# ANNUAL REPORT
# OF
# PROGRAM ACTIVITIES
# NATIONAL CANCER INSTITUTE
# Division of Cancer Treatment
# Volume II

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VOLUME II

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE CANCER THERAPY EVALUATION PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1981 - September 30, 1982

The Cancer Therapy Evaluation Program (CTEP) is responsible for the extramural clinical trials supported by DCT. Its scope of activity includes clinical evaluation of new anticancer agents and the coordination of extramural clinical research programs, principally involving the clinical cooperative groups. The current report will update these areas as well as focus on organizational changes, and activities of the Office of the Associate Director.

## I. Organizational Changes

Major organizational changes occurred within CTEP during the last year. The Radiotherapy Development Branch has been moved into the newly formed Radiation Research Program. Responsibility for coordination of extramural clinical trials in radiation remains within CTEP, with close liaison with the Radiation Research Program. The Biologics Evaluation Branch has now become the Biologics Evaluation Section within the Investigational Drug Branch. Phase I trials of biologics will be administered by the Biological Response Modifiers Program, whereas the CTEP will be responsible for their Phase II and III clinical trials.

During the past year, Dr. John Macdonald resigned as Associate Director. Dr. Daniel Kisner served as Acting Associate Director. Dr. William DeWys was appointed permanent Chief of the Clinical Investigations Branch. Dr. John Killen was appointed Head, Medicine Section within the Clinical Investigations Branch (CIB). Dr. Mario Eisenberger and Dr. Elizabeth Read were recruited to serve in the Medicine Section of that Branch. Within the Investigational Drug Branch (IDB), Mr. Clarence Fortner was recruited to head the newly created Drug Management Section and Drs. Brenda Foster and Peter O'Dwyer will serve as Drug Monitors in the Drug Evaluation and Reporting Section of IDB.

## II. Activities of the Office of the Associate Director

The Office of the Associate Director (OAD) is responsible for integrating the efforts of both program branches. This is primarily achieved through participation of the Associate Director (AD) in the branches' activities and through twice-monthly staff meetings and weekly branch chief meetings.

Major responsibilities and achievements of the OAD have been: A) program supervision and budgetary allocation, B) review and development of therapeutic strategies, C) reorganization of clinical trials, and D) coordination of clinical activities in all international agreements.

The process of protocol review has been streamlined by centralizing all protocol correspondence and administration of the review procedures into a central office within the OAD.

The Clinical Oncology Review Committee (CORC) performs annual reviews of all incrementally funded clinical research and resource contracts. During FY '81 there were nine CORC meetings for review of 36 contracts and 45 proposals for 14 contract projects. Dr. DeWys is Chairman; Mrs. Carpenter is Executive Secretary.

Personnel in the OAD are as follows:

Daniel L. Kisner, M.D. - Acting Associate Director, CTEP
Vacant - Deputy Associate Director, CTEP
Mary Jane Mathews - Secretary to AD, CTEP
Judith Uyehara - Secretary to Deputy AD, CTEP
William Soper - Technical Information Specialist
Elise Mackie - Program Analyst
Ann Carpenter - Biologist
Tanya Prather - Protocol Clerk
Carolyn Barker - Contracts Technical Assistant
Eileen Steinman - Secretary

A. Program Supervision and Budgetary Allocation

A series of orientation sessions is instituted every July for the staff. In addition, the staff is encouraged to attend similar sessions in the Clinical Oncology Program. These sessions constitute a period of training for new staff of the CTEP. The Office of the AD is also responsible for supervising the management of all program areas in concert with the branch chiefs. Budgetary allocations to each area within the program are worked out by direct coordination with program directors, project officers in each of the branches, and the Administrative Officer. These are subsequently discussed with the Director, DCT and decisions are transmitted back to the entire staff of the CTEP.

B. Development and Progress of Specific Therapeutic Strategies and Program Highlights

1) Phase II trials of interferon were initiated in conjunction with the Biological Response Modifiers Program. Phase II studies in breast and ovarian carcinoma were initiated in the cooperative groups this year.

2) An RFA was issued for the purpose of studying patients with Kaposi's sarcoma. This will include a component of clinical research directed at elucidating not only etiology and immunologic disorders, but also new treatment approaches.

3) NCI's distribution of THC under its Group C program continues. During Fiscal 1981-1982 more than 200,000 capsules have been distributed to over 600 pharmacies and 2,000 physicians. It is estimated that more than 12,000 patients have benefitted from this program.

4) A program directed at stimulating research in surgical oncology began during this fiscal year. Specifically, five institutions were granted planning funds for the purpose of developing grant proposals for research in surgical oncology.

5) The CTEP will expand its efforts in monitoring of clinical trials. Following approval by the DCT Board of Scientific Counselors, the CTEP has organized a program of periodic site visits to each institution performing clinical trials on investigational drugs for the purpose of verifying the accuracy of the clinical data. This project will involve site visits conducted by teams of peers supplemented by NCI staff.

6) Progress was made toward the implementation of an information system for CTEP with the award of a contract towards the end of the fiscal year. This system is expected to be operational in FY '83. It will provide the staff of CTEP with a computerized information resource with which to develop forward planning in specific therapeutic areas.

7) New drugs introduced into Phase I clinical trials during the current year included tricyclic nucleotide, homoharringtonine, echinomycin, N-methylformamide, and dihydroazacytidine. Two drugs came on the market for general availability for treatment of cancer patients. FDA approved New Drug Applications (NDA's) for streptozotocin and ifosphamide, thus making them generally available for cancer patients.

C. Organization of Clinical Trials

During the previous year, CTEP had proposed that all research clinical trials be transferred to a cooperative agreement funding mechanism. Cooperative agreements are similar to grants in regard to review processes and define carefully the relationship between the cooperative agreement holder and the Government. During the current year all existing clinical cooperative groups were converted to cooperative agreements. Furthermore, most of the clinical trials contracts supported by contracts were also converted to cooperative agreements. It is expected that this process will be completed in the early part of the upcoming year. Only Phase I studies will be supported by contract in the future.

The process of development of regional groups came to fruition during this year. The Piedmont Oncology Association and the Mid-Atlantic Oncology Group became the first regional groups funded as a result of this new initiative.

During this year more than 25,000 patients were entered onto research clinical trials. Added to the 46,000 patients in followup from previous years, the clinical trials resources of the DCT followed more than 71,000 patients during the current fiscal year.

D. Representation in International Activities

CTEP is responsible for providing clinical input for treatment research activities of the DCT involving international agreements. NCI - PAHO (Pan American Health Organization) treatment research programs, which were initiated in 1978, continued in the current year. The program is reaching is reaching a more mature state with a streamlining and reduction to approximately 20 active protocols.

EORTC (European Organization for Research on Treatment of Cancer) protocol activities were also similarly reviewed. A representative of CTEP serves on its protocol review committee.

Participation in the U.S - French, U.S. - U.S.S.R., U.S. - Italy, U.S. - Germany, and U.S. - Japan agreements also took place during 1981-82, with major emphasis on new drug testing.

III. Publications


1. Ardalan, B., Glazer, R.I., Kensler, T.W., Jayaram, H.N., Van Pham, T., Macdonald, J.S., and Cooney, D.A.: Synergistic effect of 5-fluorouracil and N-(phosphonacetyl)-L-aspartate with ribonucleic acid synthesis in a human mammary carcinoma. Biochem. Pharmacol. 30:2045-2049, 1981.

2. Ardalan, B., Macdonald, J.S., Cooney, D., Lippman, M., and Schein, P.S.: The potential for clinical application of in vitro assays predicting 5-fluorouracil sensitivity in man. Cancer Treat Rep. 65 (Suppl. 3): 57-62, 1981.

3. Brennan, M. and Macdonald, J.S.: Cancer of the endocrine system: Carcinoid tumors. In DeVita, V., Hellman, S., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Company, 1982, pp. 1019-1024.

4. Brennan, M. and Macdonald, J.S.: Cancer of the endocrine system: The endocrine pancreas. In DeVita, V., Hellman, S., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Co., 1982, pp. 1001-1019.

5. Bruno, S., Creaven, P., Ledesma, E., Poster, D., Yoon, J. and Mittelman, A.: Phase II study of 3-deazauridine in advanced colorectal adenocarcinoma. Am. J. Clin. Oncol. 5:69-71, 1982.

6. DeWys, W.D. and Kisner, D.L.: Principles of nutritional care of the cancer patient. In Carter, S.K., Glatstein, E., and Livingston, R.B. (Eds.): Principles of Cancer Treatment. New York, McGraw-Hill Book Co., 1982, pp. 252-259.

7. Dimitrov, N.V., Macdonald, J.S., and Hoth, D.F.: Toxicity of newer modalities of antitumor treatment. In Creaven, P.J. (Ed.): Toxicology of Antitumor Agents, New York, Grune & Stratton (in press).

8. Gisselbrecht, C., Smith, F.P., Macdonald, J.S., Boiron, M., Woolley, P.V., and Schein, P.S.: The effect of sequential addition of the nitrosourea, chlorozotocin, to the FAM combination in advanced gastric cancer. Cancer (in press).

9. Goldin, A., Chirigos, M.A., Macdonald, J.S., Fefer, A., and Mihich, E.: Biological response modifers and adjuvant chemotherapy: Consideration of selected preclinical investigations in relation to clinical potential. In Mathe, G. and Muggia, F.M. (Eds.): Recent Results in Cancer Research, Vol. 80. Berlin, Heidelberg, Springer-Verlag, 1982, pp. 351-356.

10. Hubbard, S.M. and Macdonald, J.S.: An introduction to current controversies in cancer management: Stage I testicular cancer, a case in point. Cancer Treat. Rep. 66:1-3, 1982.

11. Jasmin, C., Mori, K.J., Hayat, M., Macdonald, J.S., and Mathe, G.: In vivo study of chronic hematotoxicity of three nitrosoureas, chlorozotocin (chloro-2-ethyl)-ribofuranosyl-3-nitrosourea and (chloro-2-ethyl)-1-ribopyranosyl-3-nitrosourea. In Serrou, B., Schein, P.S., and Imbach, J.L. (Eds.): Nitrosoureas In Cancer Treatment. Amsterdam, New York, Oxford, Elsevier/North Holland Biomedical Press, 1981, pp. 193-206.

12. Killen, J.Y. and Macdonald, J.S.: Investigational new drugs in the treatment of small cell bronchogenic carcinoma. In Greco, A., Oldham, R.K., and Bunn, P. (Eds.): Small Cell Lung Cancer. New York, Grune and Stratton, 1981, pp. 399-412.

13. Kisner, D.L.: Malnutrition in lung cancer. In Mathe, G. and Muggia, F.M. (Eds.): Recent Results in Cancer Research, Vol. 80. Berlin, Springer-Verlag Publishing Co., 1981, pp. 240-245.

14. Kisner, D.L.: Nutrition of the cancer patient: An introduction. Cancer Treat. Rep. 65(Suppl. 5):1-3, 1981.

15. Kisner, D.L.: Reporting treatment toxicities. In Buyse, M., Staquet, M., and Sylvester, R. (Eds.): Cancer Clinical Trials: Design, Practice and Analysis. Oxford, Oxford University Press (in press).

16. Kisner, D.L.: Summary of discussions on the host/tumor metabolism session of the Conference on Nutrition and the Cancer Patient. Cancer Treat. Rep. 65(Suppl. 5):85-86, 1981.

17. Kisner, D.L. and Brennan, M.F.: Malnutrition and nutritional support in cancer management. In Wiernik, P.H. (Ed.): Supportive Care of the Cancer Patient. Mt. Kisco, N.Y., Futura Publishing Company (in press).

18. Kisner, D.L. and Macdonald, J.S.: Chemotherapy of metastatic gastrointestinal cancers: Prospects for future adjuvant systemic therapies. In Mathe, G. and Muggia, F.M. (Eds.): Recent Results in Cancer Research, Vol. 80, Berlin, Springer-Verlag Publishing Co., 1981, pp. 291-295.

19. Kisner, D.L. and Macdonald, J.S.: The impact of chemotherapy on the treatment of gastric cancer. In Muggia, F.M. (Ed.): Cancer Chemotherapy Martinus Nijhoff Publishers, Hingham, Massachusetts, (in press).

20. Kisner, D.L. and Macdonald, J.S.: Mitomycin-C in the treatment of gastric and pancreatic carcinomas. In Rozencweig, M. (Ed.): Proceedings of the Third NCI-EORTC Symposium on New Drugs in Cancer Therapy (in press).

21. Kisner, D.L., Schein, P.S., and Macdonald, J.S.: Recent results of clinical therapeutic trials for gastrointestinal malignancies conducted in the United States. In Klein, H.O. (Ed.): Recent Results in Cancer Research, Vol. 79. Berlin, Springer-Verlag Publishing Co., 1981, pp. 28-40.

22. Macdonald, J.S.: Therapy of Gastrointestinal Cancer. Br. J. Cancer 43:707-708, 1981.

23. Macdonald, J.S.: Summary of session on diagnostic procedures, histopathology, and prevention for the UICC International Conference on Clinical Oncology. UICC Bulletin (in press).

24. Macdonald, J.S., Gunderson, L., and Adson, M.: Cancer of the hepatobiliary system. In DeVita, V., Hellman, S., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Company, 1982, pp. 590-615.

25. Macdonald, J.S., Gunderson, L., and Cohn, I.: Cancer of the pancreas. In DeVita, V., Hellman, S. and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Company, 1982, pp. 563-589.

26. Macdonald, J.S., Gunderson, L., and Cohn, I.: Cancer of the stomach. In DeVita, V., Hellman, S., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Company, 1982, pp. 534-562.

27. Macdonald, J.S., Haller, D., and Kisner, D.: Adjuvant chemotherapy in colon and gastric cancer. In Mathe, G. and Muggia, F.M. (Eds.): Recent Results in Cancer Research, Vol. 80. Berlin, Heidelberg, Springer-Verlag, 1982, pp. 284-290.

28. Macdonald, J.S., Marsoni, S., Bruno, S., and Poster, D.: Current status of clinical trials of M-AMSA, dihydroxyanthracenedione, and deoxycoformycin. In Mathe, G. and Muggia, F.M. (Eds.): Recent Results in Cancer Research, Vol. 80. Berlin, Heidelberg, Springer-Verlag, 1982, pp. 323-330.

29. Macdonald, J.S., Weiss, R.B., Poster, D., and Hammershaimb, L.: Subacute and chronic toxicities associated with nitrosourea therapy. In Prestayko, A.W., Crooke, S.T., Baker, L.H., Carter, S.K., and Schein, P.S. (Eds.): Nitrosoureas: Current Status and New Developments. New York, Academic Press, Inc., 1981, pp. 145-154.

30. Mihich, E., Macdonald, J.S., Oettgen, H., Waldmann, T., Jasmin, C., Serrou, B., Grimm, E., Rosenberg, S., and Blomgren, H.: Symposium on Cancer Immunochemotherapy: Clinical Studies. In Proc. of the 12th International Congress of Chemotherapy, 1981 (in press).

31. Muggia, F.M., Carter, S.K., and Macdonald, J.S.: The Cancer Therapy Evaluation Program. Semin. Oncol. 8:394-402, 1981.

32. Penta, J.S., Poster, D., Bruno, S., and Macdonald, J.S.: Clinical trials with antiemetic agents in cancer patients receiving chemotherapy. J. Clin. Pharmacol. 21:11S-22S, 1981.

33. Piccart, M., Rozencweig, M., Abele, R., Cumps, E., Dodion, P., Dupont, D., Kisner, D., and Kenis, Y.: Phase I clinical trial with ametantrone (NSC 287513). Eur. J. Cancer Clin. Oncol. 17(7):775-779, 1981.

34. Piccart, M., Rozencweig, M., Dodion, P., Cumps, E., Crespeigne, N., Makaroff, O., Atassi, G., Kisner, D., and Kenis, Y.: Phase I clinical trial with alpha 1,3,5-triglycidyl-s-triazinetrione (NSC-296934). Eur. J. Cancer Clin. Oncol. 17(12):1263-1266, 1981.

35. Poster, D.S., Bruno, S., Penta, J., Neil, G., and McGovern, P.J.: Acivicin: An antitumor antibiotic. Cancer Clin. Trials 4:327-330, 1981.

36. Poster, D.S., Bruno, S., Penta, J., Pinna, K., Vilk, P., and Macdonald, J.: Current status of chemotherapy, hormonal therapy, and immunotherapy in the treatment of renal cell carcinoma. Am. J. Clin. Oncol. 5:53-60, 1982.

37. Poster, D.S., Penta, J.S., and Bruno, S.: PCNU: A new nitrosourea in clinical oncology. Am. J. Clin. Oncol. 5:9-12, 1982.

38. Poster, D., Penta, J., Bruno, S., and Macdonald, J.S.: ICRF-187 in clinical oncology. Cancer Clin. Trials 4:143-146, 1981.

39. Schein, P.S., Winokur, S., Macdonald, J.S., and Woolley, P.V.: Long term complications of cytotoxic and immunosuppressive chemotherapy. In Holland, J. and Frei, E. III (Eds.): Cancer Medicine, Second Edition, Philadelphia, Lea & Sebiger, 1982, pp. 759-774.

40. Smith, F.P., Kisner, D.L., and Schein, P.S.: Nutrition and cancer: Prospects for clinical research. Nutr. Cancer 2:34-39, 1980.

41. Smith, F.P., Schein, P.S., Macdonald, J.S., Woolley, P.V., Ornitz, R., and Rogers, C.: Fast neutron irradiation for locally advanced pancreatic cancer. Internat. J. Radiation Biol. Oncol. Phys. 7:1527-1531, 1981.

42. Sugarbaker, P., Gunderson, L., and Macdonald, J.: Cancer of the Anal Region. In DeVita, V., Hellman, S., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Company, 1982, pp. 724-731.

43. Sugarbaker, P., Macdonald, J.S., and Gunderson, L.: Colorectal cancer. In DeVita, V., Hellman, S., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Company, 1982, pp. 643-723.

44. Weiss, R.B. and Macdonald, J.S.: Toxicities associated with nitrosourea treatment. In Serrou, B., Schein, P.S., and Imbach, J.L. (Eds.): Nitrosoureas In Cancer Treatment. Amsterdam, New York, Oxford, Elsevier/ North Holland Biomedical Press, 1981, pp. 295-304.

45. Weiss, R.B. and Poster, D.S.: The renal toxicity of cancer chemotherapeutic agents. Cancer Treat. Rev. 9:37-56, 1982.

46. Weiss, R.B., Poster, D., and Penta, J.: The nitrosoureas and pulmonary toxicity. Cancer Treat. Rev. 8:111-125, 1981.

CLINICAL INVESTIGATIONS BRANCH (CIB)
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The Clinical Investigations Branch (CIB) is responsible for the scientific administration of the national cooperative clinical trials groups (the Cooperative Group Program); for scientific monitoring of the Phase II/III contracts, the disease-oriented contracts, an individual investigator-initiated clinical oncology grant program, a nutrition grant program, the interagency agreements, the surgical oncology grant program, and the Intergroup Testicular study; and for scientific administration of the Program Project grants in clinical cancer treatment.

During this fiscal year the cooperative group grants and the majority of the research contracts were converted to cooperative agreements. In this annual report they will be listed as grants or contracts, but in subsequent years, will appear combined under the heading, cooperative agreements.

1.0 Personnel

1. William D. DeWys, M.D - Chief, CIB, and Head, Nutrition Section
2. Edwin M. Jacobs, M.D. - Associate Chief
3. Richard S. Ungerleider, M.D. - Head, Pediatric Section
4. John Y. Killen, Jr., M.D. - Head, Medicine Section
5. Bimal C. Ghosh, M.D. - Head, Surgical Oncology Section
6. Gary B. Witman, M.D. - Senior Investigator
7. Freddie Ann Hoffman, M.D., Senior Investigator
8. Mario A. Eisenberger, M.D. - Senior Investigator (beginning 5/15/82)
9. Elizabeth I. Read, M.D. - Senior Investigator (beginning 7/25/82)
10. Brenda Edwards, Ph.D. - Statistical Consultant
11. Barbara Shepherd - Secretary to the Chief and Grants Assistant
12. Elaine Lewis - Secretary to the Associate Chief
13. Wilma Kline - Secretary to Drs. Ghosh and Hoffman
14. Sandra Downes - Secretary to Drs. Killen, Ungerleider, Witman and Eisenberger
15. Mira Milic - Stay-in-School (prior to 6-5-82)
16. Helen Bradley - Stay-in-School
17. Colleen Cleary - Summer Student
18. Charles Pruet, M.D. - Guest Worker, Surgical Oncology

Dr. DeWys is responsible for the overall administration of the Branch and coordination of its activities with the Cancer Therapy Evaluation Program, the Grants Administration Branch, the Cancer Clinical Investigation Review Committee (CCIRC), and the National Cancer Advisory Board. He also supervises the Project Officers on clinical contracts, and the Program Directors on grants and cooperative agreements. He is Chairman of the Clinical Oncology Review Committee (CORC) and Head of the Nutrition Section, CIB. He also is the coordinator of an intergroup protocol for Stage I and II testicular cancer, and a member of the National Prostatic Cancer Cadre.

Dr. Jacobs serves as Associate Chief of the CIB and Program Director for the Clinical Cooperative Group program. He coordinates the program review with the Executive Secretary of the CCIRC, the review body for the Cooperative Group Program. He is administrator for the Group protocols. He is also the Project Officer for the Memorial Hospital Phase II/III contract.

Dr. Ungerleider is Head of the Pediatric Section, CIB, and the Project Officer for the Phase II pediatric task order contracts. He assists the Program Director in the administration of the R01 grants, and is Program Coordinator for the Cooperative Groups which are conducting studies of pediatric cancers.

Dr. Killen is Head of the Medicine Section of the CIB. He is Project Officer for the Ovarian Cancer Study contract, the Gastrointestinal Tumor Study Group contract, the Kaposi's Sarcoma RFA, the Phase II GI contract, and the Emmes Corporation Statistical contract. Dr. Killen is also Program Director for the R01 Clinical Treatment grants, and the Regional Group grants.

Dr. Ghosh is Head of the Surgery Section, CIB, Project Officer for the Head and Neck contracts, and Program Director for the P20 and R01 Surgical Oncology grant programs. He also works in the Surgery Branch, especially with patients with esophageal cancer.

Dr. Witman was (to 8-1-82) Project Officer for the Mayo Clinic, Wayne State, M.D. Anderson, and the University of Michigan Phase II/III contracts, the Lung Cancer Study Group contracts, the Cervix Chemoprevention contract, and the Phase II task order contract. Additionally he was Program Director for the Program Project (P01) grants and coordinated review of these grants with the Clinical Cancer Program Project Review Committee

Dr. Hoffman is Program Director for both the R01 and P01 Nutrition grant program, and assists the Program Director in the administration of the Clinical Treatment R01 grants. She is Project Officer for the Nutritional Assessment contracts, the Small Cell TPN contracts, and the Immunotherapy contracts.

Dr. Eisenberger will be taking over much of Dr. Witman's area of responsibility. He is Program Director for the P01 Program Project grants, and Project Officer for the Lung Cancer Study Group and the Phase II/III contracts. He is providing program liaison with the National GI System Program (formerly Colon & Pancreas Organ Site) and the Gynecologic Oncology Group. He will also be co-Project Officer on the PAHO contract.

Dr. Read has recently joined the CIB and will become Program Director for the R01 grants, the Hepatic Artery grants, and the Bone Marrow Transplant registry. She will be Project Officer for the Milano Breast contract and the National Surgical Adjuvant Breast Project, and will provide liaison with the Breast Cancer Task Force.

Dr. Brenda Edwards spends 2/5 of her time within CTEP and divides this time between participation in protocol review and general consultation on projects. She is a principal reviewer on the majority of the Phase III protocols. She also has participated in the development of intergroup protocols in soft tissue sarcoma and malignant melanoma.

Mrs. Shepherd acts as administrative secretary for the Branch and is the grants administration coordinator for the R01 grant program (Clinical Treatment, Nutrition, and Surgery), and the P01 Program Project grants.

Ms. Lewis is secretary to the Associate Chief, and coordinates cooperative group protocol review and R10 grant administration.

Ms. Kline and Downes serve as secretaries to senior staff.

Ms. Bradley and Cleary have replaced Ms. Milic and assist senior secretaries in the Branch as needed.

Dr. Pruet is stationed at the National Naval Medical Center, and provides expertise to the CIB in surgical oncology, particularly in head and neck surgery. He reviews protocols and attends weekly protocol review.

## 2.0 Grant Programs

### 2.1 Clinical Cooperative Groups

The Cooperative Group Program was initiated by the Cancer Chemotherapy National Service Center to test the new agents from the NCI drug development program (1955-66). The program underwent several administrative changes and most recently has been in the Division of Cancer Treatment (1975 - present) where the major emphasis has been on combined modality approaches to cancer treatment.

The accomplishments of the Cooperative Groups were reviewed by the DCT Board of Scientific Counselors in March 1979. Much of the material presented by the groups has been published (see Hoogstraten et al (Eds): Cancer Research, Impact of the Cooperative Groups. New York, Masson Publishers, 1980.

