Lymphokine Section, LMI (F. W. Ruscetti); Clinical Investigations Section, BTB, NCI (K. A. Foon).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.0	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 have initiated studies on the expression and regulation of receptors for human interferons (IFNs) on normal and malignant peripheral blood leukocytes. For these studies we radioiodinated recombinant IFN-α A and IFN-γ by techniques which preserved the biological activity of the molecules. The binding of the iodinated IFNs to enriched and highly purified populations of peripheral blood cells from normal donors and from patients with lymphoproliferative diseases has been examined.

We have observed that highly purified resting T lymphocytes and large granular lymphocytes from normal donors constitutively express receptors for IFN-α and IFN-γ and we have measured the number of IFN receptors per cell and the affinities of the IFN-receptor interactions. The level of IFN receptor expression has varied with the activation state of the normal T lymphocytes. Proliferating normal lymphocytes express 3 to 5 fold more receptors for IFNα than resting lymphocytes. In contrast the number of receptors for IFNγ was transiently decreased by 10 fold by 18 hrs after activation of lymphocytes by lectins.

We have observed that the cells from IFN-α A responsive hairy cell leukemia patients expressed more IFN-α A receptors than the cells from the nonresponsive chronic lymphocytic leukemia patients. However, due to differences in cell size, the receptor density on these two types of leukemic cells was similar. Taken together, our data suggest that although it may not be sufficient as a predictive parameter for clinical responsiveness to IFN, the absolute number of receptors per cell may be an important criterion in the antiproliferative response to IFN.

PROJECT DESCRIPTION

PERSONNEL

Joost J. Oppenheim	Chief, LMI	BS LMI NCI
Gerald L. Princler	Chemist	BS LMI NCI

OBJECTIVES

To characterize the expression and regulation of receptors for IFN-α and IFN-γ on peripheral blood cells from normal donors and from patients with lymphoproliferative diseases. To determine the role of IFN receptor expression in clinical responsiveness to IFN therapy. To elucidate the molecular events occurring immediately after the binding of IFN to its receptor which transduce the signal from the cell surface and result in the pleiotypic activities of IFN.

METHODS EMPLOYED

Radiolabeling of IFNs with ^{125}I followed by gel filtration chromatography. Analysis of biological activity of unlabeled and radiolabeled IFNs with an antiviral assay. Ficoll-Hypaque separation of peripheral blood mononuclear cells (PBMC). Further separation of T lymphocytes and large granular lymphocytes (LGL) by nylon wool depletion of contaminant adherent cells followed by centifugation through Percoll gradients. In vitro activation of lymphocytes with lectins, lymphokines and other cell activators. Daudi, HeLa and WISH cells are grown in tissue culture. Measurement of cell volumes with a celloscope. Binding assays to cell populations using radiolabeled and unlabeled IFNs in competitive displacement experiments followed by analysis of the data according to the method of Scatchard.

MAJOR FINDINGS

Highly purified T lymphocytes (>95%) and enriched populations of LGL (>80%) from normal human donors constitutively expressed receptors for IFN-α and IFN-γ. Resting T lymphocytes had approximately 200 IFN-α and 500 IFN-γ high affinity receptors per cell, whereas the LGL had approximately twice this number of receptors. Interaction of IFN-α and IFN-γ with receptors on fresh normal lymphocytes were of high affinity, similar to the affinities on established tissue culture cell lines. However, in addition to high affinity binding, there was also a lower affinity interaction of IFN-α A with T lymphocytes with 1000 receptors/cell that has not usually been observed on established cell lines. The significance of this lower affinity binding on the fresh lymphocytes is not presently known.

Although freshly isolated T lymphocytes constitutively express receptors for IFN-α and IFN-γ, the level of expression varied with the state of activation and proliferation of the cells. T lymphocytes proliferating in response to interleukin-2 expressed approximately four times more IFN-α receptors per cell than the resting cells. However, since the proliferating cells were considerably larger, the cell surface IFN-α receptor density was similar on resting and proliferating T cells. Receptors for IFN-γ were

regulated differently. Within 18 hours following a lectin stimulus, the ability of T cells to bind IFN-γ decreased 10-fold but returned to a level equal to or greater than on resting cells after 3 days in culture.