#### 2.11 Listing of the Cooperative Groups

Multimodality Multidisease Groups

Cancer and Leukemia Group B (CALGB)
Eastern Cooperative Oncology Group (ECOG)
North Central Cancer Treatment Group (NCCTG)
Northern California Oncology Group (NCOG)
Southeastern Cancer Study Group (SEG)
Southwest Oncology Group (SWOG)

Multimodality Groups Devoted to a Major Oncologic Area

Children's Cancer Study Group (CCSG)
Gynecologic Oncology Group (GOG)
Pediatric Oncology Group (POG)

Single Modality Group

Radiation Therapy Oncology Group (RTOG)

Single Disease Groups

Intergroup Ewing's Sarcoma Study (IESS)
Intergroup Rhabdomyosarcoma Study (IRS)
National Surgical Adjuvant Breast and Bowel Project (NSABP)
National Wilms' Tumor Study Group (NWTG)
Polycythemia Vera Study Group (PVG)
Radiotherapy Hodgkin's Disease Group (RHDG)

Special Activities Groups

European Organization for Research on Treatment for Cancer (EORTC)
Operations and Statistical Office
Lymphoma Pathology Reference Center (LPRC)
Radiologic Physics Center (RPC) (see Radiation Research Program)
Quality Assurance Review Center (QUARC)

2.12 Description of the Cooperative Groups

The Cancer and Leukemia Group B (CALGB), founded in 1955, studied primarily hematologic malignancies until the 1970's when it also developed multimodal studies in solid tumors. It has made major contributions in the chemotherapy of breast cancer as well as the leukemias and lymphomas. The group elected a new chairman and narrowed their scientific scope to the treatment of leukemia, lymphoma, lung, and breast cancer. Plans were formulated to move the statistical office, and the group elected a new statistician.

The Eastern Cooperative Oncology Group (ECOG), founded in the 1950's, developed and remains committed to multimodal solid tumor studies, but has increased studies in the hematologic malignancies. The group has made major contributions in breast and gastrointestinal malignancies.

The North Central Cancer Treatment Group (NCCTG) is recently organized and consists of the Mayo Comprehensive Cancer Center and ten clinics in the North Central region. The objectives of the group are to make the most promising cancer research accessible to patients in their region, and to conduct clinical research of high quality in a community setting.

The Northern California Oncology Group (NCOG) has recently been developing multimodal programs in brain tumors, high LET radiation and radiosensitizer studies, and are participating in the head and neck contract. They represent a regional type cooperative group. The group elected a new chairman, and currently they are evaluating their scientific direction.

The Southeastern Cancer Study Group (SEG), previously involved in hematology studies, underwent reorganization and have now developed multimodal studies in lung cancer and melanoma, and have major potential in genitourinary cancers. They have initiated a new information system which utilizes minicomputers in clinical protocols.

The Southwest Oncology Group (SWOG) has been involved in multimodal programs. One of the largest groups, with an annual accrual of about 3000 patients, it has the resources to rapidly complete Phase II and III studies. They have made significant contributions in AML, myeloma, lymphoma, and breast cancer.

The Children's Cancer Study Group (CCSG) is a multimodality organization concerned exclusively with pediatric malignancies. They have initiated major Phase II and III studies in hematologic and solid tumors, and have begun to collect information about the long term effects of cancer therapy. They recently decided to give more emphasis to innovative pilot studies (e.g., bone marrow transplantation) among selected institutions while maintaining their interest in group-wide studies.

The Gynecologic Oncology Group (GOG) has involved the specialties of gynecology, radiation, medical oncology, and pathology for research in gynecologic cancers. They have done a systematic analysis of Phase II drug activity in several gynecologic malignancies as well as Phase III studies in early ovarian and cervical cancers.

The Pediatric Oncology Group (POG) is a newly formed multimodality organization composed of former members of the pediatric divisions of SWOG and CALGB. While continuing to accrue patients on a few former SWOG pediatric protocols, they have initiated several new Phase II and III studies, with a major interest in the classification of childhood leukemias through the use of cell markers. They have recently launched a randomized trial of adjuvant therapy in osteogenic sarcoma.

The Radiation Therapy Oncology Group (RTOG) has protocols exploring the methodology and technique of radiation therapy as applied in various tumor types, and disease-oriented studies exploring more than one modality of treatment using radiation therapy as a primary focus for study. Studies include time-dose relationships, the use of radiosensitizers, high LET radiation, hyperthermia, and a study of the late effects of radiation therapy. The statistical operation was transferred to the RTOG Headquarters, and a new group statistician was appointed.

The Intergroup Ewing's Sarcoma Study (IESS) is conducted by members of the two pediatric cooperative groups, CCSG and POG. Since its inception in 1973 it has accumulated the largest group of patients with Ewing's sarcoma in any study in the world. IESS-1 demonstrated that addition of either adriamycin or bilateral pulmonary irradiation to chemotherapy with VAC improves response and survival of children with nonmetastatic disease. IESS-2 has as its objective improved survival and relapse-free survival rates with the fewest long-term complications. Because of lack of new directions the IESS is on phase-out funding while innovative studies will be pursued by POG and CCSG independently.

The Intergroup Rhabdomyosarcoma Study Group (IRS) is composed of members of POG and CCSG. Their first study developed a staging system, demonstrated varied prognoses depending on site, and evaluated the effect of multimodal therapy. Their current study has incorporated special treatment considerations relating to primary site of disease. A third study

is currently being devised for initiation within the next 12 months, and will tentatively involve a trial of intensive multidrug chemotherapy versus total body irradiation with autologous bone marrow infusion.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) is a pioneer multimodality group. In the past, it focused exclusively on primary treatment of breast cancer, but now it is also involved in studies of primary colorectal cancer. Major contributions to our theory and practice of adjuvant chemotherapy have been accomplished by this group. A current major study compares segmental mastectomy with or without radiotherapy to total mastectomy. Recently they have developed protcols to study adjuvant chemotherapy in Stage I breast cancer.

The National Wilms' Tumor Study Group (NWTG) is an intergroup organization incorporating the pediatric cooperative groups along with several independent investigators. Their third study (NWTS-3) is primarily concerned with refinement of therapy. NWTS-1 and 2 conclusively demonstrated that most children with this tumor can now be expected to survive if they are managed by combined modality therapy from the outset, and that prognosis is closely related to histopathologic findings. They are also closely monitoring the late effects of cancer therapy in young children.

The Polycythemia Vera Study Group (PVSG) has protocols to determine the natural history, course, and optimum therapy of polycythemia vera. Currently it is funded for followup and final analysis of their primary protocol which has shown an increase in incidence of leukemia in patients treated with chlorambucil as compared to radioactive phosphorous or phlebotomy.

The Radiotherapy Hodgkin's Disease Group (RHDG) has studied whether survival in localized Hodgkin's disease was different when patients received involved fields of radiation, or extended fields. The trial is presently in followup.

The following special activities groups provide support services for groups:

The Operations and Statistical Office of the EORTC is funded by the DCT.

The Lymphoma Pathlogy Reference Center (LPRC) provides expert review of pathological material for the groups performing therapeutic research in malignant lymphoma.

The Quality Assurance Review Center (QUARC) provides radiation therapy quality control for three national cooperative groups (CCSG, CALGB, POG) and two pediatric intergroup studies (IRS, NWTSG).

## 2.13 Summary of the Cooperative Groups

Phase I and broad Phase II trials comprised a substantial effort in the past, but now their emphasis has expanded to include Phase III and combined modality studies with curative intent (adjuvant studies).

Change in direction is also indicated by specialties represented by group members. There has been a steady increase in the groups' medical oncolgists and pediatric oncologists over the years, and the large increase in numbers of pathologists, radiotherapists, surgeons, and other physicians in the past three years is impressive and is a direct reflection of the move toward the multidisciplinary clinical research of cancer. Innovative pilot Phase I and Phase II studies open to one or a few institutions have become a formal part of cooperative group programs.

The scientific progress of the groups is reflected in publication of numerous papers and abstracts.

Some specific areas where noteworthy contributions have been made by the cooperative groups include:

1) Improved statistical methods for conducting clinical trials.
2) Definition of prognostic factors in childhood leukemia.
3) Progressively improved therapy in leukemia with improved survival.
4) Intergroup trials in Wilms' tumor widely extending the benefit of combined modality therapy.
5) Combined modality therapy of Ewing's sarcoma, rhabdomyosarcoma, and osteosarcoma with improved survival in these tumors.
6) Delineation of the natural history of polycythemia vera.
7) An understanding of cell kinetics and tumor burden in myeloma.
8) Further refinement of the combined modality and chemotherapy of Hodgkin's disease.
9) Development of testing of combination chemotherapy of non-Hodgkin's lymphoma.
10) Large scale adjuvant trials in operable breast, colon, and rectal cancer which should define the current promise of long-term chemotherapy of micrometastases.
11) Evaluation of adriamycin and daunorubicin in AML with improved therapeutic results.
12) Demonstration of the role of l-asparaginase in ALL treatment.
13) Large scale trials of combined mdality therapy of small cell lung cancer.
14) Conducted studies where exaggerated results of preliminary studies were refuted by carefully-done randomized multiinstitutional trials.
15) Initiation of an Intergroup (national) Stage I and II testicular cancer trial.
16) Initiation of an Intergroup Mesothelioma study.
17) Introduction of in vitro assay of new agents in large clinical trials.
18) Introduction of cell surface markers and other assay techniques in large clinical trials.

## 2.2 Program Project Grants (PO1)

At the present time there are 31 active program project grants with a total expenditure of $23,130,500. Program project grants provide research support for broadly based programs that blend pre-clinical and clinical activities. In addition to the Clinical Oncology program project grants, a Program Announcement was issued this year for PO1 applications for surgical oncology research. Three responses were

received, one of which is to be funded.

Each grant involves a number of investigators each of whom conducts a research project designed to elucidate one or more aspects of a common goal. These efforts are conducted in an organized fashion in order to facilitate the interactions of these participating investigators. This approach is designed to acquire knowledge more effectively than would a simple aggregate of research projects operating without organization and thematic integration.

Historically, the program has supported highly successful research projects that have made significant contributions. By bringing together basic and clinical investigators, the program has been able to provide excellent patient care and also explore basic elements in tumor biology.

Although clinical research is the main thrust of all programs, substantial efforts in more basic elements are present. These activities include drug development and pharmacology, cell kinetics, immunobiology, marrow transplantation, histopathology and hematology.

The development of potentially curative strategies based on investigation of the kinetic basis for drug responsiveness of common tumor types has been a high priority. A variety of new methods have been developed and some older techniques have been used in new or different ways to predict kinetic patterns.

The aggressive use of bone marrow transplantation as an adjunct to other forms of treatment continues. Monoclonal antibodies directed against human hemopoietic and immunologic precursors and leukemic cell antigens are being used as research tools. Progress has been made in efforts to define the cellular and clinical characteristics of malignant lymphomas and related leukemias in terms of T and B lymphocyte systems. These efforts have lead to new studies on control mechanisms in lymphoma induction and progression, membrane and cytoplasmic markers, and cell surface receptors and antigens.

In vitro assays using explanted tumor tissue in culture to measure the effectiveness of drugs and other types of treatment methods have been correlated with in vivo activity. Overall, many of these grants are supporting investigative teams which are demonstrating that the whole can be greater than the sum of its parts.

## 2.3 RO1 Grant Programs

### 2.31 Clinical Oncology

Description

The thrust of this program is to foster development and evaluation of techniques for treating cancer patients. This includes the use of chemotherapy, radiation therapy, immunotherapy, and surgery alone, or in combination. Improved experimental designs and statistical analyses are integral to the program. The program also supports investigators who are searching for improved methods

of supportive care, protected environments, and bone marrow transplantation. 76 grants are included in this program.

Accomplishments

A number of clinical trials or preclinical feasibility studies have been completed or are well underway. A few examples are:

1. DeGowan, et al, have demonstrated that an extramedullary tumor in mice induced a microenvironmental lesion in bone marrow that inhibited blood production, and that this bone marrow failure might be mediated by prostaglandin-E. They have evidence that bearing a small tumor for several days enhances the radiosensitivity of mouse bone marrow cells, and inhibits repopulation and recovery of these vital cells after treatment. Recognition of the mechanisms which produce marrow failure may permit the design of less suppressive treatment regimens.

2. Herzig and associates have documented the ability of cryopreserved marrow to restore hematopoiesis after lethal total body irradiation, and have determined the maximum tolerated dose of total body irradiation and concomittant cyclophosphamide. On this regimen approximately 15% of patients with refractory leukemia and lymphoma have achieved unmaintained remissions of more than one year.

3. Halberg and associates have evidence in animals that timing of drug administration is highly significant in both toxicity and antitumor effects. Preliminary studies in patients suggest that myelosuppression resulting from adriamycin can be significantly reduced by evening administration, and that nephrotoxicity resulting from cisplatinum is lessened when it is given in the morning.

4. Buchner, et al have further refined immunoabsorbent columns for the removal of anti-A or anti-B red cell antibody from patients prior to ABO-incompatible bone marrow transplants. ABO incompatibility between donor and recipient constitutes an obstacle to marrow transplantation unless some method of removing the risk of hemolysis is utilized. The refinement consists of the development of columns biocompatible with whole blood, which simplifies the immunoabsorption technique by eliminating the need for a blood cell centrifuge for plasma separation. These columns have been successfully tested in dogs, and will be tested in humans.

5. Enneking, et al have demonstrated a unified clinicopathologic staging system for musculoskeletal sarcoma which is statistically valid. Their investigations have shown that low grade (Stage I) lesions may require only a wide margin for local control, and adjunctive chemotherapy is not indicated because of the low risk of metastasis. Stage II disease requires a radical margin for local control but incurs a significant risk of metastasis that may be independent of local control. Local control appears to depend on the margin and not the procedure.

6. Fisher and colleagues have found that removal of primary tumor in mice causes changes in kinetic factors (increased labeling indices indicating increased cell turnover) which may afford opportunities to design more successful chemotherapeutic regimens.

7. Ensminger, et al, have demonstrated that a totally implanted hepatic arterial drug delivery system, when combined with nuclide angiography for correct catheter placement, represents an extremely reliable method for producing a high response rate in treating hepatic metastases from colorectal cancer or carcinoids tumors. This delivery system represents an important mechanism for testing new drug regimens.

8. Palmieri and colleagues have demonstrated a correlation between hydroxyproline and bone metastases in breast cancer. A newer, simpler assay for this marker is being evaluated to replace the cumbersome twenty-four hour urine collection.

9. In a continuation of studies begun with Dr. Cronlund, Dr. Rickles is investigating the mechanisms of activation responsible for the "hypercoagulable" state noted in cancer patients. A rise in fibrinopeptide A (FPA) levels, which may represent ongoing fibrinogenolysis, occurs prior to clinical evidence of disease. The use of the FPA assay in determining the presence of microscopic disease is now being explored, along with the role of anti-coagulants in the treatment of malignancy.

10. Moran et al. are exploring the cytotoxic determinants and selectivity of methotrexate alone and with leukovorin or thymidine rescue. A microassay for $H_4$PteGlu and its oligoglutamyl congeners has been developed, as well as other improvements in the methods employed to elucidate the mechanisms of these commonly used agents.

11. Dr. Park and associates are continuing to improve the usefulness of their clonogenic assay for human acute nonlymphocytic leukemia. The effect of chemotherapeutic agents observed in vitro in this assay appears to correlate to the in vivo situation. This is one of the best worked-out systems which may potentially lead to the prediction of drug sensitivity in the clinical setting.

12. Dr. Tu et al. are conducting a clinical trial investigating the use of a 2'deoxycoformycin, a potent inhibitor of adenosine deaminase, in the treatment of T-cell leukemia. These investigators are refining the use of this T-cell specific agent by monitoring both in vivo and in vitro metabolic changes resulting from therapy.

## 2.32 Nutrition

### Description

A separate grant program was created and an RFA released in September 1979. Grants may include both clinical and preclinical studies encompassing areas such as pathophysiology of malnutrition, nutritional assessment, and nutritional intervention of the tumor-bearing host.

Accomplishments

Current grants are exploring various aspects of the relationship between nutrition and malignancy.

1. Holroyde, et al. have observed significant deviations from the normal metabolic response folllowing protein ingestion by cancer patients with weight-loss. Abnormalities in glucose and lactate metabolism are also known to be commonly associated with the malignant state.

2. Cancer patients have also been found to be hyprrmetabolic, when evaluated by indirect calorimetry. Heber and colleagues have compared the basal metabolic rates of cancer patients and normal controls.

3. In an animal model, Dr. Meadows, et al are studying the effects of nutritional depletion in the treatment of melanoma. This project combines two established anit-tumor approaches in a novel fashion and has a high likelihood of clinical applicability.

2.33 Surgical Oncology

Description

This program was established to stimulate and support surgical oncology research efforts throughout the country and a Program Announcement for R01 grant applications was issued this year to encourage these efforts.

Accomplishments

54 R01 grants were submitted for the October 1981 deadline in response to this program announcement. These grants were reviewed by Special Study Sections and ten of them received sufficiently good priority scores for funding.

2.4 P01 Nutrition Grants

Two core grants are currently supporting the development of Clinical Nutritional Research Units in major cancer centers. These programs combine the resources of both clinical and basic laboratories in geographically related hospitals and unversities. A variety of projects are currently underway, which include nutritional intervention and rehabilitation of patients with head and neck cancer, the effects of vitamins and minerals on immunity and cell surface membrane receptor expression, and the development of assays and methods to better assess the status of the nutritionally depleted patient.

2.5 P20 Surgical Oncology Grants

An RFA for Surgical Oncology Planning Grants (P20) was published, and

28 applications were received in response. Of these, five will be funded.

2.6 Cooperative Agreements

2.61 Regional Cooperative Groups

In addition to the two existing regional clinical trials groups, the CTEP plans to establish additional groups during FY '82. An RFA has been released and a total of 17 applications were received and have undergone peer review and approval by the NCAB. New groups which will receive Cooperative Agreements during the summer of 1982 are the Piedmont Oncology Group and the Mid-Atlantic Oncology Project.

2.62 Acquired Immunodeficiency, Opportunistic Infectious and Kaposi's Sarcoma in Homosexual Males

Since June, 1981, the Centers for Disease Control in Atlanta have identified a syndrome of immunodeficiency, opportunistic infections and Kaposi's sarcoma concentrated in male homosexuals and strikingly clustered in several geographic locations. The etiology and pathogenesis of the syndrome remain obscure but the implications, both from a public health viewpoint and from the viewpoint of the etiology of cancer are enormous. The staff of the CIB, in conjunction with the NCI's Division of Cancer Cause and Prevention, are in the process of preparing an RFA to fund research into all aspects of this syndrome. It is projected that a multidisciplinary working group of clinicians and basic researchers will be formed. Funding will be by the Cooperative Agreement mechanism, and will begin in early FY '83.

3.0 Contract Programs

3.1 Medicine Section

3.11 Gastrointestinal Tumor Study Group (GITSG)

The GITSG continues to show benefit for adjuvant therapy of rectal and gastric cancers. This consortium consists of nine active member institutions which have contracts for the treatment of gastric, pancreatic and colorectal cancer and a statistical support contract (Emmes Corp.). The six gastric and pancreatic contracts were recompeted in 1980. The colorectal project was recently recompeted and awards were made to the following six institutions: Hawaii Medical Association, New York University, University of Wisconsin, Georgetown University, Wayne State University and Roswell Park Memorial Institute. Important in the past year were the following:

(1) New generations of gastric and colorectal studies, activated during the previous year continue to accrue at acceptable rates. These include studies for adjuvant therapy and therapy of advanced disease.

(2) A study for locally advanced gastric cancer, was activated. It will address questions of the value of radiation therapy and nutritional support.

(3) A new protocol for adjuvant therapy of pancratic cancer is being designed, following closure of protocol 9174. The latter suggest that postoperative radiation plus 5-FU is superior to surgery alone.

(4) New protocols for advanced pancreatic cancer are being designed.

3.12 National Surgical Adjuvant Breast and Bowel Project (NSABP)

NSABP Breast Contract

This contract supports the study of adjuvant treatment in breast cancer. Currently, protocols B-04, B-05, B-07, B-08, B-09 and B-10 are closed to patient accrual although all patients are still being followed. Protocol B-06 (segmental mastectomy) is still open for patient accrual. During the past year two new adjuvant studies for stage II breast cancer have been activated. These test the value of Adriamycin in the adjuvant setting. In addition, studies for stage I disease have also been activated, as has a trial of interferon in advanced disease. Preliminary data from protocol B-09 indicate that Tamoxifen adds to the efficacy of the combination of LPAM plus 5-FU in the subset of postmenopausal women with positive estrogen receptors.

NSABP Colo-rectal Contract

This contract is designed to support randomized controlled studies using adjuvant therapy in treatment of colo-rectal cancer. Present studies include C-01 which randomizes patients having Stage B+C colon cancer between no treatment, chemotherapy, and immunotherapy and R-01 which randomizes patients having Stage B+C rectal cancer between nontreatment, radiotherapy and chemotherapy. Protocol C-01 will complete accrual in the near future and a replacement study is currently being designed.

3.13 Phase II-III Drug Evaluation Contracts

This contract is for Phase II-III studies to detect useful therapeutic effects of new drugs alone and in various combinations in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, ovary, endometrium, cervix, head and neck, stomach, pancreas and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. At each of the 4 participating institutions a minimum of 175 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immnotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

3.14 Lung Cancer Study Group

This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study completed patient accrual in October, 1980 and has been supplanted by a trial evaluating surgical lobectomy vs. wedge resection for peripheral Stage I disease. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III epidermoid carcinoma, the role of post operative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cis-platinum) is being evaluated for incompletely resected advanced Stage III disease and in partially resected locally advanced non-small cell cancer.

3.15 Istituto Nazionale por le Studio e la Cura dei Tumori

A major effort in breast cancer has been through this contract. It has dealt primarily with adjuvant therapy of resectable disease, and its results have received world-wide attention. The Istituto has recently shown an improved overall survival for premonopausal patients treated with CMF. They also recently reported that 12 months of CMF is no more effective than 6 months. Studies testing the value of non-cross resistant drug regimens in the adjuvant setting are currently in progress.

3.16 WHO Melanoma

This contract provides for data management and statistical operations for the clinical trials of the WHO International Melanoma Group. This group initially demonstrated, in a prospective randomized study, that prophylactic node dissections have no therapeutic value for clinical Stage I malignant melanomas. During the last 5 years the WHO melanoma Group has performed studies to determine: (1) the value of DTIC, BCG or DTIC plus BCG as adjuvant therapy of Stage II melanoma, and (2) the value of DTIC versus DTIC plus BCG versus C. parvum for metastatic melanoma. The last two studies have been performed under an NCI contract which provides support for the Group's Secretariat but no patient care costs. Each study has been closed to further patient accrual and results will be published when suitable followup is available.

In the two past years the Group has initiated two new studies: (1) a study to determine whether 4-5 cm margins are necessary in resecting primary melanomas, and (2) a study of intralymphatic BCG for treatment of regional lymphatic disease. The previous adjuvant therapy trial will be replaced by a study of poly A-poly U versus a control group. The WHO melanoma group is currently on phase-out funding due to budgetary restrictions.

3.17 Phase II Gastrointestinal Cancer

This contract is designed to carry out Phase II studies in gastric, pancreatic, and colonic cancer. There are two participants. The Georgetown University program is responsible for a systematic investigation of new agents in gastric and pancreatic cancer and the development of new combinations of agents. The Mayo Foundation is evaluating new drugs placed in clinical trials in patients having advanced gastrointestinal cancer. The Human Clonogenic Stem Cell Assay (HCSCA) is being analyzed in some of the 175 colon cancer patients placed on studies as a prospective means of selecting chemotherapy.

3.18 Chemoprevention of Cervical Cancer

This contract is supporting a Phase I evaluation of topical retinoids, a vitamin A analog, applied directly onto the surface of the cervical mucosa in patients with severe dysplasia. After a careful evaluation of toxicities, a Phase III trial will be initiated, which will attempt to identify the role of vitamin A analogs as chemoprevention agents. Women with abnormal pap smear cytology will be prospectively analyzed in a double blind trial to see if retinoids can improve dysplastic cervical morphology. This chemopreventive trial will try to reproduce the finding in laboratory animals that cellular differentiation and maturation can be induced by vitamin A analogs. The clinical trials are ongoing at the University of Arizona and Albert Einstein College of Medicine. Pathologic support is through a Central Pathology Unit at Georgetown University.

3.19 Immunotherapy Contracts

A contract with Mount Sinai, "Chemoimmunotherapy of Acute Myelocytic Leukemia," is showing a survival advantage for patients treated with chemotherapy plus allogeneic neuraminidase-treated myeloblasts over chemotherapy alone. Additional patient accrual is needed to obtain statistical significance.

The UCLA study of "Specific and Non-specific Immunotherapy as an Adjunct to Chemotherapy in Skeletal and Soft-tissue Sarcoma" has been phased out, and the project is continuing under a PO1 grant. Also phased out was a UCLA three-armed study evaluating BCG in melanoma patients. At median followup survival was longer for patients treated with BCG and a tumor cell vaccine than either controls or those receiving BCG alone.

A Yale study of "Intratumoral BCG Immunotherapy Prior to Surgery for Carcinoma of the Lung" is expected to continue accrual through next spring.

Memorial Sloan-Kettering's study of the effects of BCG immunotherapy in the treatment of recurrent superficial bladder carcinoma has been completed. Patients with carcinoma in situ responded more

favorably to BCG treatment than those receiving fulguration alone. Another Memorial contract which involved a Phase I trial of the effects of immune stimulants in the human immune response has expired. Intravenous BCG, endotoxin, and Poly IcLc and their effects on neutrophils, complement factors, and lymphocytes were evaluated.

A Health Research, Inc. contract studying adjuvant tumor specific active immunotherapy of squamous cell lung cancer has been phased out.

The Albany Medical College study of "Intrapleural BCG after Primary Surgery for Lung Cancer is treating Stage I patients with resectable disease. So far, the BCG arm appears to be showing a negative correlation with survival.

An M.D. Anderson project involving a series of Phase I trials studying the effects of immunotherapy and biologic response modifiers on various human malignancies has been phased out and will be continued as a P01 grant.

3.2 Pediatric Section

Pediatric Phase I-II Task Order

This task order contract is for Phase I and II testing of new agents in pediatric oncology patients. There are 9 master contractors who constitute a pool of investigators to study new agents as selected by CTEP. This contract is currently in its third year, with Phase I studies of the following drugs being conducted: DON (M.D. Anderson Hospital) and Mitoxantrone (Illinois Cancer Council). Phase I studies of indicine-n-oxide (Children's Cancer Research Foundation) and AZQ (Children's Hospital of Los Angeles and Memorial Sloan-Kettering Cancer Center) were completed this year.

3.3 Nutrition Section

Three contract projects are currently active and one has recently been completed. The ongoing TPN Small Cell Lung Cancer group, comprised of five institutions, has now followed 99 patients for over one year. Patients who received TPN demonstrated better hematologic status and fewer chemotherapeutic delays, than did controls. This is the largest randomized study of the value of agressive nutritional support in cancer patients. In another contract, patients with various malignancies are being studied by direct calorimetry. A third project was recently launched, involving three institutions, which will explore, longitudinally the effects of three levels of nutritional intervention in advanced non-small cell lung and colorectal cancer patients. In this study, newer techniques in nutritional assessment will be evaluated. A series of contracts evaluating methods for the assessment of the nutritional status of patients with cancer recently completed work and data is being analyzed.

3.4 Surgical Section

Head and Neck Contracts Program:

This is a collaboration of six institutions and two cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the additon of a six month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy.

The participating institutions are University of South Florida, University of Texas-Galveston, University of Cincinnati, University of Maryland, Memorial Sloan-Kettering Hospital, University of Michigan, Radiation Therapy Oncology Group, Northern California Oncology Group. This study was activated October 28, 1978, and patient accession was discontinued on April 30, 1982 with a total accrual of 462 patients. Followup will continue for two years.

4.0 Miscellaneous

4.1 Intergroup Testicular Study:

This is a collaboration between five cooperative groups and three large institutions having an interest in testicular cancer. The protocol is (1) a randomized controlled study of adjuvant chemotherapy of stage II resectable testicular cancer and (2) a monitoring of stage I testicular cancer. For stage II the study compares the disease-free and overall survival for surgery alone (with combination chemotherapy for relapses) versus surgery plus early adjuvant chemotherapy. Stage I patients are registered and monitored to identify prognostic variables which may predict recurrence in this group. The protocol also includes important biologic studies such as histologic typing, serum marker studies, and studies of the accuracy of lymphangiograms, CT scans, and ultrasonography. Accrual statistics include:

| Stage | 4-14-80 | 5-12-81 | 6-1-82 |
|---|---|---|---|
| I | 18 | 79 | 156 |
| II | 37 | 100 | 148 |
| Total | 55 | 179 | 304 |

At this rate of patient entry the original accrual objectives will be met with one additional year of patient entry. To date 38 relapses have been recorded, 11 in stage I and 27 in stage II. Four patients have died, but in each case there was deviation from the protocol followup, or from administration of full-dose chemotherapy.

4.2 Extramural Clinical Trials Office (ECTO) - EMMES

This contract provides administrative support to contract-funded clinical research projects: Lung Cancer Study Group, Gastrointestinal Cancer Study Group, Head and Neck Contract Project, the Intergroup Testicular Study and the Kaposi's Sarcoma Study. The services provided by ECTO include: assistance in protocol and forms design; patient randomization; quality control of data; coordination of scientific activities of clinical investigators, statisticians, and project officers; planning of meetings and preparation of agenda, minutes, and reports; communications (telephone and correspondence); and related administrative tasks.

4.3 NCI-Pan American Health Organization: Collaborative Cancer Treatment Research Program (PAHO:CCTRP)

The continuing collaboration between US-Latin American investigators in the development of clinical studies resulted in an accrual of 448 patients in Phase III studies and 166 patients in Phase II pilot studies for a total of 614 patients (total accrual on these studies now stands at 6118 as of 1982). Nine Latin American and nine sister institutions in the United States participate in this collaboration. Two new gastric and two cervical studies have been activated. Eight Phase II trials in penile, vulvar, cervical and gastric carcinomas, and osteosarcoma are in final stages of activation. Additionally, two master protocols in gastric and cervical carcinoma have been generated and approved.

A number of Phase III studies continue to accrue patients and will soon generate important data in Hodgkin's disease (CUPP $\pm$ radiation) and non-Hodgkin's lymphomas (BACOP vs. C-MOPP). A study of intraarterial cis-platinum will be piloted prior to design of a multimodality adjuvant trial in osteosarcoma.

4.4 VA Surgical Oncology Group (VASOG)

The VA Surgical Oncology Group is receiving phase-out support via an Interagency Agreement. The agreement supports collection and analysis of final protocols and publications of results.

4.5 Patient Accrual - 1981

Total patients entered on Phase III, IV (adjuvant) studies: 17,120
(Cooperative groups: 15,113; contract projects: 2,007)

Total patients entered on Phase, I, II, and pilot studies: 7,544
(Cooperative groups: 4,591; contract projects: 2,953)

Total patients entered: 24,664

Total patients on study and in followup: 71,913

Compared to 1981 accrual figures, accrual decreased in all categories except one--Phase I, II, and pilot studies accrual of contract projects

showed a slight (68) increase. Total patients entered in 1981 showed a 7,000 decrease and total on study and in followup decreased about 8,000 from 1980.

## 5.0 Protocol Review

Protocols are reviewed weekly by the full CTEP staff under the chairmanship of the Chief, CIB. Following review a consensus of the review is transmitted to the study chairman with a recommendation either for activation or revision. Maximum turnaround time from receipt of a protocol to completion of review is 30 days.

## 6.0 Staff Publications


DeWys, W.D.: Pathophysiology of cancer cachexia: current understanding and areas for future research. Cancer Res 42: 721s-726s, 1982.

DeWys, W.D.: Urologic and male genital malignancies. In Skeel, R.T. (Ed.) Manual of Cancer Chemotherapy. Boston, Little Brown, 1981 (in press).

DeWys, W.D.: Weight loss in cancer patients: Prognostic and pathophysiologic considerations. In Kluthe, R. (Ed): Nutrition and Metabolism in Cancer International Workshop. Stuttgart, Georg Thieme Verlag, 1982, pp. 8-16.

DeWys, W.D.: How to evaluate a new treatment for cancer. Your Patient & Cancer 2(5): 31-36, 1982.

DeWys, W.D.: Summary of discussions on the anorexia session of the conferrence on nutrition of the cancer patient. Cancer Treat Rep 65: 53, 1982.

DeWys, W.D.: Summary of discussions on the nutritional support and cancer therapy session of the conference on nutrition and the cancer patient. Cancer Treat Rep 65: 157, 1982.

DeWys, W.D., Begg, C.B., Band, P.R., Tormey, D.C.: The impact of malnutrition on treatment results in breast cancer. Cancer Treat Rep 65: 87-92, 1982.

DeWys, W.D., Costa, G., Henkin, R.: Clinical parameters related to anorexia. Cancer Treat Rep 65: 49-52, 1982.

DeWys, W.D., Curran J., Henle, W., Johnson, G.: Report on a workshop on Kaposi's sarcoma. Cancer Treat Rep 66: 1387-1390, 1982.

DeWys, W.D. and Kubota, T.: Enteral and parenteral nutrition in the care of the cancer patient. JAMA 246: 1725-1727, 1981.

Donaldson, S.S., Wesley, M.N., DeWys, W.D., Suskind, R.M., Jaffee, N., van Eys, J.: A study of the nutritional status of pediatric cancer patients. Am J Dis Child 135: 1107-1112, 1981.

Donaldson, S.S., Wesley, M.N., Ghavimi, F., Shils, M.E., Suskind, R.M., DeWys, W.D.: A prospective randomized clinical trial of total parenteral nutrition in children with cancer. Med Pediatr Oncol 10: 129-139, 1982.

Glaubiger, D.L., Von Hoff, D.D., Holcenberg, J.S., Kamen, B., Pratt, C., and Ungerleider, R.S.: The relative tolerance of children and adults to anticancer drugs. Frontiers of Radiation Therapy and Oncology 16: 42, 1982.

Ghosh, L., Nassauer, J., Faiferman, I., Ghosh, B.C.: Pathology of metastizing tumors in nitrosomethyl urea-induced rat mammary carcinoma. J Surg Onc 18: 21-26, 1981.

Ghosh, L., Nassauer, J., Faiferman, I., Ghosh, B.C.: Ultrastructural study of membrane glycocalyx in primary and metastatic human and rat mammary carcinoma. J Surg Onc 17: 395-401, 1981.

Killen, J.Y.: Systemic therapy of colorectal carcinoma. Current Concepts in Oncology. 3 (3): 17-22, 1981.

Killen, J.Y., Holyoke, E.D., Mittleman, A., et al: Adjuvant Therapy of Adenocarcinoma of the Colon Following Clinically Curative Resection: An Interim Report from the Gastrointestinal Tumor Study Group (GITSG). In Salmon, S.E. and Jones, S.E. (Eds.): Adjuvant Therapy of Cancer III. New York, Grune and Stratton, 1981, pp. 527-538.

Killen, J.Y., Hoth, D.F., Smith, F.P., Shein, P.S., Woolley, P.V.: Phase II studies of methyl-glyoxal-bis-guanylhydrazone in carcinoma of the colon and lung. Cancer (in press).

Killen, J.Y., Macdonald, J.S.: Investigational New Drugs in the Treatment of Advanced Small Cell Carcinoma. In Greco, F.A. and Bunn, P.A. (Eds.): Small Cell Lung Cancer. New York, Grune and Stratton, 1981, pp. 399-412.

Mednieks, M.D., Jungmann, R.A., DeWys, W.D.: Cyclic AMP-dependent phosphorylation and the control of leukemia L1210 cell growth. Cancer Res (in press).

Mitchell, E.P., Killen, J.Y., Smith, F.P., Willis, L.L., Schein, P.S., Woolley, P.V.: A phase II study of PCNU in colorectal carcinoma. Cancer Treatment Rep 65 (11-12): 1127-1129, 1981.

Muggia, F.M., DeWys, W.D.: Staging in testicular teratomas. In Whitehouse, J.M.A. and Williams, C.J. (Eds). Recent Advances in Clinical Oncology (in press).

Poplack, D.G., Sallan, S.E., Rivera, G., Holcenberg, J., Murphy, S.B., Blatt, J., Lipton, J.M., Venner, P., Glaubiger, D.L., Ungerleider, R.S. and Johns, D.: Phase I study of 2'Deoxycoformycin in acute lymphoblastic leukemia. Cancer Research 41: 3343, 1981.

Tormey, D.C., Gelman, R., Band, P.R., Sears, M., Bennett, J.M., DeWys, W., Perlia, C., Rice, M.A.: Comparison of induction chemotherapies for metastatic breast cancer: An Eastern Cooperative Oncology Group trial. Cancer (in press).

Ungerleider, R.S.: An introduction to cell markets in leukemia. Cancer Research 41: 4751, 1981.

Ungerleider, R.S., DeWys, W.D., and Fink, D.J.: Pediatric cancer and nutrition workshop: Introductory comments. Cancer Research 42: 698, 1982.

Weiss, R.B., Poster, D.S.: The renal toxicity of cancer chemotherapeutic agents. Cancer Treat Rev 9: 37-56, 1982.

Weiss, R.B., Poster, D.S., Penta, J.: The nitrosoureas and pulmonary toxicity. Cancer Treat Rev 8: 111-125, 1981.

Witman, G., Cadman, E.; Kapp, D; Wagner, F: Treatment of malignant glioma with cisplatin: Case report and therapeutic implications. Journal of Medical Hypothesis (in press).

Zwelling, L.A., Michaels, S., Erickson, L.C., Ungerleider, R.S., Nichols, M. and Kohn, K.W.: Protein associated DNA single-strand breaks in L1210 cells treated with the DNA intercalating agents, 4'-(9-Acridinylamino)-Methanesulfon-m-Anisidide (m-AMSA) and Adriamycin. Biochemistry 20: 6553, 1981.

7.0 Staff Presentations

William D. DeWys, M.D.

1. Nutrition in the Care of the Cancer Patient. American Cancer Society Symposium. Baltimore, Maryland, October 3, 1981.

2. Current Questions and Problems in Nutrition for the Patient with Cancer. Pediatric Oncology Branch Seminar. Bethesda, Maryland, January 19, 1982.

3. Chemotherapy of Head and Neck Cancer. University of Miami Symposium on Head and Neck Cancer. Miami, Florida, March 5, 1982.

4. Treatment of Prostatic Cancer. NCI - Navy Medical Oncology Branch Seminar. Bethesda, Maryland, April 8, 1982

5. Clinical Studies in Stage I and II Testicular Cancer. Northern California Oncology Group Semi-annual Meeting. Palo Alto, California, April 16, 1982.

6. Testicular Cancer: The Remaining Clinical Challenges. NCI Clinical Oncology Program Combined Rounds. Bethesda, Maryland, May 19, 1982.

7. Treatment of Head and Neck Cancer. NCI - Navy Medical Oncology Branch Seminar. Bethesda, Maryland, May 20, 1982.

8. Treatment of Prostatic Cancer. Walter Reed Army Hospital. May 24, 1982.

9. Diagnostic and Prognostic Studies in Stage I and II Testicular Cancer. 13th International Cancer Congress. Seattle, Washington, September 11, 1982.

10. Management of Anorexia. 13th International Cancer Congress. Seattle, Washington, September 11, 1982.

11. Nutritional Support of Cancer Patients. Symposium on "Medical and Social Considerations in the Treatment of Cancer Patients." Washington, D.C., September 29, 1982.

Edwin M. Jacobs, M.D.

1. Effect of TMCA (Trimethylcolchicinic Acid Methyl Ether d-Tartrate) On Hodgkin's and Non-Hodgkin's Lymphoma. Conference on a New Look At Older Drugs in Cancer Treatment. National Institutes of Health, Bethesda, Maryland, June 8, 1982

John Y. Killen, Jr., M.D.

1. Bladder Cancer Prevention: Thiotepa and Retinoids. U.S.-Japan Meeting on the Treatment of Bladder Cancer. Tokyo, Japan. November 16-18, 1981.

2. Pitfalls in Clinical Trials Design. U.S.-Japan Meeting on the Treatment of Bladder Cancer. Tokyo, Japan. November 16-18, 1981.

3. Carcinoma of the Colon. Medical Grand Rounds, Episcopal Hospital. Philadelphia, Pennsylvania, February 26, 1982.

4. The Current Status of Investigational Drugs at the National Cancer Institute. EORTC Early Clinical Trials Group. Amsterdam, The Netherlands, June 10-11, 1982.

Gary B. Witman, M.D.

1. Autologous Bone Marrow Transplantation. Medical Grand Rounds, The University of Ottawa, Ottawa General Hospital. July 25, 1981.

2. NCI Drug Development Program. National Cancer Institute of Canada. Kingston, Ontario, November 2, 1981.

Bimal C. Ghosh, M.D.

1. Current Concepts in Management of Malignant Melanoma. Howard University. Washington, D.C., July 1981.

2. Current Concepts in Management of Breast Carcinoma. Medical College of Pennsylvania. Philadelphia, Pennsylvania, December 1981.

3. Decisions in Surgical Oncology. 38th Annual Midwest Clinical Conference. Chicago, Illinois, March 1982.

4. Chairman, Scientific Program, and Moderator, Surgical Oncology, University of Calcutta Medical Association 6th Annual Meeting at Lake Ozark, Missouri, August 1982.

5. Immunohistochemical and Ultrastructural Study of Hormones, Enzyme and Protein in Primary and Metastasizing Breast Carcinoma. 13th International Cancer Congress. Seattle, Washington, September 1982.

Richard S. Ungerleider, M.D.

1. Suggested Agents for Phase II Studies in Children with Cancer. Children's Cancer Study Group, Montreal, Canada, October 12, 1982.
2. Suggested Agents for Phase II studies in Children with Cancer. Pediatric Oncology Group, Kissimmee, Florida, March 7, 1982.

8.0 Conferences

1. Kaposi's Sarcoma Workshop, 9/15/81

2. Surgical Oncology Workshop in Melanoma, 12/4/81

3. Adult Acute Non-Lymphocytic Leukemia, 12/10-11/81

4. Hepatic Artery Infusion Workshop, 1/22/82

5. Mechanism of Metastasis and Surgeon's Role, 6/2/82

6. Workshop on the Role of Computers in Cancer Clinical Trials, 7/8/82

7. Local Therapy of Malignancy Confined to the Liver, 9/28/82

# INVESTIGATIONAL DRUG BRANCH

The Investigational Drug Branch has the mission of sponsoring new investigational drugs for clinical trials and of evaluating them for antitumor efficacy. It does this by pursuing several objectives: (1) obtaining Investigational New Drug exemption (IND) authorization from the Food and Drug Administration (FDA), (2) sponsoring Phase I trials of new agents developed by the DCT, (3) planning with the Clinical Investigations Branch of CTEP Phase II trials in specific tumor types and subsequently monitoring the results of the clinical trials, (4) meeting FDA regulatory requirements for all active IND's, (5) regulating the distribution of investigational new drugs, and (6) maintaining close contact with the pharmaceutical industry participating in the development of new investigational drugs.

During this last year the Investigational Drug Branch underwent substantial reorganization. The Branch is now divided in five sections. Two medical sections, one for the cytotoxic agent and one for the biologic response modifiers are concerned with the clinical aspects of the drug development process; a Drug Regulatory Affairs Section provides a constructive interaction with the Food and Drug Administration; the Drug Management Section regulates the distribution of investigational new drugs to all NCI sponsored investigators, and finally a Quality Assurance and Compliance Section has the mission of monitoring all investigators performing clinical trials with NCI sponsored agents in order to assure data quality and investigator compliance to FDA and HHS regulations. The professional staff of the Branch includes six physicians, one pharmacologist, one biochemist, and two pharmacists.

During the past year monitoring of some 1300 protocols for more than 90 INDs continues. Seven new drugs entered Phase I testing.

Projects continuing since last year include the THC Group C distribution the award of the Phase I contracts (the new Phase I contractor list is given on the following page) and the Clinical Trial Monitoring Service. Details on the THC distribution are provided below. The CTMS is a computerized data base project which manages all clinical data on NCI Phase I trials. The data base is now available on-line to both the investigator performing the clinical trials and to the IDB. This system now contains data on more than 2000 patients.

The following major projects have been initiated this year. An application to designate m-AMSA in Group C for acute leukemia has been approved by the FDA. The distribution of the drug is now underway. The system for reporting of adverse drug reactions has been streamlined. During the current year the professional staff of the IDB has evaluated more than 300 adverse drug reactions. As a part of the CTEP a Phase II computerized data base has been developed which captures summary information on Phase II protocols sponsored by NCI. This resource will provide a significant increase in IDB's ability to manage clinical trials with investigational drugs. The liaison with the FDA has increased. Working meetings occur bimonthly to discuss problem areas. In addition, liaison with the Office for Protection from Research Risks has increased. Plans have

been developed to provide for routine review of informed consents in investigational drug trials by staff of the OPRR. In another major area the IDB has developed plans for site visit monitoring of all NCI investigational drug clinical trials. This will expand from the current program of site visit monitoring for Phase I trials into all Phase II and III drug trials. The purpose of this project is to assure the accuracy and quality of data submitted on investigational drug trials. To implement this a new section has been established, Quality Assurance and Compliance Section.

PHASE I/II WORKING GROUP

| Institution | Principal Investigator |
|---|---|
| Clinical Pharmacology Branch, NCI | C. Myers |
| Johns Hopkins Hospital | D. Ettinger* |
| Mayo Clinic | J. Kovach* |
| M. D. Anderson | G. Bodey* |
| Medicine Branch, NCI | R. Young |
| Memorial Sloan Kettering Cancer Center | C. Young* |
| NCI-Navy Medical Oncology Branch | J. Minna |
| Ohio State University | J. Neidhart* |
| Pediatric Oncology Branch | P. Pizzo |
| University of Maryland | D. Van Echo* |
| University of Texas, San Antonio | D. Von Hoff* |
| University of Vermont | I. Krakoff* |
| University of Wisconsin | D. Tormey* |
| Wayne State University | L. Baker* |

*Phase I funded contractors

Phase I Studies. Seven investigational drugs sponsored by the DCT were newly introduced into Phase I clinical trials.

5-Dihydroazacytidine (NSC-264880)
Homoharringtonine (NSC-141633)
Henkel's Compound (NSC-296934)
Tricyclic nucleoside (NSC-280594)
CBDCA (NSC-241240)
Echinomycin (NSC-526417)
N-Methylformamide (NSC-3051)

In addition, Phase I studies were continued from last year on the following drugs.

Cytoxan + WR-2721
PALA + Alanosine
24 Hr. m-AMSA
cis-platinum + WR-2721
Ara-A + 2'DCF
Spirogermanium 24 hr. infusion
Desmethylmisonidazole
Misonidazole + cytoxan
Bisantrene 72 hr. infusion

Four further drugs are expected to entered Phase I trials before the end of the year.

2-Fluoro-AMP
Taxol
Tiazofurin
Spirohydantoin

Approximately 120 Phase II clinical trials have been conducted under the sponorship of NCI and presented regularly at the Phase II meetings for the following drugs:

Aclacinomycin
AZQ
Mitoxantrone
Bisantrene
PCNU
Methyl-G
Alanosine
Deoxycoformycin
AT-125
Spirogermanium
Dichloromethotrexate

Activity has been defined for the following drugs in respective diseases: Mitoxantrone - Breast, Leukemias, Lymphomas, Hepatomas; AZQ Primary and Secondary Brain Tumors; Bisantrene - Breast; Methyl-G - Head and Neck, Lymphoma, 2'DCF - T-cell Lymphomas and Leukemias. Table 1 gives a summary overlook of the status of Phase II trials with the above mentioned drugs.

## DRUG REGULATORY AFFAIRS SECTION

For the fiscal year 1982, a Notice of Claimed Investigational Exemption for a New Drug (IND) was submitted to the Food and Drug Administration (FDA) for each of the following seven compounds:

| | |
|---|---|
| Tricyclic Nucleoside 5'-Phosphate | NSC 280594 |
| Homoharringtonine | NSC 141633 |
| Carboplatin (CBDCA) | NSC 241240 |
| Teroxirone (Henkels) | NSC 296934 |
| 5,6-Dihydro-5-Azacytidine | NSC 264880 |
| Echinomycin | NSC 526417 |
| N-Methylformamide | NSC 3051 |

The IND for 6-Methylmercaptopurine Riboside (NSC 40774) was reopened.

IND's for the following compounds were discontinued due to a lack of clinical activity and/or interest:

| | |
|---|---|
| TIC Mustard | NSC 82196 |
| Cycloleucine | NSC 1026 |
| D-Amygdalin (oral) | NSC 15780 |
| D-Amygdalin (IV) | NSC 251222 |

The following two IND's for compounds which are commercially available were discontinued:

| | |
|---|---|
| Diethylstilbesterol | NSC 3070 |
| Ara-C | NSC 63878 |

The Drug Regulatory Affairs Section also supported IND-related activities of the Biological Response Modifiers Program, DCT. This support included preparation and maintenance of IND's and support and monitoring of drug distribution.

The following IND's have been filed with the Bureau of Biologics for the Biological Response Modifiers Program:

| | |
|---|---|
| Interferon (Warner Lambert Leukocyte) | NSC 340855 |
| Interferon (Burroughs Wellcome Lymphoblastoid) | NSC 339140 |
| Interferon (Meloy Leukocyte) | NSC 335044 |
| Interferon (Meloy Immune) | NSC 354655 |
| T101 Monoclonal | NSC (not yet assigned) |

Distribution of Delta-9-Tetrahydrocannabinol (THC)

With the approvals of both the Oncology Advisory Committee and the Food and Drug Administration, Delta-9-Tetrahydrocannabinol (THC), NSC 134454, was placed under the NCI's Group C Distribution Mechanism for use as an antiemetic in cancer chemotherapy. Guidelines for THC use and a procedures document for distribution were developed.

Because THC is also a Schedule I drug, concurrence on distribution procedures was obtained from the Drug Enforcement Administration (DEA).

Briefly, THC is distributed only to hospital pharmacies which meet DEB standards and are specifically registered for THC distribution. To date, there are 840 hospital pharmacies registered (about 10% of all JCAH accredited hospitals) dispensing THC for 2,340 approved physicians, supplying 198,000 5 mg capsules to approximately 12,000 patients.

Distribution of THC as an antiemetic under state and individual investigator studies is also a DEB function. Currently, there are eleven state controlled studies and ten individual investigator studies in active status.

DEA also distributes marijuana cigarettes for use in cancer chemotherapy; however, requests for cigarettes have been minimal.

New Drug Studies

There are forty four Cancer Centers and thirteen New Drug Studies Groups participating in the New Drug Studies Mechanism. This represents a slight increase over last year. These institutions submitted a total of eighty eight protocols, of which sixty seven were activated in fiscal '82. Currently, there are 290 protocols in active study.

| | Acute Leukemia | Breast | Colon | Lung SC | Lung NSC | Lymphomas | Melanoma | H&N | Brain | Gastric |
|---|---|---|---|---|---|---|---|---|---|---|
| Alanosine | * | 0 | - | 0 | 0 | 0 | - | - | | 0 |
| PCNU | 0 | - | - | - | - | 0 | + | + | + | 0 |
| Spirogermanium | 0 | - | - | 0 | 0 | + | 0 | 0 | 0 | 0 |
| AZQ | - | + | - | - | + | - | - | * | + | 0 |
| AT-125 | 0 | - | * | 0 | * | 0 | 0 | | | |
| Methyl G | + | - | - | - | + | + | - | + | * | +* |
| ADAH | * | + | - | * | - | * | * | * | | * |
| DHAD | * | * | - | <5%PR | <5%PR | + | <5%PR | * | | * |
| DCF | + | 0 | 0 | 0 | 0 | +† | 0 | | | |

0 = Not Tested
+ = Tested, positive data
- = Tested, negative data
* = Ongoing studies, too early for results
If blank, not tested

† = Mycosis fungoides

| | Hepatic | Pancreatic | Renal | Bladder | Prostate | GYN | Ovarian | Myeloma | Sarcoma |
|---|---|---|---|---|---|---|---|---|---|
| Alanosine | 0 | 0 | * | 0 | 0 | | * | 0 | 0 |
| PCNU | + | + | 0 | 0 | 0 | | - | 0 | 0 |
| Spirogermanium | 0 | 0 | - | 0 | 0 | | - | 0 | 0 |
| AZQ | 0 | 0 | - | - | - | | * | * | * |
| ADAH | * | 0 | <5% | * | 0 | 0 | * | * | 0 |
| DHAD | * | - | - | 0 | * | - | - | + | <5% |
| Methyl G | 0 | + | - | - | * | *†† | * | * | * |

0 = Not Tested
+ = Tested, positive data
- = Tested, negative data
* = Ongoing studies, too early for results
If Blank, not tested

† = Active H&N, and esophagus
†† = Endometrial

STAFF PUBLICATIONS:


1. Duque-Hammershaimb, L. and Hoth, D.: Chlorozotocin: clinical trials. In Prestayko, A.N, Crooke, S.T., Baker, L.H., Carter, S.K., And Schein, P.S. (Eds.): Nitrosoureas: Current status and new developments, 1981, pp. 387-398.