In ongoing clinical trials of IFN-αA in the BRMP, patients with hairy cell leukemia (HCL) have responded well to IFN-αA therapy whereas chronic lymphocytic leukemia (CLL) patients have been poor responders. We compared the binding of radiolabeled IFN-α to baseline samples of PBMC from the leukemia patients with clinical responsiveness to IFN-αA. The PBMC from the responding HCL patients bound approxiately twice as much IFN as PBMC from CLL patients. This difference was due to more high affinity receptors per cell with no difference in the affinity of the IFN-receptor interaction. However, since PBMC from HCL patients were larger than PBMC from CLL patients, the cell surface receptor density was similar. PBMC from the single nonresponding HCL patient in this study bound less IFN than the cells from the 8 responding patients. The rapidity of response did not correlate with the level of binding of IFN to the PBMC. Our results suggest that the absolute number of IFN receptors per cell may be an important parameter in the response to IFN-αA therapy. However, more unresponsive patients with hairy cell leukemia must be studied to ascertain whether IFN receptor number has predictive value.

Using cell lines derived in Dr. Ruscetti's laboratory from hairy cell leukemia patients, we have shown that the cells in culture retained the same level of IFN-αA binding as freshly isolated cells. Moreover, these cells were growth inhibited by high doses of IFN-αA in vitro.

SIGNIFICANCE

IFNs consist of a family of proteins with antiproliferative, antiviral and immunomodulatory activities. In order to better understand the role for the IFNs in the immune system and as therapeutic agents, knowledge of the molecular events in the action of IFN is essential. Because these events begin with the binding of IFN to specific cell surface receptors, a basic understanding of the nature of IFN-receptor interactions and of the expression and regulation of IFN receptors is of significance. Identification of agents that modulate expression of receptors for IFN may provide additional means of modifying biological responses. Such information is particularly relevant to the clinical trials of IFN ongoing in the BRMP and may provide a means of predicting therapeutic efficacy of IFN.

IFN-αA has been shown to be highly effective in the treatment of certain lymphoproliferative malignancies, especially hairy cell leukemia. Our studies on patients with lymphoproliferative diseases indicate that the absolute number of IFN-αA receptors per malignant cell may provide an important parameter in the response to rIFN-αA therapy. Further studies to elucidate the molecular mechanisms for the antiproliferative action of IFN are required to provide more rational courses for IFN therapy.

PROPOSED COURSE

We plan to continue the studies on the regulation of IFN-γ receptor expression by identifying the agents and molecular mechanisms which cause the early loss

and subsequent return of IFN-γ receptors on T cells following activating stimulus. We will also examine the expression and regulation of IFN receptors on other types of peripheral blood cells and other tissues. In particular, because of the effects of IFN-γ on Ia/DR expression on macrophages studies on IFN-γ receptors on monocytes and macrophages will be undertaken.

Since very little is presently known about the receptor for human IFN-γ, we plan to study its molecular characteristics. Some of these studies can be done on crude membrane preparations. To enable more extensive studies both on the nature of the receptor and on its regulation, we plan to partially purify the receptor by affinity chromatography followed by production of antisera to the receptor. Both polyclonal and monoclonal antisera to the IFN-γ receptor will be of considerable utility in our studies on the role of IFN-γ and its receptor in the immune system.

We plan to continue the study on IFN-αA receptors on hairy leukemic cells. We are particularly interested in studying HCL patients who do not respond to IFN-αA therapy to determine whether nonresponsiveness correlates with low IFN binding in other patients. In addition, we will evaluate the IFN receptor expression on cloned hairy cell leukemic lines that are unresponsive to IFN for comparison with responsive lines.