2. Hammershaimb, L. and Witman, G.: Autologous Bone Marrow Transplantation. Workshop. J. of Nat'l. Cancer Inst., 1981 (in press).

3. Macdonald, J.S., Marsoni, S., Bruno, S., and Poster, D.: Current status of clinical trials of m-AMSA, dihydroxyanthracenedione, and deoxycoformycin. In Recent Results in Cancer Research, Springer-Verlag, Berlin, Heidelberg, New York, 1981 pp. 323-330.

4. Macdonald, J.S., Weiss, R.B., Poster, D., and Hammershaimb, L.: Subacute and chronic toxicities associated with nitrosourea therapy. In Prestayko, A.W., Crooke, S.T., Baker, L.H., Carter, S.K., and Schein, P.S. (Eds.): Nitrosoureas: Current status and new developments, 1981 pp. 145-154.

5. Penta, J.S., Poster, D., Bruno, S., and Macdonald, J.S.: Clinical trials with antiemetic agents in cancer patients receiving chemotherapy. J. Clin. Pharmacol. 21: 11S-22S 1981.

6. Poster, D.S., Bruno, S., Penta, J.S., Neil, G., and McGovern, P.J.: Acivicin: An antitumor antibiotic. Cancer Clin. Trials 4: 327-330, 1981.

CONTRACTOR PUBLICATIONS:

1. Dosik, G.M., Stewart, D., Valdivieso, M., Burgress, M.A., and Bodey, G.A.,: Phase I study of L-Alanosine using a daily X 3 schedule. Cancer Treatment Rpts., 66: 73-76, 1982.

2. Hart, R.D, Ohnuma, T., Holland, J.F., and Bruckner, H.: Methyl-GAG in patients with malignant neoplasms: A Phase I re-evaluation. Cancer Treatment Rpts., 66: 65-71, 1981.

3. Kovach, J.S., Eagan, R.T., Powis, G., Rubin, J., Creagan, E.T., and Moertel, C.G.: Phase I and pharmacokinetic studies of DON . Cancer Treatment Rpts., 65: 1031-1036, 1981.

SUMMARY REPORT
ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
October 1, 1981 - September 30, 1982

## I. Introduction

The Radiation Research Program was formally approved by the Secretary for Health and Human Services on May 17, 1982. The proposal to establish the Radiation Research Program within the Division of Cancer Treatment was based on recognition of the potential scientific advantages of coordinating radiation research activities and the need to provide an organizational context which would emphasize and encourage a more coherent approach to research in these areas.

For much of FY82, the three Branches which comprise the Radiation Research Program were located in the Office of the Director, Division of Cancer Treatment, and overall coordination of the activities of these Branches was provided by Dr. David A. Pistenmaa as the Program Director for Radiation Research. The three Branches include the Radiotherapy Development Branch (formerly located in the Cancer Therapy Evaluation Program), the Diagnostic Imaging Research Branch, and the Low-Level Radiation Effects Branch (formerly located in the Office of the Director, NCI).

The mission of the Radiation Research Program is the planning, development, administration, and evaluation of an extramural radiation research program through establishing program priorities, allocation of resources, maintaining project integration, evaluation of program effectiveness, and representing the program area in the management and scientific decision making processes of the National Cancer Institute. This requires the coordination of research program activities with related programs elsewhere at NCI and NIH, with other Federal agencies, and with national and international research organizations. The RRP also provides a focal point within NIH for extramural investigators nationally and internationally on radiation research.

The staffing of the new Program was accomplished through the transfer of staff along with the transfer of the scientific responsibilities of the three Branches.

## II. Personnel

1. David A. Pistenmaa, M.D., Associate Director
2. Ms. Bonnie R. Jenkins, Secretary to Associate Director
3. Ms. Dorothy A. Tisevich, Acting Administrative Officer
4. Ms. Louise E. Patten, Budget Assistant
5. James L. Hamner, M.D., Special Assistant
6. Victor H. Zeve, Ph.D., Special Assistant
7. Ms. Elizabeth F. Swerda, Secretary to Special Assistants
8. Ms. Wanda Baylor, Stay-in-School

III. Current Activities

A. The major activities of the OAD have been devoted to organizing radiation activities in DCT and NCI and to developing new initiatives for the Radiation Research Program.

B. Responsibilities outside of DCT are as follows:

a. Radiation Coordinating Group (RCG)

The RCG was established in February 1982 to assure an integrated NCI approach to cross-cutting radiation matters. The RCG will include the following as standing members:

David A. Pistenmaa, M.D., Chairman
Elliott H. Stonehill, Ph.D., Co-Chairman
Victor H. Zeve, Ph.D., Executive Secretary
Roger S. Powell, DIRB, RRP, DCT
Francis J. Mahoney, Ph.D., RDB, RRP, DCT
Oddvar F. Nygaard, Ph.D., LLREB, RRP, DCT
Eli Glatstein, M.D., ROB, COP, DCT
Gilbert Beebe, Ph.D., FSSP, DCCP

b. Interagency Radiation Research Committee (IRRC)

Dr. Pistenmaa serves as the Executive Secretary to this Committee which is Chaired by the Director, NIH. Dr. Zeve is his alternate and is handling the two subcommittees which are primarily concerned with 1) planning the implementation of the Federal Strategy for Research into the Biological Effects of Ionizing Radiation and the second subcommittee 2) establishing common procedures for funding and managing radiation research as well as establishing common methods of scientific review of research proposals throughout the Federal Government. The Radiation Research Program, DCT, NCI, provides staff and other resources to support these efforts.

IV. Plans

A. Personnel

Recruitment is underway for Chiefs for each of the three Branches in the RRP. In the interim Dr. Pistenmaa will serve as Acting Chief, RDB; Dr. Murray as Acting Chief, LLREB; and Mr. Powell as Acting Chief, DIRB.

Senior scientists are being recruited for temporary assignments in all three Branches. It is anticipated that the following will join RRP for one year:

1. Dr. Glenn Sheline, Deputy Director, Division of Radiation Oncology, UCSF, joins RDB in August 1982.

2. Dr. Gabriel Wilson, Chairman, Department of Radiology, UCLA, joins DIRB in September 1982.

3. Dr. Alfred Smith, Chief, Medical Physics Section, Department of Radiotherapy, UNM, joins RRP in September 1982.

4. Dr. Robert Burt, Professor of Nuclear Medicine, University of Indiana, joins DIRB in July 1982.

B. Long-Range Program Planning

The major responsibilities of the senior scientists noted above will be to develop long-range research programs for the RRP. This will include strengthening of liaison activities with other BID's in NIH, with other Federal agencies, and with private scientific research institutions and professional organizations.

# DIAGNOSTIC IMAGING RESEARCH BRANCH

## I. Introduction

The Diagnostic Imaging Research Branch (DIRB) of the Radiation Research Program was formed in October 1981 to serve as a focus within the National Institutes of Health for the development, application, and evaluation of modern technological advancements in medical imaging through the administration of a wide variety of grant- and contract-supported extramural programs of basic and applied research.

## II. Personnel

1. Roger S. Powell, M.S., Acting Chief and Program Director for Nonionizing Radiation
2. Matti Al-Aish, M.A., Ph.D., Program Director for X-Ray Imaging
3. Ms. Bernice L. Nasoff, Secretary

## III. Research Program

The research program supported by the DIRB consists of three contracts and approximately 55 grants.

### A. Grants

The funding for grants in FY81 is distributed among the major areas of investigation as follows:

| Area of Investigation | FY81[a] |
|---|---|
| X-Ray Imaging | $3,900 |
| Nuclear Medicine | 500 |
| Imaging with Non-Ionizing Radiations | 3,015 |
| Total | $7,415 |

[a]Dollars in thousands; estimates.

### B. Contracts

The three contracts supported by DIRB are as follows:

1. University of Kansas - Development of Contrast Agents for Use in Clinical Ultrasonic Diagnosis

2. New York State University - Development of Electrophoretic Display Cell for Clinical X-Ray Imaging

3. Stanford University - Microbubble Contrast Agents in Ultrasonic Imaging of Tumors

## IV. New Initiatives

Rapid progress has been made in the creation and improvement of imaging systems using the techniques of nuclear magnetic resonance, computer assisted tomography, positron emission tomography, ultrasound, thermography, nuclear medicine, and others. These new imaging improvements are aimed at earlier and more accurate detection and diagnosis of diseases of all kinds, including cancer; the screening of asymptomatic subjects; in interventional radiology; and as aids in the planning and assessment of treatment.

Most of these research programs have been carried out under grant support. However, new initiatives planned for FY83 will be supported by contract programs as follows:

A. Comparative Clinical NMR Imaging Studies

An evaluation of the emerging technology of nuclear magnetic resonance (NMR) imaging will be accomplished by comparing the clinical application of NMR imaging with the results of examinations with other modalities such as ultrasound, positron emission tomography, or CAT scanning.

B. Development of New and Improved Nonionizing Imaging Systems for the Detection of Breast Cancer

The development of new and improved imaging instruments optimized specifically for breast examination utilizing nonionizing techniques, including ultrasound, nuclear magnetic resonance imaging, thermography, and diaphanography, will be carried out at a number of institutions.

# LOW-LEVEL RADIATION EFFECTS BRANCH

## I. Introduction

The Low-Level Radiation Effects Branch (LLREB) is concerned with research that will provide new and relevant information on the molecular and cellular processes leading to mutagenesis, cell transformation, and carcinogenesis by ionizing radiation, in particular at low doses or low dose rates. Both cellular and whole animal studies are used, but fundamental biophysical and radiochemical investigations are also supported as well as selected epidemiological studies. The LLREB is the direct successor of the low-level radiation office originally established in the Office of the Director in 1979 in response to Public Law 95-622.

## II. Personnel

1. James L. Murray, D.V.M., M.S., Acting Chief
2. Oddvar F. Nygaard, Ph.D., Radiation Biologist
3. Kenneth L. Mossman, Ph.D., Radiation Biologist
4. Paul W. Todd, Ph.D., Intermittent Expert, Biophysics
5. Ms. Devirah Goodrich, Secretary

## III. Low-Level Radiation Effects Research Program

The LLRE research program is composed of both contracts (including interagency and intraagency agreements) and conventional investigator-initiated research grants. Since prior to becoming established as a Branch the low-level radiation program did not have the facilities and manpower to handle grants, the current program consists primarily of contracts. A better balance is expected to be achieved in the near future as a result of favorable responses to a recent program announcement and to the transfer to the LLREB of selected low-level radiation-oriented grants from other branches and divisions.

### A. Contracts

The bulk of the contracts issued by the LLREB are interagency agreements with the Department of Energy (DOE) awarded to investigators carrying out research of several of the National Laboratories. Two projects are also supported by intraagency agreements with FDA/Bureau of Radiological Health.

Brief summaries of the currently active contracts are given below:

ENERGY, DEPARTMENT OF (Y01-CO-00320) Start Date 6/20/80

The primary objective of this Interagency Agreement is to measure the late effects of low doses of ionizing radiation in a large, relatively long-lived animal, the dog, to aid in assessing hazards and understanding mechanisms of radiation damage in man. These studies address two basic problems associated with exposure to low doses of ionizing radiation: 1) obtaining reliable data on biological responses at low dose rates when exposures are protracted and 2) extrapolation of data from experimental animals to man. The studies use beagles to test whether the results from studies with shorter-lived, smaller, and more radiation resistant rodents do, in fact, establish a constant radiation injury parameter, at low doses and dose rates that is characteristic for all mammalian species. The data from these studies will help determine whether species differences in sensitivity (the dog is 3 times as sensitive as the mouse at high doses and dose rates) are a consideration in extrapolation to man. This study will be completed in about six years.

ENERGY, DEPARTMENT OF (Y01-CO-00321) Start Date 9/22/80

This Interagency Agreement is for support of the Tritium Toxicity Program at Brookhaven National Laboratory. The objective of this program is to evaluate the genetic and late somatic effects resulting from long-term ingestion of low concentrations of tritiated water. Tritium produces beta particles with a spectrum of energies and has an estimated physical half-life of 12.3 years. Previous studies indicate that the relative biological effectiveness (RBE) of tritium varies from about 1 to 3. The world inventory of tritium is expected to increase in future years and the dose rate to human tissues, although still below 1 mrem/year, is expected to increase steadily. The program involves chronic exposures to mice with tritiated water and studies of cytogenetics, reproductive efficiency, somatic effects, RBE of tritium, biochemistry, and microdosimetry. This project will be completed at the end of FY82.

FOOD AND DRUG ADMINISTRATION (Y01-CO-10700) Start Date 2/1/81

This is an Intraagency Agreement with FDA/Bureau of Radiological Health to provide support for their study entitled "Follow-up Study of Patients Who Had 131-Iodine and Other Diagnostic Procedures During Childhood and Adolescence." This is a nationwide study in cooperation with at least 20 medical centers to follow children exposed to diagnostic levels of 131-iodine between 1946 and 1967. The goal is to determine if there is an increased risk of thyroid neoplasia in these people associated with their exposures. Endpoints other than neoplasia are being examined, and a dose-response analysis will be made. This study will cover approximately 6,000 exposed patients and two controls per patient. This support agreement will end in FY82, and the data analysis should be completed by BRH/FDA in FY83.

ENERGY, DEPARTMENT OF (YO1-CO-10711) Start Date 9/20/81

This Interagency Agreement supports research at Brookhaven National Laboratory which is designed to describe quantitatively and to understand the shapes of dose response curves for genetic effects of ionizing radiation of different linear energy transfers (LET) radiations at low doses or at high doses and low dose rates. The cells studied will be those of the stamen hairs of the plant Tradescantia, a system for which the genetics are well understood. The fraction of cells that change color from blue to pink as a result of irradiation can be measured with high precision at low doses (1 rad or less of gamma rays). No other biological system has this sensitivity. These experiments should give a firm experimental and theoretical base to the effects of low levels of environmental radiation in producing genetic, and presumably carcinogenic, effects in higher eukaryotic systems. The duration of this study will be four years.

ENERGY, DEPARTMENT OF (YO1-CO-10712) Start Date 9/20/81

This Interagency Agreement is for support of research at Brookhaven National Laboratory. The experimental protocol to irradiate different strains of mice with single or repeated doses and with a wide range of dose rates to determine 1) the incidence of leukemia at low average dose rates; 2) the presence of preleukemic cells in the mice as a function of total dose and dose rate; 3) the number of preleukemic cells initiated by radiation; 4) the relative degree of "repair" following single, repeated, and continuous exposure; and 5) if there remains a fraction of the effects of low LET radiation that is nonrepairable or comparable to the "single hit" damage and effects seen with high LET radiation. The study is also designed to measure the incidence of preleukemic cells rather than waiting for the development of overt leukemia. Preleukemic cells can only be detected early by injection into a lethally or sublethally irradiated mouse, whereas the frankly leukemic cells grow later but equally well in normal or irradiated hosts. An aim is to determine if preleukemic cells are present at very low average dose rates, whereas frank leukemia may not be detectable perhaps because of statistical limitations. The duration of this study will be five years.

ENERGY, DEPARTMENT OF (YO1-CM-20111) Start Date 3/1/82

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Radiation-induced Myelogenous Leukemia." This project is concerned with the dose-response relationships and the mechanisms of neutron radiation-induced myelogenous leukemia in mice. Neutrons and other high-LET radiations are considered more effective for induction of tumors than low-LET radiations. Since survivors of the atomic bomb at Hiroshima were exposed to both gamma rays and neutrons, attempts have been made to deduce the leukemogenic effects of neutrons from the Japanese data, but the neutron dose estimates for Hiroshima have recently been questioned and may be revised. It is essential to obtain data for neutron radiation-induced leukemia in experimental animals. This study will be completed in four years.

ENERGY, DEPARTMENT OF (YO1-CM-20112) Start Date 3/1/82

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Extrapolation of Radiation Risk." This study will address a question of fundamental importance to the understanding of mechanisms of carcinogenesis and to risk estimation; namely, whether the natural incidence of cancer influences the susceptibility to induction by radiation or by other carcinogenic agents. The investigators will determine whether the natural incidence of specific cancers influences the susceptibility to induction by gamma radiation in two different strains of mice. This study will be completed in five years.

ENERGY, DEPARTMENT OF (YO1-CM-20113) Start Date 3/1/82

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Co-Carcinogenesis: Ionizing and Ultraviolet Radiation." This study will determine quantitatively the interactions of ultraviolet radiation and ionizing radiation (x-rays and fission neutrons) in terms of production of skin cancer in hairless mice. The persistence of initiated cells in skin will be investigated after exposure to doses of ionizing radiation that alone do not result in an increase in the incidence of skin cancer. These cells may be promoted later to cancer cells by other agents. This study will be completed in five years.

FOOD AND DRUG ADMINISTRATION (YO1-CM-20115) Start Date 6/16/82

This Intraagency Agreement is for partial support of a continuing study of malignancy as a cause of death in beagle dogs given whole body irradiation during development. This is part of a comprehensive study of the long-term effects of prenatal and postnatal gamma radiation exposures conducted at the Collaborative Radiological Health Laboratory at Colorado State University and sponsored by the FDA Bureau of Radiological Health. This partial support is currently scheduled to continue for two years.

UNIVERSITY OF UTAH (NO1-CO-23917) Start Date 6/30/82

This is a new research contract for "Assessment of Leukemia and Thyroid Disease in Relation to Fallout in Utah." The purpose of this project is to conduct a detailed reassessment of the possible long-term effects of radioactive fallout resulting from atmospheric weapons testing at the Nevada Test Site between 1950 and 1962. This contract includes 1) a study of milk consumption patterns in relation to milk sources and fallout exposures, 2) a case-control study of thyroid cancer in Utah, 3) a cohort study of malignant and benign thyroid disease, 4) a case-control study of leukemia in Utah, and 5) a cohort mortality study of leukemia in Utah. This project will be completed in five years.

B. Grants

As a part of the reorganization of radiation research within NCI into a coherent Radiation Research Program, the portion of the existing radiation biology grant portfolio dealing with mutagenesis, transformation, and carcinogenesis will be transferred to the LLREB. In addition, a number of responses have been received to the LLREB-generated Program Announcement entitled "Experimental Research Related to Biological Effects of Low Doses of Ionizing Radiation" published September 4, 1981. For the November 1, 1981 and the March 1, 1982 application deadlines 7 and 14 direct responses were received, respectively, with three grants awarded from the first group and several more likely to be awarded from the second group. A number of additional high-quality applications relevant to the LLRE research program were also received by NIH for these review cycles. Furthermore, new applications are still being received in response to the original Program Announcement.

In the absence of firm figures, we estimate that grants assigned to the LLREB by the end of FY82 will total approximately $7 million.

IV. Committees and Working Groups

A. Interagency Radiation Research Committee (IRRC). Dr. Nygaard is designated by NIH as Special Staff Consultant to this Committee. In addition, both Dr. Nygaard and Dr. Mossman are identified as alternates (for Dr. Pistenmaa) on the Radiation Research Strategy Implementation Subcommittee of the IRRC. Finally, Dr. Nygaard is the Chairman of the IRRC Three-Mile Island Followup Research Subcommittee.

B. NIH Plan for Response to PHS Call For Assistance In Technological Emergencies and Disasters. Dr. Nygaard has been designated as the NCI coordinator and focal point for radiation emergencies (with Dr. Pistenmaa as alternate).

C. NCI Conference on "Radiation Carcinogenesis - Epidemiology and Biological Significance" (sponsored by DCCP/NCI May 24-26, 1982). Dr. Nygaard (and Dr. Pistenmaa) was a member of the Conference Organizing Committee.

D. "First Conference on Radioprotectors and Anti-Carcinogens" at National Bureau of Standards, June 21-24, 1982. Dr. Nygaard is designated as Co-Chairman of the Conference and will be senior editor of the published proceedings.

V. Workshop

The LLREB has brought together experts in various areas of the fields of radiation biology and related sciences for the purpose of identifying areas of research that may offer opportunities for new initiatives relevant to the program of the Branch. In FY82 the following workshop was held:

| | | |
|---|---|---|
| June 27-29, 1982 | Mammalian Mutagenesis of Low Doses of Ionizing Radiation | Argonne National Laboratory, IL |

VI. New Initiatives

Plans for new initiatives in low-level radiation research in FY83 include the following:

A. "Neoplasia in Beagle Dogs After Acute Fission Neutron Irradiation" (RFP)

B. "Carcinogenesis in Small Animals Irradiated In Utero" (RFA)

C. "Oncogene Products in Irradiated Cells and Tissues" (Program Announcement)

D. "Specific Radiation-Induced Chromosomal Abnormalities and Cancer" (Program Announcement)

RESEARCH BUDGET OF LOW-LEVEL RADIATION EFFECTS BRANCH[a]

| | |
|---|---|
| Grants to be Transferred (estimate) | $ 7,000,000 |
| Grants - New | 386,000 |
| Contracts | 3,061,000 |
| Total Research Budget | $10,447,000 |

[a]Dollars in thousands

# RADIOTHERAPY DEVELOPMENT BRANCH

## I. Introduction

The Radiotherapy Development Branch (RDB) is concerned with the basic, applied (pretherapeutic), and clinical development of cancer treatment modalities utilizing ionizing and nonionizing radiations and the investigation of means of enhancing the biological effects of these radiations. The RDB supports the basic and applied pretherapeutic research and development activities by grants or contracts and uses similar funding mechanisms to introduce new or improved conventional radiotherapeutic modalities or techniques into clinical trials.

## II. Personnel

1. David A. Pistenmaa, M.D., Ph.D., Acting Chief
2. Francis J. Mahoney, Ph.D., Deputy Chief/Program Director for Radiation
3. Thomas A. Strike, Ph.D., Project Officer for Brain Tumor Study Group
4. Richard L. Cumberlin, M.D., Cancer Expert
5. George A. Alexander, M.D., Cancer Expert
6. Ann L. Huang, Ph.D., Assistant Program Director
7. Ms. Janet W. Johnson, Technical Assistant
8. Ms. Maureen L. Volz, Statistical Assistant for Brain Tumor Study Group

## III. Radiotherapy Development Branch Research Program

The Radiotherapy Development Branch research program is composed of individual investigator grants, program project grants, cancer research emphasis grants, and contracts. The total costs of this program in FY 1981 and FY 1982 are shown in Table 1.

### A. Contracts

A brief summary of contracts and FY 1982 awards are as follows:

1. Phase II Study of Photoradiation Therapy-Roswell Park Memorial Institute (NO1-CM-97311) and University of California(NO1-CM-27483)

   These contracts were recompeted early this year and two awards were made. These contracts will evaluate the use of photoradiation as a local treatment modality for various malignancies. Twenty patients per year are to be accrued to a Phase II clinical trial for the next three years, and the scope and limitations of photoradiation therapy will be evaluated. The progress of both contractors this year has been satisfactory.

2. Radiosensitizers and Radioprotectors

a. Radiosensitizer Synthesis and Testing-Institute for Cancer Research Sutton, England (NO1-CM-17502) and Stanford Research Institute (NO1-CM-17485)

The objective of these contracts is to develop new and/or improved radiosensitizers. One of the compounds, the most optimal of the nitroimidazole class, SR-2508, will complete toxicology and be put into the clinic early next year. Several other potential radiosensitizers developed under these contracts will undergo in vivo testing in the radiosensitizer screen. The emphasis of these contracts is to move away from the nitroimidazole class of radiosensitizers. Both contracts are exploring and synthesizing drugs in several other classes for testing as radiosensitizers. The progress of both contractors has been highly satisfactory during the first year of a three-year award that resulted from recompetition of these contracts last year.

b. Screening of Radiosensitizers-Arthur D. Little, Inc.(NO1-CM-07257)

The objective of this contract was to identify new hypoxic cell sensitizers or other types or classes of radiosensitizers which act by a different mechanism. Compounds may undergo in vitro and/or in vivo testing after various physical-chemical properties are determined and evaluated. During this reporting interval, 71 compounds were sent to the contractor for evaluation. Physicochemical characterization was completed on 41 of those compounds and 12 were selected for in vitro screening. In addition, 8 compounds with known radiosensitizer properties (cell and animal models) were sent to the contractor for in vivo testing. This testing is necessary to evaluate these compounds in three different tumor systems with three different endpoints, a requirement of the linear array for moving these compounds into the decision network. Progress on this contract has been outstanding during this interval. This contract is in the final year and will be recompeted.

c. Screening of Radioprotectors-Fox Chase Cancer Center(NO1-CM-07330)

The objective of this contract was to identify compounds or classes of compounds which increase the therapeutic ratio of radiotherapy more than the reference radioprotector WR-2721 or compounds which protect tissues not protected by the reference compound. Various in vitro and in vivo testing is utilized in this screen to further evaluate promising compounds. A total of 15 compounds were entered into the screening program during this period. To date, none of the compounds tested are better than WR-2721 and none afford any unique type of protection. The major problem with this contract is still the ability to provide sufficient amounts of drug for testing. The contractor is investigating a red-cell model which may resolve this problem. Progress on this contract has been satisfactory during this interval. Next year is the final year for the current contract, and recompetition will take place at that time.

3. Clinical Neutron Therapy Program

a. Construction of the facility at Fox Chase Cancer Center (NO1-CM-97314) was completed in October 1981. The DT generator tube was shipped to Philadelphia in April 1982. The first beam was achieved on May 5, 1982. On May 6, 1982, the pressure in the DT generator tube began to rise indicating a leak. The tube was returned immediately to Berkeley. The leak was a minor one and the tube was returned to Philadelphia on May 25. Protocols for physics checkout and radiobiological testing of the neutron beam were completed in July 1982. The treatment of patients will begin in August 1982.

b. UCLA has identified the necessary funds required to proceed with construction of the facility. The Cyclotron Corporation estimates that the cyclotron should be completed on 11/7/82. Inplant testing will begin in September 1982. Assuming that inplant testing goes well, the unit should be ready for shipment about 1/1/83. There will be a site visit at The Cyclotron Corporation facility in Berkeley, California, between 8/25/82 and 9/15/82 to insure that the cyclotron will be delivered on time. Patient treatment should begin in late 1983.

c. The construction at the University of Washington (NO1-CM-97282) started this spring and will be completed this winter. Fabrication of the cyclotron and patient treatment system is proceeding well in Europe. The isocentric gantry will be delivered on or about 9/20/82. The cyclotron and other components will be moved into position in late November. The system will be assembled in Seattle in December 1982. Patient treatment is expected to start in the spring of 1983--six months ahead of schedule.

B. Clinical Research Activities

1. Brain Tumor Study Group

a. The Brain Tumor Study Group (BTSG), a contract-supported clinical trial cooperative effort consisting of 7 medical centers, evaluates multimodality treatments of malignant brain tumors. Patient accrual into Phase II and Phase III protocol studies continued to be excellent during this period. Approximately 250 newly diagnosed patients having malignant gliomas were randomized into the Phase III protocol (BTSG 80-01). This protocol was activated in November 1980 and has accrued 440 patients to date. An additional 120 patients with recurrent malignant gliomas were randomized to the Phase II protocols (BTSG 78-20 and 81-20). The latter protocol was activated on 1/4/82. Analysis of the previous Phase III protocol (BTSG 75-01) has been completed and accepted for publication in Cancer Clinical Trials. FY83 is expected to be a "phase-out" year for this cooperative clinical trial group because of budget constraints.

Brain Tumor Study Group [Indiana University NO1-CM-17475; New York University (NO1-CM-17473); Memorial Sloan-Kettering Cancer Center (NO1-CM-17348); University of Tennessee (NO1-CM-17472); Montefiore Hospital - University of Pittsburgh (NO1-CM-17474); University of Iowa (NO1-CM-17476); University of North Carolina (NO1-CM-17471)]

b. Neuropathology Coordinating Center [Duke University (NO1-CM-17477)]

This contract provides neuropathology support for the clinical trials being conducted by the Brain Tumor Study Group. The contractor provides a coordinating center where the surgical and autopsy pathology specimens from patients randomized to BTSG protocols are read and diagnosed. Pathology reports from the neuropathology coordinators constitute the final pathology for study patients. During the past year the contractor continued to read the pathological material submitted and render a report in an expeditious manner. In addition, the contractor has investigated and published the relationship of various pathological characteristics of brain tumors on survival.

c. Data Management and Statistical Support for the Brain Tumor Program [Information Management Services, Inc. (NO1-CM-17349)]

During this period the contractor updated the Brain Tumor Study Group data files prior to generating the multitude of data reports required for the BTSG Fall 1981 meeting. All required lists, tables, figures and charts were submitted to the Project Officer in a timely way so that the preparations for this meeting proceeded smoothly. Following the BTSG meeting, the contractor continued to update the protocol data files by keypunching the recently submitted reporting forms and editing the data before adding it to the permanent data base. Ad hoc data requests by the Project Officer were handled in an expeditious fashion.

2. Radiation Therapy Oncology Group

The Radiation Therapy Oncology Group is a grant-supported clinical cooperative group which emphasizes radiation therapy clinical research. Although protocols do involve chemotherapy and/or surgery, the primary thrust of the RTOG is radiation therapy-related research. Major areas of effort include 1) improvement of low LET radiotherapy through radiosensitizers and radioprotectors as well as hyperthermia and 2) high LET radiotherapy research. The latter activity is supported in part through a separate P01 grant. The clinical high LET radiotherapy research program is integrated insofar as possible into the overall plan for the Group. The Program Director is Dr. Edwin M. Jacobs, CTEP.

3. Radiological Physics Center [Shalek; (5 U10 CA 10953-14)]

The Radiological Physics Center, well established as a most important resource for the clinical cooperative groups, checks calculations and treatment plans and assists in the calibration of radiotherapy machines and other equipment in radiotherapy facilities.

C. New Initiatives in FY 1982

a. Phase I Evaluation of Equipment for Hyperthermic Treatment of Cancer [University of Arizona (NO1-CM-17522); Massachusetts Institute of Technology (NO1-CM-27525); M. D. Anderson Hospital and Tumor Institute (NO1-CM-17524); University of Utah (NO1-CM-17523); and Stanford University (NO1-CM-17480)]

During the first year of this effort the contractors have developed the approach they will utilize for the various equipment they have available for testing. Many of the problems were discussed at the two group meetings held during this year. Committees were organized to resolve some of the existing problems; i.e., forms for data collection, data storage, standardized approaches to thermometry, etc. Progress on these contracts is satisfactory.

b. Intra-Agency Agreement: [Food and Drug Administration, Bureau of Radiological Health (YO2-CM-20107)] Technical Support for Evaluation of Hyperthermia Equipment

Under this agreement the Bureau of Radiological Health (BRH) will provide advice in the selection of heat generating and thermometry equipment to be evaluated by the five contractors selected to make these evaluations. The BRH shall perform sufficient measurements on all types of heat generating equipment (ultrasound, radiofrequency, or microwave) which are selected for study to fully characterize and calibrate the equipment in terms of heat generation, leakage radiation, thermal fields, energy requirements and reproducibility. The BRH shall perform measurements to calibrate thermometry equipment to be used in the project and provide administrative support for the receipt and shipping of equipment from investigators to BRH laboratories in Rockville, Maryland, and their return. The BRH shall provide technical support and consultation in the evaluation of reports prepared by contractors. This intra-agency agreement is for five years and will be active during the period the hyperthermia equipment is being evaluated by the five existing contractors.

c. Intraoperative Radiotherapy [Massachusetts General Hospital (NO1-CM-17481); Mayo Clinic (NO1-CM-27528); Howard University (NO1-CM-27543)]

This project consists of two tasks. Task A is the investigation of the role of intraoperative radiotherapy in the treatment of intraabdominal malignancies according to carefully defined surgical, pathological, and radiotherapy criteria and the development of guidelines for intraoperative radiotherapy techniques and their use for the irradiation of intraabdominal malignancies. Task B is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy in the treatment of intraoperative malignances. The contractors, along with representatives from the Radiation Oncology Branch, COP, held working group meetings in December 1981 and June 1982 to coordinate activities.

d. Evaluation of Treatment Planning for Particle Beam Radiotherapy [M. D. Anderson Hospital (NO1-CM-27531); Massachusetts General Hospital (NO1-CM-27532); University of Pennsylvania (NO1-CM-27529); and Lawrence Berkeley Laboratory (YO1-CM-20110)]

This project consists of two tasks. Task A is treatment planning for particle beam radiotherapy in each major anatomic site (brain, head and neck, lung, mediastinum, upper abdomen, pelvis, trunk, extremities, and superficial and deep lymph nodes) utilizing state of the art imaging and computer treatment planning systems. Task B is the evaluation of particle beam treatment capabilities which will involve dosimetry and microdosimetry measurements in patients or in phantoms to confirm the treatments planned in Task A. Participants include institutions with capabilities for radiotherapy with protons, heavy ions, and cyclotron- and DT generator-produced neutrons. Although not funded by contract, the Los Alamos pion therapy group is participating in this effort. Working group meetings were held in January and May 1982.

## IV. Working Groups and Committees

### A. Radiosensitizer/Radioprotector Working Group

This Working Group consists of 12 representatives from the scientific community, most of whom are experts in the development of and/or investigation (both basic and clinical) of radiosensitizers and/or radioprotectors. In addition, there are basic scientists who provide guidance in the pharmacological aspects of sensitizers and protectors. The Working Group held a meeting in Park City, Utah, on April 17, 1982.

### B. Radiosensitizer/Radioprotector Analog Committee

This Committee is composed of 18 members who are experts in radiation biology, radiotherapy, drug development, and/or closely related fields. This group serves as an advisory body to the Associate Director of the Radiation Research Program in formulating and conducting the efforts to identify radiation sensitizer and radiation protector compounds using in vivo and in vitro screening systems. The final evaluation of potential radiosensitizer and radioprotector compounds will be made in clinical trials. This Committee met on February 18, 1982 to review progress in the development of radiosensitizers and radioprotectors. Dr. David Davidson, Walter Reed Army Institute of Research, gave an update of the U. S. Army's radioprotector program. He also gave an overview of the 13th meeting of the NATO Panel on Chemoprophylaxes, held at Walter Reed on November 2-5, 1981. The Committee also decided to make a strong effort to put the 10-12 radiosensitizers that are currently being investigated by the scientific community into the in vivo portion of the radiosensitizer screen. In this way, the data needed to put these compounds into the NCI Decision Network would become available. This approach was subsequently approved by the RS/RP Working Group.

## V. New Initiatives

### A. Quality Assurance Program for Clinical Hyperthermia

Interest in clinical applications of hyperthermia alone, with radiotherapy, and with chemotherapeutic agents has increased markedly and chaotically in the past few years. The physical and physiological challenges of heat generation and thermometry are enormous. It is essential that a mechanism similar to the Radiological Physics Center be established for quality assurance in Phase I/II as well as Phase III hyperthermia studies if this adjuvant modality is to be evaluated expeditiously. This contract will build upon the other two hyperthermia contracts. Award of this contract is expected in early FY83.

### B. Dose Calculations for Treatment with Radioactively Labelled Antibodies

It is anticipated that clinical studies with radioactively labelled anti-tumor antibodies will literally explode over the next several years because of the theoretical advantages of depositing radiation within the tumor in a highly specific manner. At this time, dose calculations for treatment in this manner are very crude estimates. In order to interpret the results of treatment with this modality over the next several years, it is essential that we develop criteria and guidelines for calculating doses to tumors and normal tissues. This RFP addresses that question specifically.

### C. Low LET Treatment Planning

Recent advances in computerized treatment planning in conjunction with "state-of-the-art" tumor localization at present with CT and ultrasound devices and in the near future with nuclear magnetic resonance and advanced radioisotopic scanning systems offer radiotherapists an opportunity to deliver radiation optimally to almost all patients. Although considerable effort has been devoted by manufacturers and by individual institutions to treatment planning, there has been no coordinated effort to evaluate the numerous types of hardware and approaches in software for treatment planning systems in a systematic fashion. This proposed RFP will provide an opportunity to consolidate these recent advances in treatment planning and make them available to all radiotherapists, not just those participating in clinical trials.

### D. Collaborative Effort to Develop Guidelines for the Use of Interstitial Irradiation Alone or in Conjunction with External Beam Radiotherapy or Hyperthermia

There has been increased interest in interstitial irradiation in the past decade and almost as many approaches have been taken as there are physicians using interstitial irradiation. This RFP is designed to bring together the best implant teams to develop criteria and guidelines in order to improve the quality and safety of that treatment method.

Table 1

RADIOTHERAPY DEVELOPMENT BRANCH

Dollars in Thousands

| GRANTS | FY 1981 | FY 1982 |
|---|---|---|
| Individual Investigator Grants (R01, R23, etc.) | $17,798 | $23,785 |
| Program Project Grants (P01) | 23,471 | 20,589 |
| CREGS | 1,452 | |
| RTOG (including RPC) | 3,155 | 3,471 |
| GRANT TOTAL COSTS | $45,876 | $47,845 |
| **CONTRACTS** | | |
| Radiosensitizer/Radioprotector Development | $ 910 | $ 914 |
| Clinical Neutron Research | 1,544 | 2,330 |
| Photoradiotherapy (RPMI)[a] | 3[b] | 134 |
| Brain Tumor Study Group[c] | (967) | (421) |
| Hyperthermia Research[a] | 576 | 866 |
| Particle Beam Radiotherapy Treatment Planning | | 292 |
| Intraoperative Radiotherapy[a] | 47 | 266 |
| CONTRACT TOTAL COSTS | $ 3,080 | $ 4,802 |
| OVERALL TOTAL COSTS | $48,956 | $52,647 |

[a]One or more contracts awarded in early FY82 rather than late FY81
[b]Forward funded during FY80
[c]Funded by CTEP

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1981 - September 30, 1982

The Clinical Oncology Program of the National Cancer Institute conducts clinical and laboratory investigations into the etiology, diagnosis, and treatment of human cancer. The Program is composed of seven Branches, which carry out independent research efforts and conduct clinical studies either independently or in collaboration with the other Branches of the Program. During the past year significant advances have been made in the treatment of several malignancies including ovarian cancer, soft tissue sarcomas, acute leukemia of childhood, and small cell carcinoma of the lung. In addition, significant research advances have occurred in the development of monoclonal antibodies to small cell carcinoma of the lung, in the clarification of mechanisms of hormone resistance in human breast cancer; in describing cytogenetic abnormalities associated with cancer; in the cloning and proliferation of cytotoxic human T-cells, in analyzing the role of immune response genes; in the characterization of important new metabolites of methotrexate; in evaluating drug-resistance mechanisms in tumor cells; in understanding the mechanisms of free radical mechanisms of tissue injury; and in understanding pharmacokinetic parameters needed to treat meningeal cancer.

## Program Accomplishments

### Biometric Research Branch: Dr. Richard Simon, Chief

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

designs and biometric methods related to the development and evaluation of new cancer treatments. The Branch provides statistical consultation and collaboration for laboratory reseach and maintains computerized data collection systems.

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

The Clinical Pharmacology Branch places its major emphasis on using the rapidly evolving techniques of basic science to improve our treatment of human cancer. The projects currently ongoing in the Branch range from those immediately applicable to patient treatment to projects whose clinical applications may be as long as 10 years away. Nevertheless, clinical utility is still the ultimate goal of all of these projects and the yard stick by which success of this work is evaluated.

There are, at present, 5 major research areas worthy of being highlighted.

1. Free Radical Mechanisms of Tissue Injury: This research is focused on two areas. One involves an investigation of how tissues defend themselves against free radical attack. This line of research has led us to describe tissue specific properties which effect radical damage. In addition, it has led to the description of a new radical defense enzyme. The other aspect of this research focuses on how adriamycin precipitates free radical damage. Over the past year, a new aspect of this problem has been uncovered, namely, that adriamycin forms iron chelates capable of catalyzing free radical injury.

2. Pharmacokinetics: The pharmacokinetics group has been very active over the past year. This work has included studies on misonidazole, cis-retinoic acid, adriamycin, BUdR, methotrexate, and ara-C. Much of this work is of considerable utility and has had a direct impact on protocol design and demand for this portion of the Branch clearly exceeds the resources currently available.

3. Polyglutamates of MTX: Work in this area continues to be of great interest. Over the past year, this group has shown that polyglutamate formation is a property of human cancer cells, that formation of the higher polyglutamates correlates with drug sensitivity, and that this is due to the fact that the higher polyglutamates do not efflux rapidly from cells. This work gives promise of having profound implications for dose and scheduling of MTX.

4. Genetics of Drug Resistance: The recent advances in molecular biology have provided, for the first time, tools which allow the investigator to study in detail the genetics underlying drug resistance. This Branch has been very active in applying these techniques to human tumor cells. Over the past year, MTX resistance in human breast cancer and lung cancer cells has been shown to be due to reduplication of the dihydrofolate reductase gene. This gene has been cloned from the resistant cells and is now being studied. Of all the areas currently being pursued by the Branch, this work is probably of broadest applicability. There will be continued major emphasis in this area.

5. Tubulin: Tubulin is a structural protein needed to maintain cell morphology. In addition, it is the major protein in the mitotic spindle. It is the target for a growing family of drugs and yet the kinetics of its synthesis in relationship to other events of the cell cycle is poorly defined. This group has produced a

fluorescent antibody against tubulin. Through the use of this antibody, Dr. Shackney's group has been able to use the fluorescent activated cell sorter to study the cell cycle from the point of view of the tubulin synthetic cycle. This work could provide a valuable window on biochemical events relating to tubulin and the effects of drugs upon this protein.

Medicine Branch: Dr. Robert C. Young, Chief

A broad range of clinical trials continue. The most important results include:

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, soft tissue sarcomas, cervical carcinoma, and brain tumors.

Phase I-II clinical trials have been completed this year on the following new experimental agents or combinations: AMSA, 13-cis-retinoic acid, Interferon. Phase II trials continue on AZQ, intraperitoneal chemotherapy of adriamycin and Interferon. New phase I studies include Aclacinomycin A and a new platinum derivative DCBDA.

The following clinical areas are of note:

Non-Hodgkin's Lymphoma:

The activity of ProMACE-MOPP induction therapy in advanced diffuse large cell lymphoma has been established. Seventy-four patients with advanced disease have been treated. The complete remission after restaging is 74%. Only 18% of the patients have relapsed with follow-up now in excess of 24 months. The median survival of the entire group of patients has not yet been reached, but 62% of all patients remain continuously disease-free after therapy. These results are the best yet reported for the treatment of this disease. Continuous disease-free survivals from all previous studies have been approximately 30-35%. ProMACE-MOPP appears to double the cure rate in this disease.

Certain patients with CLL have high EBV titers, and in some patients it has been possible to identify the viral genome in the DNA of CLL cells. In about 5% of patients active viral shedding occurs from cells in culture and these observations have led to the initiation of a clinical trial using Acyclovir to treat such CLL patients. Preliminary evidence suggests that the drug completely eliminates the virus but exerts no appreciable effect on the CLL.

Second malignancies in non-Hodgkin's lymphoma after treatment: A comprehensive analysis of 515 patients treated at the NCI has been completed. Results show a significant increase in second malignancies specifically in acute leukemia in patients requiring long-term therapy to control their disease. The risk appears in the "indolent" histologies (NPDL or DWDL) where continuous therapy is required. Paradoxically, those patients with aggressive lymphomas (DHL and DML) who are cured with short-term therapy are not at increased risk.

Testicular Carcinoma:

A new 4-drug combination ($PV_EBV$) composed of high dose cis-platinum, velban, bleomycin, and VP-16 appears to have an extremely high (89%) complete remission rate in patients with poor prognosis advanced non-seminomatous testicular carcinoma. Standard regimens in the past had been able to produce only a 40-60% complete response rate in this group of patients. This improved complete response rate has been accomplished without significant renal toxicity by using hypertonic saline as a vehicle for the cis-platin infusions. If the extremely high activity of this regimen is confirmed in larger numbers of patients, it will resolve one of the last major questions in testicular cancer; namely, how does one improve the survival in poor prognosis patients.

Ovarian Cancer:

A new clinical trial in advanced disease has been established (CHIPS) in which alternating sequences of chemotherapy and radiation therapy are employed. Initial therapy with cyclophosphamide and hexamethylmelamine is followed by total abdominal irradiation, intraperitoneal (I.P.) misonidazole, and systemic cis-platinum therapy. The latter three agents all show synergistic anti-tumor effects when used in animal systems. Toxicity is minimal compared to previous therapies for advanced ovarian cancer. There are no deaths in the patients entered on study so far.

Large volume I.P. chemotherapy with several agents is feasible and produces a 25-300 fold excess of drug within the peritoneal space compared to plasma concentrations. In the adjuvant 5-FU study in patients rendered disease-free with intensive induction chemotherapy, 2 patients without intraperitoneal therapy have relapsed at 8 and 17 months. One patient in the I.P. 5-FU group has relapsed at 24 months. The phase II I.P. adriamycin trial continues. Seven patients have been entered; two patients have thus far had objective regression of disease.

Extensive experience with the human ovarian cancer clonogenic cell assay has been completed. Over 160 patients have been studied. Approximately 80% of samples from ascites, pleural fluid and peritoneal washings can be successfully cloned, and about 40% have >30 colonies per plate to allow some drug testing. However only about 15% have >100 colonies which would allow extensive drug testing. Clinical correlation with assay findings exceeds 90% for inactive agents and exceeds 64% for those deemed active in the assay. A dose response relationship between adriamycin concentrations and ovarian cancer cells has been demonstrated which gives direct rationale to the intraperitoneal use of the drug in selected patients. Further improvement in the assay is required to allow more effective clinical application. However, focus in our laboratories is being redirected toward the study of mechanisms of drug resistance and the use of human ovarian cancer cell lines.

Approximately 147 patients have now been randomized to the Ovarian Tumor Study Group/GOG study on early ovarian cancer initiated by the Medicine Branch. Preliminary results from the Stage Ia and Ib study suggest that there will be few relapses in carefully staged patients regardless of initial adjuvant therapy.

Hodgkin's Disease

A comprehensive review of the remaining challenges in Hodgkin's disease therapy was completed this year in order to highlight new research directions. Important research areas remain and include: a) the optimal management of early stage disease; b) the role of alternating sequence combination chemotherapy for advanced disease; c) the optimal approach to massive mediastinal Hodgkin's disease; d) the minimization of the long-term toxicities including sterility, second malignancies, and prolonged immunosuppression.

The Branch explored the lack of influence of drug dose or timing on response to MOPP in previously untreated advanced Hodgkin's disease. Previous investigations had suggested that dose or dose rate might be an important influence on ultimate response and/or survival. Review of 156 patients in the MOPP study revealed that nearly all of our patients received over 75% of the projected dose of chemotherapy, and there were no effects of nitrogen mustard or procarbazine dose or timing on treatment outcome.

The Branch also explored the long-term effect of MOPP therapy on ovarian function of women cured of Hodgkin's disease. Twenty-seven women were studied. Persistent amenorrhea occurred in 46% and was age related. Ovarian failure is often gradual in onset, but to date, children born to women treated with MOPP have all been normal.

The Branch is investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer. It is studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous diseases, melanoma, colon carcinoma, ovarian cancer, and male breast cancer. It has developed assays for specific gene products (thymidylate synthetase, aspartate transcarbamylase dihydrofolate reductase), and is studying the effects of steroid hormones on the activities and synthesis of these proteins.

The Branch is studying intracellular pharmacokinetics of estrogen and anti-estrogen metabolism and efflux from human breast cancer cells using perfusion systems. These studies have led to new insights into hormone receptor interactions with the genome. Specifically, we have discovered that intranuclear estrogen receptors are changed over time to a less easily extractable form associated with the onset of steroid induced effects. This "processed" receptor appears tightly bound to DNA, is extractable by nuclease digestion, and may be the proximate receptor form involved in gene regulation.

The Branch is studying the interactions of novel ligands with human estrogen receptors. The goal is to develop better assays and evaluate cytotoxics linked to the hormone moiety. It has shown that $^{125}I$ 16 iodoestradiol can be used to selectively kill human breast cancer cells and has used this technique to develop resistant variants.

The Medicine Branch has made a major commitment to immunologic research and has made the following observations.

1. Acquisition of T cell repertoire in nude mice: Peripheral T cells from

thymus-grafted nude mice appear from this work to have acquired the capacity to recognize antigen in association with thymus MHC gene products but not nude host MHC gene products. Analysis of the thymus itself in such animals reveals that the cells are not responding to host type MHC gene products in the autologous MLR but acquire the phenotype of recognizing thymus type MHC gene products as self.

2. Gamma interferon production by T cells: Antigen-specific lyt 1+ T cell clones make high titers of gamma interferon upon exposure to the antigen for which they are specific on the appropriate antigen presenting cell. This interferon production begins about 4 hours after exposure to antigen and peaks at 48 hours after stimulation. While the kinetics of the response differ from the kinetics of antigen-specific proliferation, the amount of interferon produced parallels proliferation and the affinity of the receptor for the antigen controls both the amount of proliferation and the amount of interferon production. These results demonstrate that single antigen-specific helper T cell is capable of providing interferon helper factors as well as to the cells with which it interacts.

3. Fine specificity of T cell recognition: This group has analyzed the fine specificity of antigen and MHC recognition by pigeon cytochrome c-specific T cell clones from two responder strains. The response of T cells from both strains is mapped to the same region of cytochrome c and antibodies directed at shared MHC determinants block the response in both strains. Since clones from both strains recognize similar antigen and MHC determinants, they were analyzed for the ability to recognize antigen on the other responder haplotype antigen presenting cell (APC). About 90% of T cell clones from each strain could recognize antigen only on self APC, but 10% of the clones responded to antigen on either responder MHC. Some of the clones manifested both antigen specificity and allogeneic reactivity against the other responder haplotype. These results tend to support the notion that the T cell receptor is a single (rather than a dual) receptor with antigen specificity derived from recognizing antigen as an altered self structure.

Cytogenetic research continues as a major area, and the following projects were completed in the past year.

1. Cytogenetic studies in lung cancer: 38 bone marrows from patients with active small cell lung cancer were analyzed. Of the 30 successful specimens, 5 had abnormal keryotypes, all of which included a deletion of chromosome 3 involving bands 3p(14-23). The presence of this marker in direct marrow preparations is further evidence of its specificity for this tumor.

2. Cytogenetic studies of ovarian cancer: A total of 72 patients were studied by direct and/or short-term (1-3 day) culture of ascites, pleural fluid, or tumor; 44 patients were successfully analyzed by the chromosome banding technique. All patients were aneuploid and 39 showed structural abnormalities, most frequently involving chromosomes 1, 3, 2, 4, 9, 10, 15, 19,, 6, and 11; the least involved chromosomes were #21 and #5. Clone formation and the number of chromosomes involved in structural abnormalities increased with duration of disease and were more extensive in patients treated with surgery and chemotherapy than in patients treated with surgery alone. Our data did not show a deletion of chromosome 6 (6q-) to be specific for ovarian cancer.