The molecular events which transduce the signal from cell surface IFN receptors to the rest of the cell in order to establish the pleiotypic biological responses to IFN are not yet understood. We plan to examine whether IFN affects calcium fluxes or phosphatidylinositol metabolism and whether these events may function as signalling mechanisms for IFN, as they do for many other polypeptide cellular effectors. Many of the studies on signal transduction will utilize Daudi or HeLA cells in culture since the IFN receptors and responses to IFN are well characterized in these cells. As mechanisms of signal transduction are elucidated in cells in tissue culture, we will also study these events in peripheral blood cells from normal donors and from patients with lymphoproliferative diseases to determine whether clinical responsiveness correlates with the degree of signal transduction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 09287-01 LMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interleukin 1: Mechanisms of Production/Anti-tumor Effects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. K. Durum	Sr. Staff Fellow	LMI, NCI
Others:	B. Tartakovsky	Visiting Fellow	LMI, NCI
	L. Takacs	Guest Researcher	LMI, NCI

COOPERATING UNITS *(if any)*

Biological Therapeutics Branch, NCI (E.Gorelik).

LAB/BRANCH
Laboratory of Molecular Immunoregulation

SECTION
Immunobiology Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

Interleukin 1 (IL 1) is an important mediator of immune and inflammatory processes. We are examining the mechanisms of production of IL 1 during immune responses. Macrophages (Mø) release potent levels of IL 1 in response to a wide variety of immune and inflammatory stimuli. However, in the presence of anti-Ia antibodies, IL 1 is still synthesized by murine Mø, but fails to escape from the cell. Biochemical studies suggest that Ia participates in the processing of the IL 1 precursor accompanying the release process. Hence, a new concept is developing - Ia control of processing and export of IL 1.

Another new concept has recently evolved concerning IL 1 production by murine B cells. Unlike Mø, which produce IL 1 in response to a wide variety of stimuli, B cells required contact with T cells to produce IL 1. Also unlike Mø, which release IL 1 into the surrounding milieu, B cell IL 1 remained cell associated. These observations provide new insights into the complex bi-directional mechanisms of T-B interactions.

We are embarking on studies aimed at exploiting the ability of IL 1 to enhance immune responses against tumors. IL 1 powerfully promotes weak immune responses and many tumors suceed in vivo because of an ineffective immune response. It is therefore compelling to explore applications of IL 1 as an anti-tumor agent.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION

PERSONNEL

Scott K. Durum	Senior Staff Fellow	IS LMI NCI
Boris Tartakovsky	Visiting Fellow	IS LMI NCI
Laszlo Takacs	Guest Researcher	IS LMI NCI

OBJECTIVES

The mechanism of IL 1 production is being studied in several ways. 1) Ia molecules may control the release of IL 1 from macrophages. We are examining the mechanism of this control process. Does Ia regulate IL 1 production at the level of transcription or translation, or does Ia affect the way IL 1 peptides are processed, transported or secreted? 2) A second aspect of IL 1 production concerns IL 1 synthesis by B cells. What stimuli elicit its synthesis and what is the nature of the IL 1 produced? 3) We are initiating studies to assess the usefulness of IL 1 as an anti-tumor agent by promoting anti-tumor immunity.

METHODS EMPLOYED

To study mechanisms of IL 1 production, cells are exposed to a stimulus (e.g., murine Mø are stimulated with LPS or silica whereas B cells are stimulated with an alloreactive T cell line. Anti-Ia monoclonal antibodies are included during stimulation to define the role of Ia on IL 1 release. The IL 1 producing cells are analyzed for the ability to synthesize IL 1 (cells are disrupted and the intracellular activity is analyzed) and to release IL 1 (the supernatants of intact cells are analyzed). Molecular characterization of the various preparations of IL 1 is conducted by size analysis by liquid chromatography using Sephacryl S200 or by HPLC using TSK 3000 columns. To quantitate IL 1 all current methods rely on bioassay - we use the D10G4.1 murine T cell line for which IL 1 acts as a comitogen (in conjunction with ConA).

For measuring effects of IL 1 on anti-tumor immunity, we are using two different murine tumors chosen for their different mechanisms of evading immune rejection: B16 melanoma demonstrates a low level of immunogenicity whereas a variant of the 1591 fibrosarcoma induces suppressor T cells.

MAJOR FINDINGS

I. Ia molecules control IL 1 release. We previously observed that IL 1 release from murine Mø is controlled by Ia molecules, based on the following: 1) Helper T cells stimulate IL 1-release from Mø. This process is H-2-restricted between T cells and Mø and is blocked by monoclonal anti-Ia. 2) In the presence of concanavalin A (ConA) the H-2 restriction between T cells and Mø is overcome, but anti Ia still blocks IL 1-release. 3) LPS or silica are T cell-independent stimuli of IL 1 production by Mø anti-Ia also inhibits IL 1 release stimulated by these agents. More recently, we observed that anti-Ia treatment did not inhibit synthesis of IL 1, but rather inhibited its release from the Mø. This "trapped" IL 1 exists as a higher molecular weight intracellular form and is probably a precursor to the low molecular weight (17 kd) species found outside

the Mø plasma membrane. Thus, Ia molecules apparently control the release and accompanying proteolytic cleavage of IL 1.

II: B cell IL 1. In the course of studying T-B interactions, we observed that B cells could present antigens to the D10G4.1 line. This helper T cell line is widely used as an IL 1 assay, prompting us to examine B cells for IL 1 production. This proved to be an elusive property. Whereas Mø could be stimulated to produce IL 1 in response to a wide variety of soluble and particulate agents, murine B cells failed to produce IL 1 in response to most well-known B cell stimuli (LPS anti Ig, T cell lymphokines or combinations of these agents). However, we finally determined that B cells, during the course of their interaction with intact T cells, are induced to produce an IL 1-like activity; this IL 1 was not released from B cells, unlike Mø, which release 25-90% (depending on the stimulus) of the IL 1 they produce. The B cell IL 1 remained associated with cells and could only be detected in extracts of B cells.

SIGNIFICANCE

IL 1 is an important mediator of inflammation, and it is also required of several stages of the immune response. Hence, understanding the mechanism of IL 1 production and release is important in its own right and may contribute to means of controlling inflammatory and immune processes. Elucidating the role of Ia molecules in IL 1 release will help establish a quite novel function for products of the histocompatibility genes and explain the part these molecules play in interactions among cells of the immune system. B cell IL 1, its production and utilization during interaction with T cells, is fundamental to unravelling the complex bidirectional signals exchanged between T and B cells.

PROPOSED COURSE

1) Ia regulation of IL 1 release will be studied further at the biochemical level. Since Ia appears to be involved in cleavage and release of IL 1 from Mø, we will search for macromolecular (especially those with proteolytic activity) associated with Ia. 2) B cell IL 1 will be analyzed biochemically and compared to Mø IL 1. Since the IL 1 activity is not released from the cell, we will determine its subcellular location and biological role. 3) Anti-tumor effects of IL 1 will become one major focus for the laboratory. Currently we are at the initial stages of establishing appropriate experimental systems for testing in vivo effects of IL 1 on tumor rejection. The studies will be designed in consideration of two important observations that encourage the use of IL 1. 1) IL 1 is necessary for most immune responses. Thus, we have chosen a tumor known to be of low immunogenicity (a B16 murine melanoma line); in collaboration with Dr. E. Gorelik we will examine the effect of IL 1 (recombinant DNA produced, gift of Hoffman-La Roche) ontumor growth in vivo. 2) IL 1 alters the balance between helper and suppressor T cells. We observed that immune responses usually dominated by suppressor cells can be reversed by IL 1. Thus, we have chosen tumors known to induce suppressor T cells (two clones of the 1591 murine fibrosarcoma provided by Dr. Patrick Flood, Yale University). Experiments are in progress using both types of tumors, administering IL 1 using various sites and dosage regimes, and measuring tumor growth and mortality.

PUBLICATIONS

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