3. Development of a rapid, simple technique for producing high-resolution chromosome banding: The technique presently in use (introduced by Yunis) is both tedious and time consuming (22 1/2 hours). With a new method, the cells are exposed to 1:1 0.075 M 2-mercaptoethanol: 0.075M Kcl for 20 minutes with 0.05 ug/ml colcemid added during the final 10 minutes. The results are equivalent to those obtained with the Yunis technique.

4. Immuno-biotin complex study: This procedure was combined with the use of anti-thymidine and anti-guanidine to produce differential staining of the centromeres of mouse and human chromosomes. This technique will facilitate the identification of the human or mouse cells in hybridized cells.

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

The NCI-Navy Medical Oncology Branch (NNMOB) is an intramural adult oncology program of the NCI's Clinical Oncology Program of the Division of Cancer Treatment.

The major research projects are a direct continuation of studies initiated while this Branch was at the Veterans Administration Medical Center in Washington, D.C., and include the following major studies.

Small cell lung cancer limited stage disease: This is a unique randomized trial comparing combination chemotherapy with or without simultaneous chest radiotherapy conducted in collaboration with the Radiation Oncology Branch. Preliminary results suggest benefit of combined modality therapy.

Small cell lung cancer extensive stage disease: This is a unique single-arm trial of combination chemotherapy followed by "intensification" with new high dose chemo-radiotherapy at week 15 and autologous bone marrow support. It is conducted in collaboration with the Radiation Oncology Branch and the Pediatric Oncology Branch, and preliminary results suggest no striking benefit from intensification therapy.

Non-small cell lung cancer (NSCLC) all stages: The protocol for this trial is in the developmental stages. Fundamentally, the trial will include NSCLC patients of all stages. Patients will be staged, their tumors biopsied, and tumor cell lines initiated for drug and radiation sensitivity testing. Patients will receive "standard" initial treatment and then be randomized on the basis of their therapy sensitivity testing to receive standard or assay "selected" therapy. This will require collaboration with thoracic surgery, pulmonary medicine, and radiation oncology as well as the research laboratory.

Mycosis fundoides - Sezary syndrome: This protocol is a unique randomized trial comparing conservative, topical "watch and wait" therapy to aggressive combined modality chemo-total body electron beam therapy. It is conducted in collaboration with the Radiation Oncology Branch. It is too early to make specific conclusions.

Several exciting new initiatives are underway including studies testing clinical applications of monoclonal antibodies. Protocols for the clinical application

of monoclonal antibodies are under development. The first protocol will be a phase I-II trial of an anti-T lymphocyte monoclonal (Anti-T101) in mycosis fungoides/Sezary syndrome and CLL in collaboration with the Biologic Response Modifiers Program. Subsequent studies planned include use of anti-breast cancer monoclonals developed by Dr. Schlom's group and anti-lung cancer monoclonals developed by this group in nuclear medicine scanning and ultimately in targeting of radiotherapy, drugs, and toxins.

Laboratory investigation includes production and characterization of monoclonal antibodies with specificity for human lung cancer or their products. These include studies of heterogeneity and homogeneity of antigen expression; antigen biochemical characterization; distribution on normal tissues and immune histochemical stains of normal and tumor tissue. A panel of 81 selective antibodies is under investigation. These antibodies promise to be of potentially great importance in the diagnosis, staging and treatment of lung cancer. These studies include preliminary steps necessary to bring the Ab's to clinical application using in vitro and nude mouse heterotransplant models. They include collaboration with cooperative groups for performing immune histochemical retrospective studies for clinical correlations, to collaborations with the Radiation Oncology Branch chemists, radiation biologists, and radiochemists to develop immune vehicles to deliver radiotherapy. In addition to lung cancer cells per se, we are also preparing monoclonal antibodies against defined antigens such as peptide hormones produced by lung cancer cells, particularly hormones we can show are required for the growth of lung cancer cells.

Drug and radiation sensitivity testing of human tumor cell lines: The Branch is conducting detailed studies of chemo-radiotherapy sensitivity of human tumor cell lines, short-term cultures, and direct tumor samples, particularly lung cancer, and correlating this with the clinical response in patients. At present the in vitro and in vivo results seem quite well correlated. The tumor cell lines provide excellent material to characterize the biochemical pharmacology of drug and radiation resistance and serve as DNA donors for isolating drug resistance genes. The Branch is trying to directly test the utility in prospective clinical trials by : 1) screening for new drugs active against the tumor lines in vitro and then testing them in patients; 2) selecting therapy for individual patients based on the in vitro assays.

Study of the biology of lung cancer: Following clues from normal bronchial epithelial tissues and normal fetal development, the Branch has been identifying biochemical and immunologic markers associated with human lung cancer. In particular, it was learned that a series of APUD markers and peptide hormones specific for small cell lung cancer exist. The Branch is now developing similar markers for NSCLC. These "normal" markers are of potential great use for tumor typing, staging, and following the response to therapy. They also provide clues for how to better grow and regulate the lung cancer cells. In addition, the Branch is establishing tumor cell lines representing different steps in differentiation with the ultimate aim of isolating and characterizing the tumor stem cells. It was found that some of the cells blocked early in differentiation and are more malignant. The understanding of the clinical biology in patients will depend on studying these stem cells.

Identification of growth factors for human tumor cells: Lung cancers grow well in patients but are hard to clone or establish as cell lines in vitro. The

ability to do this is at present a prerequisite of many clinical applications such as therapy sensitivity testing. The Branch has developed the strategy of working out the serum-free growth factor supplemented media requirements of human tumor cell lines and then testing these on fresh clinical tumor specimens. This works and selectively allows the growth of human tumor cells considerably better than in serum supplemented media. The Branch is extending the results in SCLC and adenocarcinoma of the lung to other lung cancer types and human breast cancer. In addition, by cloning in serum-free medium it is possible to identify more growth factors. In this way, the Branch has shown that human lung cancer cells produce peptide hormones such as AVP and bombesin (as well as novel peptides) required for their own growth in an autocrine fashion. This also provides new approaches to hormonally manipulate lung cancer cells.

This Branch is using a variety of approaches to study the genetics of lung cancer and drug resistance. These include cytogenetic studies with Dr. J. Whang-Peng of the Medicine Branch, which have identified a specific acquired chromosomal defect (deletion 3p(14-23)) associated with SCLC. This Branch continues to study this defect as well as to identify others associated with NSCLC.

Another approach is to transfect via isolated DNA or hybrid cells the malignant phenotype to non-malignant cells and subsequently isolate specific genes by recombinant DNA techniques. In addition, the recent findings by others of the potential role of viral Sarc genes (Kirstin virus) with NSCLC has prompted workers in the Branch to look at expression of p 21 Sarc protein in collaboration with Dr. Scolnick and the status of the viral Sarc genes in lung cancer DNA using existing probes. A central question is, is the same or different transforming genes active in SCLC as in some NSCLC, and do all NSCLC tumors have the same active genes. Using the available recombinant DNA probes related to Sarc genes, the molecular cytogenetics in lung cancer cells is accessible. These studies are of fundamental importance to understanding the biology of lung cancer.

The same transfection technique will be used to isolate and characterize genes for chemotherapy resistance by transfecting DNA from lung cancer lines selected for drug resistance in patients into sensitive rodent cells. These transfectants should allow isolation of DNA probes specific for drug resistance and the preparation of antibodies to their products. Such reagents will provide entirely new ways to type tumors for their chemotherapy sensitivity or resistance.

Pediatric Oncology Branch: Dr. Philip A. Pizzo, Chief

The Pediatric Oncology Branch (POB) conducts research involving children with cancer.

In the study of acute lymphoblastic leukemia (ALL), the POB has investigated the efficacy of a high-dose protracted intravenous methotrexate infusion as an alternative to the conventional utilization of cranial irradiation plus intrathecal methotrexate to achieve central nervous system prophylaxis. An additional aim of this study is to assess the utility of an intensified systemic maintenance schedule which alternates standard maintenance treatment with periodic induction-type chemotherapy schedules. Accordingly, it is hoped that the application of

this intensified maintenance will improve the prognosis for patients with "high risk" ALL. To date, 139 patients have been randomized on this study. Forty-six of these were randomized to receive the conventional cranial irradiation plus intrathecal methotrexate, while 93 have been randomized to the high-dose methotrexate infusion arm (the randomization is weighted on a 2 to 1 basis). To date there is no significant difference in the efficacy of CNS prophylaxis between these arms, but it appears that the high-dose methotrexate arm may be leading to a prolonged disease-free bone marrow remission. While this study is still ongoing (with current accrual it will take approximately another year to complete) the regimen appears to be the best yet achieved in the management of high-risk leukemias.

For the treatment of patients with established central nervous system leukemia, it was demonstrated that high-dose methotrexate infusions administered systemically are capable of maintaining a cerebrospinal fluid (CSF) methotrexate level of $1 \times 10^{-5}$ molar without apparent systemic or neurologic toxicity. Moreover, it was determined that the ventricular and lumbar CSF : Plasma methotrexate ratios are similar, suggesting that systemic methotrexate administration has the advantage of providing consistent drug concentrations throughout the CSF. This technology has been applied effectively to the treatment of patients with established central nervous system disease.

As part of the study of surface and enzymatic markers which distinguish subtypes of leukemia cells, it was demonstrated that patients with T-cell leukemia have significantly elevated levels of the enzyme adenosine deaminase (ADA). Concomitant studies with the inhibitor of adenosine deaminase (2'-deoxycoformycin or DCF) has suggested the possibility that this agent may be effective in selectively treating patients with T-cell leukemia. Accordingly, 26 patients with refractory ALL were treated with 2'-DCF, and of these, two achieved complete remissions while partial responses were observed in another 4 patients. This encouraging result has now led to a current study which is exploring the interaction of 2'-DCF with adenosine arabinoside as therapy for patients with refractory acute leukemia.

The therapy for patients with undifferentiated lymphomas (both Burkitt's and lymphoblastic types) employs alternating cycles of high-dose methotrexate infusions with CHOP, administered on approximately 10-day schedules without delays for neutropenia. To date, 66 patients have been entered into this protocol, and the overall survival is 66% at three years. Excellent results have been obtained for patients with the lymphoblastic type, and a modification of the central nervous system prophylactic arm has led to improvement in central nervous system relapse.

For the treatment of patients with metastatic Ewing's sarcoma, the POB has utilized a regimen of intensive combination chemotherapy in conjunction with total body irradiation and autologous bone marrow reconstitution. This regimen is yielding a better result than any other previous NCI protocol, with approximately 30% of the patients entered into it now surviving. In order to improve these results, the POB is presently collaborating with the Radiation Oncology Branch to utilize a higher dose of total body irradiation (800 rads), and the pilot study of 3 patients has shown the feasibility of this regimen.

To assess the utility of adjuvant intensive combination chemotherapy following total resection of pulmonary metastases in patients with relapsed osteogenic sarcoma, they have evaluated a high-dose multi-agent chemotherapy schedule following total resection and have observed in 24 patients an actuarial continuous disease-free survival of 44% at three years. This result appears to be somewhat superior to studies utilizing only surgical extirpation of pulmonary metastases.

To assess the need for synergistic or additive combination antibiotic therapy in the management of the granulocytopenic patient who becomes febrile, the standard triple-drug antibiotic combination (cephalothin, gentamicin, carbenicillin: KGC) has been compared to a new third-generation cephalosporin (Ceftazidime) as the sole antibiotic for initial empiric therapy. With over 60 patients randomized to date, the efficacy of the single antibiotic appears to be comparable to that of the triple antibiotic regimen for patients with unexplained fever. Additional study is necessary to assess this regimen for patients with a variety of documented infections.

For the patient with persistent fever and granulocytopenia after a week of empiric antibiotic therapy, the benefits of adding empiric antifungal therapy in a randomized prospective trial has been demonstrated. These results are now being extended by comparing our standard antifungal agent (amphotericin B) to a new imidazole antifungal agent (ketoconazole). To date, 15 patients have been randomized to this study, and the results so far are comparable in both arms of the study.

In an attempt to prevent infections in patients who become granulocytopenic after chemotherapy, a double-blind randomized trial has been conducted in which antibiotic prophylaxis with trimethoprim/sulfamethazole plus erythromycin was compared to a placebo. Analysis of the 150 patients entered into this study indicates that the antibiotic prophylaxis exerts a significant benefit for patients with leukemia in reducing the incidence of fever and infections. However, this difference was dependent upon the degree of patient compliance in taking the antibiotic regimen, further elucidating the importance of compliance as both a dependent and an independent variable in effecting outcome, an observation which has important implications to other clinical trials.

In studies of acute leukemia, 5 purine pathway enzymes (adenosine deaminase, 5'-nucleotidase, purine nucleoside phosphorylase, terminal deoxynucleotidyl transferase, and acid phosphatase) to generate a biochemical profile of acute leukemia cells as an adjunct to defining rational schemes for specific therapy has been utilized. In concert with these studies the POB has also developed hybridoma antibodies (in collaboration with Drs. Ronald Billings and Paul Terasaki) which are being studied to both classify leukemia cells, as well as to be utilized for in vitro or in vivo immunotherapy. In addition, current studies on pharmacokinetics have demonstrated that the commonly used maintenance drug, 6-mercaptopurine (6-MP) has a surprisingly low bioavailability when administered by the oral route. This observation has important implications in evaluating response and failure in children with acute leukemia, and may be of benefit in modifying therapy for patients who achieve low serum levels with this agent.

Utilizing the subhuman primate model which we have developed for studying CSF pharmacokinetics, we have further extended our observations in using systemic high-dose methotrexate infusions as an alternative to intrathecal delivery and have substantiated the superiority of this route of administration. In addition, this model has been utilized to study new chemotherapeutic agents which may be of benefit in the treatment of meningeal leukemia or brain tumors, including AZQ, dihydroxyanthracenedione, aclacinomycin, cytosine arabinoside, L-asparaginase, m-AMSA and 6-mercaptopurine. In addition, this Branch has utilized this model to further study the interaction of irradiation with chemotherapeutic agents in producing leukoencephalopathy and have demonstrated unequivocally the importance of irradiation to this toxic effect.

The POB has developed a large number of cell lines from American and African Burkitt's lymphoma and have compared these lines utilizing a battery of biochemical, biophysical and immunologic techniques. In these studies it was demonstrated that the American Burkitt's lymphoma cell lines secrete greater quantities of IgM as compared to those of African origin and this IgM is polyvalent and contains the J-chain. In addition, the presence of monoclonal IgM has been found in the serum of patients with undifferentiated lymphoma, and appears to serve as a marker for evaluating their clinical course. Furthermore, the POB has been able to demonstrate the presence of antigens on American Burkitt's lymphoma cell lines which are not present on African lines and have characterized these antigens as being of probable glycolipid origin.

The POB has modified the human clonogenic tumor stem cell assay to measure tritiated thymidine incorporation as an alternative to colony counting. With this, they have been able to measure the sensitivity of human tumor cells from primary explants in 5 days as compared to the 2-3 weeks required by the normal counting assay, and have demonstrated a concordance between these assays of 95%. Utilizing this assay, they are able to measure both chemotherapy sensitivity and resistance as well as x-ray sensitivity and are utilizing this methodology to further define the approach to clinical tumors such as Ewing's sarcoma and neuroblastoma.

Utilizing tritiated anthracycline probes, the POB has been able to demonstrate a difference in uptake with daunomycin between anthracycline sensitive and resistant murine leukemia cell lines. These differences further appear to correlate with the overall lipid composition of the plasma membrane of these cells, suggesting that resistance may be due to the relative lipid solubility of these drugs. This observation has obvious important implications for studying resistance of tumor cells in general.

In order to accelerate granulocyte recovery following cytotoxic chemotherapy, this Branch has been studying a variety of immunoregulatory agents which may enhance granulopoiesis. It was demonstrated with in vitro and in vivo murine studies that a cyanoaziridine derivative, azimexon, produces a dramatic dose response increment of both white blood cells, granulocytes, and CFUc, in in vivo and in vitro murine studies. They are in the process of further refining the mechanism of action of this agent, as well as other agents such as lithium, as a prelude to clinical studies.

Radiation Oncology Branch: Dr. Eli Glatstein, Chief

The Radiation Oncology Branch (ROB) continues in transition. In the last two years, the turnover of staff has been virtually complete.

The three main goals of the Radiation Oncology Branch 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 and clinical questions of relevance.

3. A training program in radiation therapy, equivalent in stature to the present programs in the Medicine, Surgery, Pediatric Oncology Branches within the National Cancer Institute.

At the moment, clinical trials are progressing on a wide variety of fronts. The biology program is progressing, although it has been difficult to optimize because of problems with holding facilities for animals. The present B2 animal facility in Building 10 is not adequate for long-term experimentation, which is the main thrust of the in vivo work for the ROB. Frequency of infections that take place in the B2 facilities has meant multiple interruptions and sacrifice of ongoing experiments that are designed to last 12-24 months. It is a major limitation of the B2 facility and has caused the ROB to plan a major proportion of its future renovations on the B3 level to include an animal facility.

Concerning the training program, a provisional approval has been obtained from the AMA Residency Review Committee. This program, in conjunction with the Uniformed Services University of the Health Sciences, working through Walter Reed Army Medical Center and the National Naval Medical Center in Bethesda, consists of a three-year training program under the direction of Dr. Eli Glatstein. Essentially 18 months will be within the National Cancer Institute, one year within Walter Reed Army Medical Center and six months within Bethesda Navy Medical Center. The necessity of sharing the training experience with other medical centers is required by the fact that the Cancer Institute patient base is limited to specific diseases. The clinical material of the military centers complements the Cancer Institute material nicely, with major emphasis on gynecologic neoplasms, head and neck cancers, and genitourinary cancers. These are areas in which the Cancer Institute patient base is presently lacking.

The in vitro laboratory program includes work on low-dose rate radiation and experimentation of chemotherapeutic agents and hyperthermia as well. In addition, we hope to experiment in the area of photosensitivity.

Much of the present work has been centered on human CFUc and human tumor cell lines, in collaboration with other Branches.

The clinical program within the Radiation Oncology Branch is centered around combined modality studies. Most of these are collaborative with other Branches. The most important of these revolves around the study of small

cell carcinoma of the lung, in collaboration with the NCI-Navy Medical Oncology Branch. This study consists of a controlled prospective study of the value of radiation therapy to the chest in patients with limited small cell disease. Preliminary results suggest significant benefit can be achieved with combined modality treatment over what can be obtained by chemotherapy alone. Nonetheless, additional patients are required before a final conclusion can be made. In addition, a collaborative venture is ongoing for advanced oat cell carcinoma of the lung that includes consolidation of a chemotherapeutic response with short-term radiation therapy followed by marrow ablative cytotoxic treatment and bone marrow reconstitution. Another study in collaboration with the NCI-Navy Medical Oncology Branch revolves around electron beam treatment for mycosis fungoides. This study is well underway, although it was interrupted with mechanical problems on the linear accelerator which have now been corrected. Such treatment of whole skin electron beam treatment is carried out in only a few medical centers in the United States. There are also collaborative ventures with active participation with the Surgery Branch in soft tissue sarcomas and with the Pediatric Oncology Branch in Ewing's sarcoma and rhabdomyosarcoma. In addition, a pilot study has begun with combined modality treatment in ovarian cancer in collaboration with the Medicine Branch.

Primary ROB studies presently center around intraoperative radiation therapy. Presently, patients are operated on the 10th floor, with massive surgery carried out for carcinoma of the pancreas, stomach, or retroperitoneal sarcomas. Maximum surgery is performed and the patient is then transported through the hallways and elevators to the ROB while under general anesthesia. They are then taken to the treatment room, transposed to the treatment couch, and re-opened under anesthesia so that a large single dose of electron beam treatment can be applied intraoperatively to the tumor bed, with critical normal viscera moved out of the way. This has been done in conjunction with misonidazole, and the enthusiasm runs high for this investigational approach for these extremely difficult management problems. Randomized studies are being carried out in these diseases to delineate the benefit of this approach. At the present time, the important aspects revolve around clear delineation of the safety of this approach. Ultimately, these are seen as first steps to later studies that will incorporate chemotherapy as well.

Another series of studies within the ROB have revolved around radiosensitizing compounds. Intravenous midonidazole has been studied in some detail, and the pharmacology has been delineated. The intravenous compound was studied in carcinoma of the esophagus. Initially, patients were treated with pre-operative radiation, half the patients being randomized to receive the intravenous misonidazole with each fraction of radiation. After the first eight cases, it became apparent that the combination of pre-operative radiation followed by surgery was potentially lethal in terms of acute respiratory failure. As a consequence, the study was altered to consist exclusively of radiotherapy alone, with half the patients receiving a radiosensitizing compound. After 26 patients, the study was discontinued because the data suggested that no major benefit would accompany the use of misonidazole over that which can be obtained with radiotherapy alone.

Another major ROB study revolves around stage I and II breast cancer, comparing radical surgery to radical irradiation with preservation of the breast. This study, in conjunction with the Surgery Branch, has accrued almost 75 patients

in the first 2 1/2 years. This modest number is considered a major accomplishment, in view of the facts that no prior patient base has been recruited for such a study, and the extreme difference of the two arms makes for a difficult randomization. Nonetheless, the study appears to be accruing reasonably well, and we anticipate that the accrual will improve further with time.

A final ROB study is in progress in the treatment of locally unresectable osteogenic sarcoma and chondrosarcoma. This study deals with intravenous misonidazole, the hypoxic cell sensitizer. Despite the fact that these tumors are reported to be "radioresistant," overt tumor shrinkage has been seen in virtually all patients treated on this study, and at least one patient has been followed for three years without any growth of the tumor mass.

Under the direction of Dr. Jan van de Geijn, CT scanning has been fully incorporated into the radiotherapy treatment planning. Virtually all patients who are treated are now scanned in the treatment position, and computerized treatment plans are routinely generated, superimposed on CT sections. Dr. van de Geijn has developed a program which allows for adequate dose calculations, even when blocks are placed in the field. It is possible to also account for tissue inhomogeneity as well. The treatment plans now generated from within the ROB are extraordinarily sophisticated compared to what can be done in other medical centers with commercial units. These treatment plans are employed routinely in all curative treatments. Down time of the CT scanner itself has been the only major limitation, along with the relatively small aperture available on the CT scanner, which restricts kinds of positions that can be scanned. Fairly soon, it will be possible to scan in other planes as well, not just simply cross-sectional. The ROB has been able to interface the treatment planning system with ultrasound as well, which has certain advantages, particularly when treating the breast.

A major portion of the time has been devoted to plans for the new radiation therapy treatment facility. Extraordinarily long delays in reconstruction, along with what was felt to be an exorbitant cost estimate for completing the facility, led to the discharge of the contractor, who was more than 24 months behind on an 18-month contract. A new contractor has almost completed the building, and two units are in the process of being installed. As soon as the facility is completed, the Branch will move into its new home, after which full renovation of the present B3 facility will be made to convert it into radiation biology laboratories. Such laboratory space will consist of two major floors; one for radiologic physics and tissue culture radiobiology, and the other committed to an in vivo radiobiology program. In addition, the fourth bay within the new department will be dedicated to an intra-operative facility. At the present time, the intra-operative program can only be carried out once a week because of the disruption that it causes within the department. With one bay fully dedicated to intra-operative radiation in terms of operating space and radiation device, such procedures will be performed on a more frequent basis. The major advantages of the intra-operative program appear to be the precise localization of tumor and the ability to eliminate critical normal tissues, or at least protect them, from typical external radiation fields. In addition, it offers a unique opportunity in which to combine radiosensitizing drugs and even hyperthermia in the treatment of intra-abdominal neoplasms. It will probably prove to be an ideal approach for retroperitoneal nodes and

pelvic neoplasms. One new area of investigation has begun. A pilot study, in conjunction with the Medicine Branch, has started for advanced cervix cancer utilizing radiation therapy and adjuvant chemotherapy. Ultimately, this should be a good group of patients in which to study hyperthermia, as soon as a new cervix applicator with a built-in microwave source has arrived.

Surgery Branch: Dr. Steven A. Rosenberg, Chief

The Surgery Branch continues a number of project initiated research. Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Prospective randomized protocols in the treatment of soft tissue sarcomas have explored the role of adjuvant chemotherapy as well as the role of limb-sparing surgery. Active clinical protocols are in progress evaluating the effect of intraperitoneal chemotherapy in the treatment of colon cancer, and the effect of adjuvant chemotherapy and radiation therapy in the treatment of esophageal cancer. The role of intraoperative radiation therapy is being studied for the treatment of patients with pancreatic cancer, gastric cancer, retroperitoneal sarcomas, and bony tumors of the pelvis. The role of hepatic resection, as well as the intrahepatic infusion of chemotherapy, is being explored for the treatment of metastatic disease to the liver. Active laboratory research programs in tumor immunology, tumor immunotherapy and host-tumor metabolic interactions are also in progress. In particular, attempts to develop cytotoxic T-cell clones useful in treating certain cancers are continuing.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06308-11 BR |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biometric Research Branch

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | R. M. Simon | Chief, Biometric Research Branch | COP, DCT, NCI |
| Other: | B. K. Edwards | Expert | COP, DCT, NCI |
| | S. S. Ellenberg | Staff Fellow | COP, DCT, NCI |
| | R. W. Makuch | Senior Investigator | COP, DCT, NCI |
| | M. N. Wesley | Staff Fellow | COP, DCT, NCI |
| | R. A. Wesley | Expert | COP, DCT, NCI |

COOPERATING UNITS (if any) Clinical Oncology Program, DCT, NCI; Developmental Therapeutics Program, DCT, NCI; Cancer Therapy Evaluation Program, DCT, NCI; Clinical Branches of Division of Cancer Biology and Diagnosis, NCI.

LAB/BRANCH
Biometric Research Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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

1. Collaborative Intramural Clinical Research

The Biometric Research Branch (BRB) is organized with a designated coordinating statistician for each Clinical Oncology Program (COP) branch. Consequently, a member of the BRB participates in the development of new protocols and the interim monitoring and data collection for ongoing studies. A member of the branch also serves on the Clinical Research Panel to review all intramural clinical trials. BRB staff collaborate in clinical and laboratory studies to evaluate prognostic and treatment selection factors and elucidate tumor biology. The following is a list of studies for which major analyses have been performed in the past year and which are complete enough to be reported at meetings or in the literature.

1.1 Clinical Trials

(1) Randomized comparison of adjuvant chemotherapy versus no adjuvant, and amputation versus resection plus radiotherapy for patients with non-metastatic soft tissue sarcoma. Major improvements in prognosis for extremity patients were obtained with adjuvant chemotherapy.
(2) Evaluation of Pro-MACE-MOPP chemotherapy for diffuse lymphomas.
(3) Evaluation of prophylactic cranial irradiation for small cell lung cancer patients.
(4) Comparison of mastectomy to excision with radiotherapy via implant for patients with stage I-II breast cancer. Though this is an ongoing study, analyses have been made and reported concerning comparisons of the treatment groups with regard to ability to tolerate chemotherapy, psychosocial adjustment and complications of treatment.
(5) Randomized double blind comparison of bactrim plus erythromycin versus placebo for the prophylaxis of granulocytopenic patients.
(6) Randomized evaluation of chest and mediastinal radiation as an adjuvant to chemotherapy for patients with limited disease small cell lung cancer.
(7) Randomized evaluation of the usefulness of HLA matching in providing platelet support to leukemic patients.
(8) Evaluation of intensive combination chemotherapy for pediatric diffuse lymphoma patients.
(9) Evaluation of total parenteral nutrition as an adjuvant to the chemotherapeutic treatment of metastatic sarcoma patients.
(10) Comparison of total gastrectomy versus cimetadine therapy for patients with Zollinger-Ellison syndrome.
(11) Evaluation of intensive combination chemotherapy for patients with metastatic osteosarcoma.
(12) Evaluation of the incidence of left ventricular dysfunction in Hodgkin's disease patients who received radiotherapy to the pericardial area.
(13) Evaluation of the effect of in-vivo barbiturates on adriamycin pharmacokinetics in humans.
(14) Evaluation of a new intensive induction regimen (methotrexate, vincristine, asparaginase and dexamethasone) for the treatment of adult ALL.
(15) Evaluation of a scopolamine transcutaneous patch for the control of chemotherapy induced emesis.
(16) Evaluation of the addition of tamoxifen and premarin to the CAMF combination chemotherapy regimen for metastatic breast cancer.
(17) Double blind comparison of lysine versus placebo for the treatment of herpes.

(18) Comparison of three dosing strategies in the use of cis-retinoic acid for the treatment of acne.
(19) Randomized evaluation of the efficacy and complications of Hickman catheters.
(20) Randomized evaluation of exogenous albumin dose as part of the total parenteral nutrition of cancer patients.
(21) Major retrospective analysis of the cardiotoxicity of adriamycin in 101 soft tissue sarcoma patients treated in the Surgery Branch with cumulative doses of at least 430 $mg/m^2$.
(22) Evaluation of doses and timing of therapy on the outcome of patients with Hodgkin's disease receiving MOPP.
(23) Evaluation of EOE13 contrast media for computerized tomography visualization of liver and spleen.
(24) Evaluation of the incidence of second cancers, and its relationship to therapy, for patients with Hodgkin's disease.
(25) Evaluation of staging procedures (physical examination, gallium scan, computerized tomography and ultrasound) for pediatric B cell lymphoma patients.
(26) Evaluation of periodic bone scans for monitoring patients with Ewing's sarcoma. The average lead time achievable by such monitoring was estimated to be less than one month.

1.2 Clinical Studies of Prognostic Factors and Tumor Biology

(1) Development of a model for predicting (and thereby avoiding) misonidazole induced neuropathy based upon pharmacokinetic measurements and cumulative dose administered.
(2) Analysis of five years of febrile episodes in NCI pediatric cancer patients to define current etiologies, elucidate factors which increase the risk of infection or which differentiate fevers due to infection from those due to other causes.
(3) Evaluation of the incidence, clinical presentation, and prognostic indicators in the development of carcinomatous lepomeningitis in small cell lung cancer patients.
(4) Evaluation of the clinical correlates and prognostic significance of DNA content analysis for patients with plasma cell myeloma.
(5) Evaluation of liver function tests, radionuclide liver scans, abdominal CT scans, percutaneous liver biopsy and peritoneoscopy in the diagnosis of hepatic metastasis in patients with small cell lung cancer (SCLC). Evaluation of the prognostic significance of liver involvement for SCLC patients.
(6) Evaluation of sites of tumor involvement and immunoglobulin levels in the prognosis of patients with mycosis fungoides.
(7) Evaluation of interrelationships and prognostic value of cellular characteristics such as cell type, grade, mitoses, necrosis, cellularity, tumor front, etc. for non-metastatic soft tissue sarcoma patients.
(8) Evaluation of whether aryl hydrocarbon hydroxylase inducibility in cultured mitogen-activated lymphocytes serves as a biochemical marker for families whose children are at risk for the development of cancer. AHH inducibility in families of children with cancer was compared to inducibility in neighbors of the probands. When adjusted for effects of smoking, age and lymphocyte freeze time, there was no apparent difference between results for the populations.
(9) Evaluation of glucocorticoid receptors in the prognosis of patients with leukemia and lymphoma.
(10) Determination of factors predictive for the development of ototoxicity in granulocytopenic patients receiving ticarcillin/amikacin or moxalactam/amikacin.

(11) Evaluation of the prognostic significance of developing hepatitis for acute non-lymphocytic leukemia patients who achieve a complete remission.
(12) Evaluation of the prognostic significance of pleural effusion in patients with non-Hodgkin's lymphoma.
(13) Evaluation of prognostic factors for patients with nodular mixed lymphoma.
(14) Evaluation of relationship between body weight and prognosis for patients with breast cancer.
(15) Evaluation of the prognostic significance of CNS involvement in Burkitt's lymphoma.
(16) Evaluation of the relationship between plasma melatonin profile and hormonal receptors in patients with breast cancer.

1.3 Laboratory Studies

(1) Determination of optimal conditions for generating and expanding cytotoxic lymphoid cells in vitro directed against syngeneic lymphoma tumors.
(2) Evaluation of the influence of fat content in diet on growth rates of DMBA induced mammary tumors in rats.
(3) Evaluation of the extent and mechanism for the influence of diabetes on the rate of growth of transplanted sarcomas in Fisher rats.
(4) Evaluation of hormonal influences on time till spontaneous leukemia development in mice. Tumor development was more rapid when estrogen was administered, and this was true even of low physiologic doses.
(5) Development of an ELISA based assay for the detection of tumor associated antigens. Soluble extracts from fresh tumors are used and the assay is rapid and sensitive. The technique was used to demonstrate autologous antibodies to a sarcoma associated antigen not detectable on normal adult tissue.
(6) Development of an improved assay for defining a "rejection index" between an animal and a tumor from a syngeneic host. The assay is more sensitive than standard skin graft testing; it involves intraperitoneal injection of tumor and sampling the peritoneal cavity at later times to estimate the number of cells present.
(7) Evaluation of changes in the expression of $F_c$ receptor produced by induction of EBV in human cell lines.
(8) Analysis of Epstein-Barr-virus-induced immunoglobulin production by human B cells. Through limiting dilution experiments and developed mathematical models it was determined that individual B cell precursors secreted IgM or IgG but not both, and that infection by a single EBV virion was sufficient to produce immunoglobulin secretion.

2. Multi-Institution Clinical Studies

The BRB serves as statistical center for the following multi-institution clinical trials: (a) Two randomized national studies of the staging and treatment of early ovarian cancer. Accrual is continuing for these studies. (b) A randomized national study evaluating pre and/or post operative chemotherapy as adjuvants to standard surgery and radiotherapy for patients with head and neck cancer. Accrual for this study closed in April 1982 with 229 patients entered. (c) A randomized study of total parenteral nutrition as adjuvant to chemotherapy for patients with small cell lung cancer. Accrual closed in April 1982 with 130 patients entered. (d) A randomized study of enteral and parenteral nutrition support for patients with non-small-cell lung cancer or Duke's D colorectal cancer. This study is just beginning. A total of 350 patients will be

randomized among a control group and two nutritional treatment groups. (e) A randomized study comparing intrathecal methotrexate prophylaxis to systemic high dose methotrexate for children with non-previously-treated acute lymphocytic leukemia. This is a Pediatric Oncology Branch protocol being participated in by other institutions. (f) A randomized study being developed by the Medical Oncology Branch for the treatment of multiple myeloma.

The BRB also serves as statistical center for the working groups of the Radiotherapy Development Program. There are working groups for hyperthermia, intraoperative radiotherapy, photoradiotherapy and nuclear magnetic resonance imaging. Each working group consists of several contract institutions and data is collected centrally at NCI.

3. Preclinical Drug Discovery and Development

The Biometric Research Branch works closely with the Developmental Therapeutics Program on projects related to drug discovery and development. The three major areas of activity during the past year have been the evaluation of the tumor panel and the P388 pre-screen, the clonogenic assay screening project, and toxicology testing.

A detailed analysis of the past five years experience with over 1000 compounds completely tested in the pre-clinical tumor panel (five mouse tumors and three human xenografts) was performed by Dr. Robert Wesley in conjunction with staff of the Drug Evaluation Branch. Though complete clinical results for the prospectively selected compounds will not be available for some time, the analyses performed answered several major questions posed by the tumor panel experiment five years ago. Based upon these analyses, changes in screening procedures were proposed to the DCT Board and approved, resulting in substantial savings for the Division (about $1 million per year).

Dr. Robert Makuch is extensively involved in the clonogenic assay screening project. He serves as statistician for the project and co-project officer for the four screening contracts. The first pilot year of this project has been completed and analysis of centrally collected data is being performed to establish a protocol for large-scale screening (e.g. how many human tumors to test per compound, types of tumors required, criteria for activity, presence of positive drug controls) and the decision criteria required for accepting a compound for toxicology testing based upon in-vitro results (e.g. performance of in-vitro dose-response assays in dose ranges determined from limited toxicity testing in mice).

Dr. Robert Wesley is also collaborating with Dr. Michael Lowe of the Toxicology Branch in the design of experiments that will provide information for improving the NCI mouse toxicology protocol. These experiments are focused on relating organ system toxicity to dose, blood levels and pharmacokinetics.

In addition to the three main projects described above, there is ongoing involvement to deal with the many statistical issues related to running a large scale screening and toxicology program. For example, an analysis was recently made of an experiment jointly designed to pinpoint sources of variability in the subrenal capsule mammary xenograft. It was found that the substantial variability seen

initially for this panel tumor had disappeared both in the experiment and in production testing due to a BRB recommendation at the xenograft workshop in 1980 to increase the number of test animals at each dose from three to six.

4. Extramural Clinical Program

The Biometric Research Branch collaborates extensively with the Cancer Therapy Evaluation Program (CTEP) in the administration of the extramural clinical program. All major proposed clinical protocols funded by NCI are reviewed by the BRB for adequacy of statistical design; in the past year over 125 written reviews have been prepared, primarily for randomized Phase III studies. A member of the BRB participates in the weekly CTEP protocol review meeting, and this ensures that Phase I and Phase II studies also receive adequate evaluation. The Chief of the BRB is a member of the Clinical Oncology Review Committee, the body which reviews all clinical contracts. The BRB participates in the development of national, international and inter-group studies. In the past year, this has included an adjuvant chemotherapy study of soft tissue sarcoma, an adjuvant chemotherapy study of osteosarcoma, early stage melanoma studies, hepatic arterial infusion studies, bone marrow transplantation studies for patients with acute leukemia, studies in Kaposi sarcoma and new initiatives in surgical oncology. The Chief of the BRB serves as statistical advisor to the Pan American Health Organization Program for Cancer Treatment. The BRB has participated in trying to resolve major controversies concerning the value of adjuvant chemotherapy for gastric cancer, and the analysis of national study results concerning the efficacy of Tamoxifen in the treatment of patients with primary breast cancer. The BRB also represents the NCI on site visits to statistical centers and makes recommendations concerning funding of statistical and data management activities. A member of the BRB, Dr. Brenda Edwards, organized in conjunction with the CTEP a national Workshop On The Use Of Computers In Clinical Trials. The workshop was attended by representatives of all NCI funded cooperative groups and served as a major forum for the exchange of information in the application of rapidly developing computer technology.

5. Data Base Development

The branch has completed the development and testing of a general file management system for clinical trials. The system, CAPRI, is being utilized for intramural clinical trials. The BRB branch chief has also participated in the design of the CTEP information system and in the design and implementation of an interim data base that contains response information for all NCI sponsored Phase II studies of currently managed investigational drugs. The BRB branch chief is a member of the committee to overview the development of the Developmental Therapeutics Program Information System, the DCT Data Management Committee, and the NIH Advisory Committee On Computer Usage.

6. Development of Statistical Methodology

(1) Introduction and evaluation of composite randomization designs. These are designs in which the unit of randomization is not the individual patient; it may be all patients of a private physician, a region in a developing country, a family, etc. In some circumstances composite units are the only practical basis

for treatment assignment. In other cases they may be expedient. This material has been published and presented by invitation at the Heidelberg International Breast Cancer Conference.
(2) Development of clinical trial designs incorporating early termination criteria when initial results do not appear promising for the experimental treatment. Such early termination decisions are common, but effective statistical criteria have not previously been published. Two general designs have been developed and are applicable in many practical circumstances. One design has been used by the BRB in the decision to cease accrual to our multi-institution small cell lung cancer nutrition study. An initial paper describing one of these designs has been accepted for publication.
(3) Development of simple methods for calculating confidence intervals for the median or other percentiles of a survival distribution with censored data. Quoted medians are often misleading because of variability, and these new methods provide clinicians and statisticians with fairly simple methods for calculating confidence intervals and thereby presenting results in a much more informative manner.
(4) Study of clinical research strategies for improving treatment; the role of consecutive historical control groups and moderate-size studies.
(5) Development of methods for the evaluation and graphical representation of the prognostic importance of a time dependent covariate (e.g. attainment of remission).
(6) An assessment of randomized-consent designs for cancer clinical trials. This project discusses the theoretical issues of concern and surveys the actual trials which have utilized this design.

Publications:

1. Simon, R.: The design and analysis of clinical trials. In Levine, A.S. (Ed.): Cancer in the Young: Progress in Understanding and Management. New York, Masson Publishing, 1982, pp. 391-402.

2. Popp, M.B., Fisher, R.I., Simon, R.M., and Brennan, M.F.: A prospective randomized study of adjuvant parenteral nutrition in the treatment of diffuse lymphoma: I. Effect on drug tolerance. Cancer Treatment Rep. (in press).

3. Simon, R.: The design and conduct of clinical trials in oncology. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, Lippincott, 1982, pp. 198-225.

4. Rosenberg, S.A., Rapp, H., Terry, W., Zbar, B., Costa, J., Seipp, C., and Simon, R.: Intralesional BCG therapy of patients with primary stage I melanoma. In Terry, W.D.,and Rosenberg, S.A. (Eds.): Proceedings Second International Conference Immunotherapy of Cancer: Present Status of Trials in Man. New York, Elsevier North-Holland Publishing (in press).

5. Osborne, C.K., Norton, L., Young, R.C., Garvin, A.J., Simon, R.M., Berard, C.W., and DeVita, V.T. Jr.: Nodular histiocytic lymphoma: An aggressive nodular lymphoma with potential for prolonged disease-free survival. Blood 56: 98-103, 1980.

6. Simon, R.: Composite randomization designs for clinical trials. Biometrics 37: 723-731, 1981.

7. Nemoto, T., Horton, J., Simon, R., Dao, T.L., Rosner, D., Cunningham, T., Sponzo, R., and Snyderman, M.: Comparison of four combination chemotherapy programs in metastatic breast cancer. Cancer 49: 1988-1999, 1982.

8. Simon, R.: Patient subsets and variation in therapeutic efficacy. British J. Clin. Pharmacol. (in press).

9. Simon, R.,and Lee, Y.J.: Nonparametric confidence limits for survival probabilities and median survival time. Cancer Treatment Rep. 66: 37-42, 1982.

10. Anderson, T., Chabner, B.A., Young, R.C., Berard, C.W., Garvin, A.J., Simon, R.M., and DeVita, V.T.: Malignant lymphoma I: The histology and staging of 473 patients at the National Cancer Institute. Cancer (in press).

11. Anderson, T., DeVita, V.T., Simon, R.M., Berard, C.W., Canellos, G.W., Garvin, A.J., and Young, R.C.: Malignant lymphoma II: Prognostic factors and response to treatment of 473 patients at the National Cancer Institute. Cancer (in press).

12. Abrams, R.A., Glaubiger, D., Simon, R., Lichter, A., and Deisseroth, A.B.: Haemopoietic recovery in Ewing's sarcoma after intensive combination therapy and autologous marrow infusion. The Lancet II: 385-389, 1980.

13. Simon, R.: Imbalance functions. In Kotz, S.,and Johnson, N.L. (Eds.): Encyclopedia of Statistical Sciences, Vol. IV. New York, Wiley and Sons (in press).

14. Simon, R.: Randomized clinical trials and research strategy. Cancer Treatment Rep. 66: 1083-1087, 1982.

15. Hubbard, S.M., Chabner, B.A., DeVita, V.T. Jr., Simon, R., Berard, C.W., Jones, R.B., Garvin, J.G., Canellos, G.P., Osborne, K., and Young, R.C.: Histologic progression in non-Hodgkin's lymphoma. Blood 59: 258-264, 1982.

16. The Non-Hodgkin's Lymphoma Pathologic Classification Project: National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a working formulation for clinical usage. Cancer 49: 2112-2135, 1982.

17. Lan, G., Simon, R., and Halperin, M.: Stochastically curtailed tests in long-term clinical trials. Communications in Statistics (in press).

18. Simon, R.: Statistical regression models. In Buyse, M.E., Staquet, M.J., and Sylvester, R.J. (Eds.): Cancer Clinical Trials: Design, Practice and Analysis. Oxford, Oxford Univ. Press (in press).

19. Simon, R.: Comment on statistical defensibility. The Amer. Statistician (in press).

20. Simon, R.: An evaluation of clinical trial designs that randomize composite units rather than individual patients. In Scheurlin, H. (Ed.): Proceedings of the Second International Meeting on Clinical Trials in Early Breast Cancer. New York, Grune and Stratton (in press).

21. Makuch, R.W.,and Simon, R.M.: Sample size requirements for comparing time to failure among k treatment groups. J. Chronic Dis. (in press).

22. Glaubiger, D., Makuch, R., and Schwarz, J.: Influence of prognostic factors on survival in Ewing's sarcoma. JNCI Monograph 56: 285-288, 1981.

23. Wolf, G.T., Kerney, S., Makuch, R.W., and Chretien, P.B.: The effects of thymosin on leukocyte migration inhibition in patients with head and neck squamous carcinoma. In Serrou, B.,and Rosenfeld, C. (Eds.): International Symposium on New Trends in Human Immunology and Cancer Immunotherapy. Paris, Doin Press, 1980, pp. 773-788.

24. Cohen, M.H., Makuch, R., Johnston-Early, A., Ihde, D.C., Bunn, P.A. Jr., Fossieck, B.E. Jr., and Minna, J.D.: Laboratory parameters as an alternative to performance status in prognostic stratification of patients with small cell lung cancer. Cancer Treatment Rep. 65: 187-195, 1981.

25. Cohen, M.H., Chretien, P.B., Johnston-Early, A., Ihde, D.C., Bunn, P.A. Jr., Fossieck, B.E. Jr., Makuch, R., Matthews, M.J., Shackney, S.E., and Minna, J.D.: Thymosin fraction V prolongs survival of intensively treated small cell lung cancer patients. In Terry, W.D.,and Rosenberg, S.A. (Eds.): Second International Conference: Immunotherapy of Cancer - Present Status of Trials in Man. New York, Elsevier North-Holland Publishing (in press).

26. Von Hoff, D.D., Weisenthal, L.M., Ihde, D.C., Matthews, M.J., Layard, M., and Makuch, R.: Growth of lung cancer colonies from bronchoscopy washings. Cancer 48: 400-403, 1981.

27. Terry, W.D., Hodes, R.J., Rosenberg, S.A., Fisher, R.I., Makuch, R., Gordon, H.G., and Fisher, S.G.: Treatment of stage I and II malignant melanoma with adjuvant immunotherapy or chemotherapy: Preliminary analysis of a prospective randomized trial. In Terry, W.D.,and Rosenberg, S.A. (Eds.): Second International Conference: Immunotherapy of Cancer - Present Status of Trials in Man. New York, Elsevier North-Holland Publishing (in press).

28. Ozols, R.F., Fisher, R.I., Anderson, T., Makuch, R., and Young, R.C.: Peritoneoscopy in the management of ovarian cancer. Amer. J. Obstet. Gyn. 140: 611-619, 1981.

29. Fisher, R.I., Terry, W.D., Hodes, R.J., Rosenberg, S.A., Makuch, R., Gordon, H.G., and Fisher, S.G.: Adjuvant immunotherapy or chemotherapy for malignant melanoma: Preliminary report of the National Cancer Institute randomized clinical trial. Surgical Clinics of North America 61: 1267-1278, 1981.

30. Ihde, D.C., Makuch, R.W., Cohen, M.H., Bunn, P.A. Jr., Carney, D.N., Matthews, M.J., and Minna, J.D.: Prognostic implications of stage of disease and sites of metastases in patients with small cell carcinoma of the

lung treated with intensive combination chemotherapy. Amer. Review of Respiratory Dis. 123: 500-507, 1981.

31. Baker, S.R., Makuch, R.W., and Wolf, G.T.: Preoperative cis-platinum and bleomycin in patients with head and neck squamous carcinoma: Prognostic factors for tumor response. Archives of Otolaryngology 107: 683-689, 1981.

32. Makuch, R.W.: Adjusted survival curve estimation using covariates. J. Chronic Dis. 35: 437-443, 1982.

33. Bunn, P.A. Jr., Krasnow, S., Makuch, R.W., Schlam, M.L., and Schechter, G.P.: Flow cytometric analysis of DNA content of bone marrow cells in patients with plasma cell myeloma. Blood 59: 528-535, 1982.

34. Makuch, R.W.: Statistical guidelines for medical research reports. Cancer Treatment Rep. 66: 217-219, 1982.

35. Rosen, S.T., Aisner, J., Makuch, R.W., Matthews, M.J., Ihde, D.C., Whitacre, M., Glatstein, E., Wiernik, P.H., Lichter, A.S., and Bunn, P.A. Jr.: Carcinomatous leptomeningitis in small cell lung cancer: A clinicopathologic review of the National Cancer Institute experience. Medicine 61: 45-53, 1982.

36. Radice, P.A., Matthews, M.J., Ihde, D.C., Gazdar, A.F., Carney, D.N., Bunn, P.A., Cohen, M.H., Fossieck, B.E., Makuch, R.W.,and Minna, J.D.: The clinical behavior of "mixed" small cell/large cell bronchogenic carcinoma compared to "pure" small cell subtypes. Cancer (in press).

37. Anderson, T., Makuch, R., Bunn, P.A., Ritch, P., Radice, P., Huberman, M., and Young, R.: Peritoneoscopy utilization in evaluation of hepatic metastases. In Bleiberg, H.,and Fruhlin, J. (Eds.): EORTC Monograph on the Diagnosis of Hepatic Metastases (in press).

38. Minna, J.D., Bunn, P.A. Jr., Carney, D.N., Cohen, M.H., Cuttita, F., Fossieck, B.E. Jr., Gazdar, A.F., Ihde, D.C., Johnston-Early, A., Matthews, M.J., Makuch, R.W., Oie, H., Rosen, S., Lichter, A., and Glatstein, E.J.: Experience of the National Cancer Institute (USA) in the treatment and biology of small cell lung cancer. Bulletin des Cancer (in press).

39. Freeman, C.B., Magrath, I.T., Benjamin, D., Makuch, R., Douglass, E.C., and Santella, M.L.: Classification of cell lines derived from undifferentiated lymphomas according to their expression of complement and EBV receptors: Implications for the relationship between African and American Burkitt's lymphoma. Clinical Immuno. and Immunopath. (in press).

40. Makuch, R.W.: Planning and execution of large-scale multi-institutional adjuvant trials in head and neck cancer. In Wittes, R.E. (Ed.): Head and Neck Cancer. London, John Wiley and Sons (in press).

41. Lee, Y.,and Wesley, R.: Statistical contributions to phase II trials in cancer: Interpretation, analysis and design. Seminars in Oncology 8: 403-416, 1981.

42. Poplack, D., Reaman, G., and Wesley, R.: Treatment of acute lymphoblastic leukemia in relapse: Efficacy of a four-drug reinduction regimen. Cancer Research (in press).

43. Fisher, R., Hubbard, S., DeVita, V., Berard, C., Wesley, R., Cossman, J., and Young, R.: Factors predicting long term survival in diffuse mixed, histiocytic, or undifferentiated lymphoma. Blood 58: 45-51, 1981.

44. Burt, M., Webber, B., Flye, W., and Wesley, R.: Prospective evaluation of aspiration needle, cutting needle, transbronchial, and open lung biopsy in patients with pulmonary infiltrates. Ann. Thoracic Surg. 32: 146-153, 1981.

45. Popp, M., Fisher, R., Wesley, R., Aamodt, R., and Brennan, M.: A prospective randomized study of adjuvant parenteral nutrition in the treatment of advanced diffuse lymphoma: Influence on survival. Surgery 90: 195-203, 1981.

46. Javadpour, N., Ozols, R., Anderson, T., Barlock, A., Wesley, R., and Young, R.: A randomized trial of cytoreductive surgery followed by chemotherapy versus chemotherapy alone in bulky Stage III testicular cancer with poor prognostic features. Cancer (in press).

47. Kramer, B., Pizzo, P., Robichaud, K., Witebsky, F., and Wesley, R.: Role of serial microbiologic surveillance and clinical evaluation in the management of cancer patients with fever and granulocytopenia. Am. J. Med. 72: 561-568, 1982.

48. Wesley, R.A.,and Wesley, M.N.: A program to compute power of Fisher test at interim point in a clinical trial. Computer Programs in Biomed. 14: 67-72, 1982.

49. Eberlein, T., Rosenstein, M., Spiess, P., Wesley, R., and Rosenberg, S.: Adoptive chemoimmunotherapy of a syngeneic murine lymphoma using long term lymphoid cell lines expanded in T cell growth factor. Cancer Immuno. and Immunother. (in press).

50. Pizzo, P., Robichaud, K., and Wesley, R.: Fever in the patient with cancer: A prospective study of 1001 episodes. Medicine (in press).

51. Rosenberg, S., Tepper, J., Glatstein, E., Costa, J., Young, R., Seipp, C., and Wesley, R.: Adjuvant chemotherapy for patients with soft tissue sarcomas. Surgical Clinics of North America 61: 1415-1423, 1981.

52. Shamberger, R., Pizzo, P., Goodgame, J., Lowry, S., Maher, M., Wesley, R., and Brennan, M.: The effect of total parenteral nutrition on chemotherapy induced myelosuppression: A randomized study. Am. J. of Med. (in press).

53. Trigg, M.E., Wesley, R.A., Holiman, B.J., Cole, D.E., and Poplack, D.G.: A rapid, single step technique for determination of B and T cell surface markers in malignant lymphoid cells. Experi. Hematology (in press).

54. Dresdale, A., Bonow, R.O., Wesley, R., Palmeri, S.T., Barr, L., Mathison, D., D'Angelo, T.,and Rosenberg, S.A.: Prospective evaluation of doxorubicin-

induced cardiomyopathy resulting from post-surgical adjuvant treatment of patients with soft tissue sarcomas. Cancer (in press).

55. Rosenberg, S., Tepper, J., Glatstein, E., Costa, J., Young, R., Baker, A., Brennan, M., DeMoss, E., Seipp, C., Sindelar, W., Sugarbaker, P., and Wesley, R.: Prospective randomized evaluation of adjuvant chemotherapy in adults with soft tissue sarcomas of the extremities. Cancer (in press).

56. Rosenberg, S., Tepper, J., Glatstein, E., Costa, J., Baker, A., Brennan, M., DeMoss, E., Seipp, C., Sindelar, W., Sugarbaker, P., and Wesley, R.: The treatment of soft tissue sarcomas of the extremities: Prospective randomized evaluations of 1) limb-sparing surgery plus radiation therapy compared to amputation and 2) the role of adjuvant chemotherapy. Ann. Surgery (in press).

57. Roth, J., and Wesley, R.: Human tumor-associated antigens detected by serologic techniques: Humoral immune responses to soluble sarcoma-associated antigens demonstrated by enzyme-lined immuno-absorbant solid-phase assay (ELISA). Cancer Research (in press).

58. Schilsky, R.L., Sherins, R.J., Hubbard, S.M., Wesley, M.N., Young, R.C., and DeVita, V.T.: Long term followup of ovarian function in women treated for Hodgkin's disease. Am. J.Med. 71: 552-556, 1981.

59. Donaldson, S.S., Wesley, M.N., DeWys, W.D., Jaffee, N., Suskind, R.M., and van Eys, J.: A study of the nutritional status of pediatric cancer patients. Am. J. Dis. of Children 135: 1107-1112, 1981.

60. Gress, R.E., Wesley, M.N., and Hodes, R.J.: The role of H-2 in T cell recognition of MLS. J. Immunology 127: 1763-1766, 1981.

61. Esterhay, R., Wiernik, P., Grove, W., Markus, S., and Wesley, M.: Moderate dose Methotrexate, Vincristine, Asparaginase, and Dexamethasone for treatment of adult acute lymphocytic leukemia. Blood 59: 334-345, 1982.

62. Donaldson, S.S., Wesley, M.N., DeWys, W.D., Jaffee, N., and Suskind, R.M.: A prospective randomized clinical trial of total parenteral nutrition in children with cancer. Med. and Pediatric Oncol. (in press).

63. Weiss, J.F., Wolf, G.T., Edwards, B.K., and Chretien, P.B.: Effects of smoking and age on serum levels of immunoreactive proteins altered in cancer patients. Cancer Detect. and Prevent. (in press).

64. Wolf, G.T., Chretien, P.B., Weiss, J.F., and Edwards, B.K.: Effects of smoking and age on serum levels of immune reactive proteins. Otolaryngology-Head and Neck Surgery (in press).

65. Spiegel, R.J., Schaefer, E.J., Magrath, I.T., and Edwards, B.K.: Plasma lipid alterations in leukemia and lymphoma. Am. J. Med. (in press).

66. Vermess, M., Doppman, J.L., Sugarbaker, P., Fisher, R.I., O'Leary, T.T., Chatterji, D.C., Grimes, G., Adamson, R.H., Willis, M., and Edwards, B.K.: Computer tomography of the liver and spleen with intravenous lipoid contrast

material: A review of 60 examinations. Am. J. Radiology (in press).

67. Miliauskas, J.R., Berard, C.B., Young, R.C., Garvin, A.J., Edwards, B.K., and DeVita, V.T. Jr.: Undifferentiated non-Hodgkin's lymphomas. Burkitt's and non-Burkitt's types. The relevance of making this histologic distinction. Cancer (in press).

68. Kemeny, M.M., Sugarbaker, P.H., Smith, T.J., Edwards, B.K., Shawker, T., Vermess, M., and Jones, A.E.: A prospective analysis of laboratory tests and imaging studies to detect hepatic lesions. Ann. Surgery 195: 163-167, 1982.

69. Smith, T.J., Kemeny, M.M., Sugarbaker, P.H., Jones, A.E., Vermess, M., Shawker, T.H., and Edwards, B.K.: A prospective study of hepatic imaging in the detection of metastatic disease. Ann. Surgery 195: 486-491, 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-6402-12-CP |
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PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tumor Growth Kinetics and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | |
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| Shan-Shan Chen, Visiting Fellow | CPB, COP, DCT, NCI |
| Richard Leavitt, M.D., Sr. Investigator | BCRP, DCT, NCI |
| Bruce Conger, B.A., Medical School Student | CPB, DCT, NCI |
| William H. Schuette, Head | ACES, BEIB, NIH |

COOPERATING UNITS (if any) BCRP, DCT, NCI
Medicine Branch, COP, DCT, NCI: Western Lymphoma Group
Applied Clinical Engineering Section, BEIB, NIH

LAB/BRANCH Clinical Pharmacology Branch

SECTION Cellular Kinetics Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: 5 | PROFESSIONAL: 3 | OTHER: 3 |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Computer modeling studies have indicated that recruitment and synchronization of cells in S phase are not likely to be successful in the clinical setting. This year we have begun to shift our attention to potential targets for chemotherapeutic drug scheduling other than DNA synthesis. We have shown that there is a synthesis phase for tubulin in the cell cycle, which may be one of the major sites of action for vincristine lethality. Preliminary data suggest that cells killed by adriamycin are still capable of synthesizing a full complement of DNA, but are unable to resume RNA synthesis. Flow cytometry studies in over 300 cases of non-Hodgkins lymphomas are currently being analyzed and being prepared for publication.

1. Theoretical Studies

1.1 Computer modeling studies. The mammalian cell population kinematic simulator (MACKS)

Simulation studies of the kinetics of drug-cell interactions have been carried out at low levels this year, largely because of the prioritization of other computer related projects under fixed budgetary constraints. In view of longstanding interest in hormonal cell cycle manipulation in breast cancer both at NCI and in the NSABP, we modeled this approach using the mammalian cell population kinematic simulator (MACKS). Even a cursory examination of the problem allows us to predict with confidence that the synchronization observed in vitro will not be observed in man, whatever the clinical outcome of the trials. Kinetic studies currently in progress by the NSABP should settle this question within the next year or two.

1.2 Sezary Syndrome modeling studies

Last year we published radioautographic studies in two patients with the Sezary Syndrome (Bunn et al., Blood 57: 452, 1981) which suggested that the Sezary cells circulating in the peripheral blood may not have come from the skin, but from some other primary site of Sezary cell production. This year we analyzed these data more formally, using a three compartment flux model similar to those employed in pharmacokinetic studies. The results allow us to conclude firmly that the circulating Sezary cells could not have come from the skin. This is a prime example of the use of computer modeling to elucidate the underlying biology of human malignancy.

1.3 DNA histogram analysis

In collaboration with William Schuette, of the Applied Clinical Engineering Branch, BEIB, we have recently developed a computer-based mathematical method for increasing the resolution of DNA histograms, allowing us to distinguish minimal aneuploidy by flow cytometry. For example, we now can distinguish normal male and normal female lymphocytes in a mixture, using flow cytometry. This is an exciting new development which brings the sensitivity of FCM much closer to that of karyotype studies in the clinical detection of emerging new malignant cell clones. Since the method is quite general and since it can be applied to broad classes of scientific data, it has already attracted wide attention. We have been invited to present this work at the IEEE Computer Society's International Conference on Medical Computer Science/Computational Medicine in September, 1982.

1.4 The grain overlap problem

Early this year, in collaboration with William Schuette we solved the problem of counting overlapping grains in our image analysis system, using a

technique borrowed from probability theory. Theoretical calculations were validated by experimental results. The technique has been incorporated into our image analysis system, and we have proceeded to analyze radioautographs of lymphoma cells without difficulty (see below).

## 2. Experimental studies

### 2.1 Drug studies in vitro

#### 2.1.1 Vincristine studies in sarcoma 180

Dr. Mujagic's vincristine studies in sarcoma 180 have confirmed the findings of others that the maximum lethal effect of the drug occurs in interphase, during a period that overlaps S phase in time. In addition, flow cytometry studies demonstrate a transient $G_2$ block, and mitotic index studies demonstrate a transient block in metaphase. The metaphase block is an epiphenomenon, and not the lethal event. Cells exposed to high dose of VCR for short periods of time that are lethally affected in interphase die by cell fragmentation after mitosis. Prolonged exposure to low drug concentrations produces lethal effects by a different mechanism, namely by inhibition of cytokinesis and cell endoreduplication. Thus, there is a new rationale for exploring clinically low dose prolonged infusions of the vinca alkaloids and other agents with similar mechanisms of action.

#### 2.1.2 Adriamycin studies in sarcoma 180

Dual parameter flow cytometry studies carried out by Dr. Chen on the effects of ADR on cell RNA content showed a direct correlation between cell RNA content and Coulter volume in recovering cells. However, there was a class of cells that were large and RNA-poor that accumulated over the course of time after exposure. It is known from previous studies that lethally damaged cells accumulate with large size and 4N DNA content. It is reasonable to suppose that these large 4N DNA cells are the same as the large, RNA-poor cells. If this is so, this would mean that ADR does not prevent synthesis of a full complement of DNA in any of the cells, but does permanently inhibit RNA synthesis in lethally damaged cells. Proof of this will come later in the year from dual parameter studies which will permit us to measure DNA, RNA and cell volume reliable on the same cells.

### 2.2 The tubulin cell cycle

Dr. Mujagic has isolated and purified tubulin from bovine brain, prepared an antibody to it, conjugated it with FITC, and has examined the tubulin content histogram by flow cytometry in sarcoma 180 cells. The histogram spans a twofold range, and exhibits the same saddle shape as the DNA histogram. This implies that cell tubulin content doubles between cell divisions and halves

at mitosis, and that it is synthesized during a specific phase of the cell cycle. This phase may be the VCR sensitive phase, since it overlaps DNA S phase. These studies are being repeated to confirm our initial results.

### 2.3 Proliferation and maturation in a murine pre-B lymphoma cell line

A popular hypothesis relating morphology, phenotypic surface marker expression and biological behavior in the lymphomas is that there are discrete blockade points along the normal lymphoid maturation sequence; that all cells in a given maturation sequence may be blocked at one of these points; and that the position of the actual point of blockade in the evaluation sequence determines the biological behavior, morphology, and immunologic phenotype of the lymphoma that develops.

We have studied murine lymphoma WEHI 231, which was developed and characterized extensively by Nowell Warner at the University of New Mexico, and has been labeled a "pre-B" cell lymphoma. We have found that during the course of transition from log phase to plateau phase, the DNA histogram changes (the S fraction decreases and the $G_1$ peak increases). Dr. Mujagic has found that cell morphology changes from a primitive blastic appearance to a predominance of small lymphoid cells with a "mature" appearance. Preliminary surface marker studies indicate quantitative changes in immunologic surface marker fluorescence. Dr. Chen and Ms. Occhipinti have found that total cell RNA content decreases, and that $^3$HdR incorporation into RNA also decreases as cells progress from log phase to plateau phase.

These results suggest that cell maturation is a complex and multifaceted process. Even "pre-B cell" lymphomas that are supposedly "blocked" at an early point in the lymphoid maturation sequence, do, in fact, undergo morphological, biochemical, and perhaps immunological changes that reflect "maturation", and all are associated with a decrease in proliferative rate. These studies may prove most valuable in understanding multiparameter clinical studies in the lymphomas and other neoplasms of the immune system.

## 3. Clinical studies

### 3.1 Flow cytometry studies of the non-Hodgkin's lymphomas

We have now studied over 300 cases of non-Hodgkin's lymphoma by means of dual parameter flow cytometry (75 Medicine Branch cases and the rest from USC). The data are now being analyzed. Several publications are in preparation dealing with the biology of the lymphomas, clinical correlations, biological pathological correlations, and unusual cases. Our studies in the lymphomas are continuing, with current emphasis being placed on: a) obtaining repeat biopsies in patients already studied, and b) making additional multiparameter measurements, e.g. surface markers, RNA, and perhaps tubulin.

3.2 Image analysis studies in the lymphomas

Dr. Chen has studied several cases of lymphoma this year using image analysis techniques.Multiple correlated measurements in each cell included nuclear size, cell DNA content and radioautographic grain count. These studies have supported earlier FCM studies in demonstrating that large lymphoma cells proliferate more rapidly than small lymphoma cells, and demonstrate clearly that the onset and termination of S phase are gradual processes.

PUBLICATIONS:

Shackney, S.E., Schuette, W.H., and Lukes, R.J.: The proliferative behavior of human lymphomas. In Parker, J.W, and Lukes, R.J. (Eds.): Lymphomas Neoplastic Lymphoproliferative Disorders. New York, Churchill Livingstone, in press.

Ritch, P.S., Occhipinti, S.J., Cunningham, R.E., and Shackney, S.E.: Schedule dependent synergism of combinations of hydroxyurea with adriamycin and cytosine arabinoside with adriamycin. Cancer Research 41: 3881-3884, 1981.

Ritch, P.S., Occhipinti, S.J., Skramstad, K., and Shackney, S.E.: Increased relative effectiveness of adriamycin with prolonged drug exposure in sarcoma 180 in vitro. Cancer Treatment Report 66: 1159-1168, 1982.

Cunningham, R.E., Smith, C.E., Newburger, A.E., and Shackney, S.E.: Artifacts associated with mithramycin fluorescence in the clinical detection and quantitation of aneuploidy by flow cytometry. J. Histochem. Cytochem 30: 317-322, 1982.

Shackney, S.E., and Schuette, W.H.: Multicompartment analysis of patterns of cell proliferation and cell migration in the Sezary syndrome. J. Hematol. Oncol., 1982, in press.

Shackney, S.E.: The use of Flow cytometry studies in the diagnosis and characterization of the non-Hodgkin's lymphomas. Cytometry, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06513-06-CP |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Pharmacology of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| | Neil Clendeninn, M.D., Ph.D., Pharmacol. Res. Fellow | CPB, DCT, NCI |
| | Gregory Curt, M.D., Clinical Associate | CPB, DCT, NCI |
| | Marc Lippman, M.D., Head, Med. Breast Cancer Section | MB, DCT, NCI |
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| | Brenda Bailey, Biologist | CPB, DCT, NCI |
| | Robert Do, Student Scientist | Georgetown University |
| | Lawrence Tamarkin, Ph.D. | NICHHD, NIH |

COOPERATING UNITS (if any)

Pediatric Oncology Branch; Surgery Branch; Laboratory of Chemical Pharmacology; Medicine Branch; Navy-NCI Medical Oncology Branch; Laboratory of Medicinal Chemistry and Biology, NCI

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) During the current year we have continued to examine the properties of the polyglutamate metabolites of methotrexate (MTX). We have shown that, in human breast cancer cells, additional glutamyl residues are added in gamma-carboxyl linkage to MTX to form derivatives up to five peptides in length. The longer derivatives are retained by the cell, both in free form and bound to dihydrofolate reductase, when free extracellular drug is removed from the medium. The rate of disappearance of the polyglutamates is inversely related to their chain length. The longer polyglutamates progressively replace the parent compound as the major form of bound inhibitor. We have purified dihydrofolate reductase from human breast cancer cells to homogeneity and have begun to determine the binding affinity of the polyglutamates. (2) We have initiated studies of MTX-sensitive and -resistant human lung cancer cell lines, and have described the first case of unstable MTX resistance associated with double minute chromosomes and elevated DHFR levels in a tumor line from a patient treated with (and resistant to) high-dose MTX. (3) We have continued studies of melatonin physiology in women with breast cancer and have demonstrated the absence of a nocturnal peak in melatonin secretion in women with ER+ breast cancer.

Publications

Chabner, B.A., Donehower, R.C.,and Schilsky, R.L.: Clinical pharmacology of methotrexate. Cancer Treat. Rep. 65 (Supp. I): 51-54, 1981.

Schilsky, R.L., Bailey, B.D., and Chabner, B.A.: Characteristics of membrane transport of methotrexate by cultured human breast cancer cells. Biochem. Pharmacol. 30: 1537-1542, 1981.

Jolivet, J., and Schilsky, R.L.: High-pressure liquid chromatography analysis of methotrexate polyglutamates in cultured human breast cancer cells. Biochem. Pharmacol. 30: 1387-1390, 1981.

Chabner, B.A.: Nucleoside analogs. In Crooke, S.T.,and Prestayko, A.W. (Eds.): Cancer and Chemotherapy: Antineoplastic Agents. New York, Academic Press, 1981, pp. 3-24.

Schilsky, R.L., Jolivet, J., and Chabner, B.A.: Antimetabolites. In Pinedo, H.M. (Ed.): Cancer Chemotherapy Annual. Amsterdam, Excerpta Medica, 1981, pp. 1-31.

Cowan, K., Myers, C.E.,and Chabner, B.A.: Drug monitoring in antineoplastic therapy. In Richen, A., and Marks, V. (Eds.): Therapeutic Drug Monitoring. London, Churchill-Livingstone, 1981, pp. 471-481.

Ellims, P.H., Kao, A.Y.,and Chabner, B.A.: Deoxycytidylate deaminase: Purification and some properties of the enzyme isolated from human spleen. J. Biol. Chem. 256: 6335-6340, 1981.

Tamarkin, L., Cohen, M., Roselle, D., Reichert, C., Lippman, M., and Chabner, B.: Melatonin inhibition and pinealectomy enhancement of dimethylbenz[a]anthracene-induced mammary tumors in the rat. Cancer Res. 41: 4432-4436, 1981.

Chabner, B.A.,and Myers, C.E.: Clinical pharmacology of cancer chemotherapy. In DeVita, V.T., Hellman, S.,and Rosenberg, S. (Eds.): Cancer: Principles and Practice of Oncology. Philadelphia, J.B. Lippincott, Co., 1982, pp. 156-197.

Liang, C.M., Donehower, R.C., and Chabner, B.A.: Biochemical interactions between N-phosphonacetyl-L-aspartic acid (PALA) and 5-fluorouracil (5-FU). Molec. Pharmacol. 21: 224-230, 1982.

Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. Philadelphia, W.B. Saunders Co., 1982, 457 pages.

Chabner, B.A.: Principles of cancer therapy. In Wyngaarden J., and Smith L. (Eds): Cecil Textbook of Medicine. Philadelphia, W.B. Saunders Co., 1982, pp. 1032-1047.

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

Schilsky, R.L., Jolivet, J., Bailey, B.D.,and Chabner, B.A.: Synthesis, binding, and intracellular retention of methotrexate polyglutamates by cultured human breast cancer cells. Proc. of Workshop on Folyl and Antifolyl Polyglutamates, in press.

Jolivet, J., Schilsky, R.L., Bailey, B.D., and Chabner, B.A.: The synthesis and retention of methotrexate polyglutamates in cultured human breast cancer cells. Ann. N.Y. Acad. Sci., in press.

Jolivet, J., Schilsky, R.L., Bailey, B.D., Drake, J.C., and Chabner, B.A.: Synthesis, retention and biological activity of methotrexate polyglutamates in cultured human breast cancer cells. J. Clin. Invest., in press.

Tamarkin, L., Danforth, D., Lichter, A., DeMoss, E., Chabner, B., and Lippman, M.: Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. Science, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06515-03-CP |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Adriamycin Free Radical Biochemistry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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Clinical Pharmacology

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| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Over the past year, we have discovered a new aspect of adriamycin chemistry which may have profound implications for its action in vivo. Briefly, adriamycin binds tightly to iron to yield on adriamycin iron chelate. This chelate in turn is able to catalyze the transfer of electrons from reduced thiols such as glutathione to molecular oxygen. The products produced include superoxide, hydrogen peroxide and hydroxyl radical. Because the adriamycin iron chelate also binds to cell membranes and to DNA, it is able to cause damage to these macromolecules. Our plans over the next year include: 1) achieve an understanding as to the mechanism of the catalysis produced; 2) do structure activity studies in order to determine what other drugs may possess this mechanism of action; 3) determine what role this complex plays in vivo.

The second aspect of this work has been on the use of NAC to prevent cardiac damage after adriamycin. The original mouse work upon which our current clinical trial is based was published in the Journal of Clinical Investigation this year. The clinical trial testing whether adriamycin affects the cardiac toxicity is in its latter stages and looks as though it might be significant.

PUBLICATIONS:

Myers, C.E., Gianni, L., Simone, C.B., Klecker, R., and Green, R.: Oxidative destruction of erythrocyte ghost membranes catalyzed by the doxorubicin-iron complex. Biochemistry. 21: 1707-1713, 1982.

Myers, C.E., Katki, A., and Travis, E.: Effect of selenium and vitamin E on radiation induced tissue damage. Annals New York Academy of Science, in press.

Dresdale, A.R., Barr, L.H., Bonow, R.O., Mathisen, D.J., Myers, C.E., Schwartz, D.E., d'Angelo, T. and Rosenberg, S.A.: Prospective Randomized study of the role of N-acetyl cysteine in reversing doxorubicin induced cardiomyopathy. Cancer Clinical Trials, in press.

Myers, C.E.: Anthracyclines In Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. Philadelphia, W.B. Saunders Co., 1982, pp 416-434.

Myers, C.E.: Antitumor Antibiotics I Anthracyclines. In Pinedo, H.M. (Ed.): Cancer Chemotherapy Annals Excerpta Medica, Amsterdam, 1982, in press.

Myers, C.E.: The Role of Free Radical Damage in the Genesis of Doxorubicin Cardiac Toxicity. In Muggia, F. (Ed.): New York University Anthracyclin Symposium, in press.

Ozols, R., Myers, C.E., and Young, R.C.: Intraperitoneal Administration of Adriamycin. In Muggia, F. (Ed.): New York University Anthracycline Symposium, in press.

Ozols, R.F., Young, R.G., Speyer, J.L., Sugarbaker, P.H., Greene, R., Jenkins, J., and Myers, C.E.: Phase I and pharmacologic studies of adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res., in press.

Myers, C.E.: The biochemical basis for selective free radical injury. UCLA Symposium Series, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06516-01-CP |
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PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Drug Resistance in Human Tumor Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | |
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| Desmond Carney, M.D., Visiting Scientist | Navy, MOB, DCT, NCI |
| A.W. Nienhuis, M.D., Chief | CHB, NHLBI |
| M.F. Lippman, M.D., Head, Medical Breast Section | MB, COP, DCT, NCI |

COOPERATING UNITS (if any)

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CHB/NHLBI

LAB/BRANCH

Clinical Pharmacology Branch

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| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This laboratory is involved in research in understanding the mechanisms whereby human tumor cells develop resistance to cytotoxic agents. In particular, we are concerned with identifying the genetic mechanisms involved in the development of drug resistance.

We have isolated human breast cancer and human small cell lung cancer cells which are resistant to a number of cytotoxic agents including methotrexate, PALA, azauridine, pyrazofuran, and cadmium. Characterization of these resistant cells have revealed that, at least, in the first two instances ($MTX^R$ and PALA) drug resistance in human tumor cells isolated in vitro is frequently associated with over-production of the target enzyme (DHFR and ATCase respectively), increased levels of messenger RNA coding for the target enzyme, and increased copies of the genes which code for these proteins. In the $MTX^R$ breast cancer cells the amplified DHFR genes have apparently undergone a uniform gene rearrangement in the 5' (upstream) flanking sequences during the course of gene amplification.

Ongoing studies include: 1) cloning and structural analysis of the amplified DHFR and comparison with the parental DHFR gene; 2) studies on the mechanisms of regulation of DHFR levels by estrogen and by MTX; 3) studies on

the factors which affect the frequency of drug resistant in tumor cells; 4) analyses of the mechanisms of resistance to MTX in human tumor cells obtained directly from patients; 5) the transfer of drug resistant genes to other human cells; 6) analysis of the mechanisms of cross resistance to other unrelated cytotoxic agents.

## 1. $MTX^R$ Resistance in Human Tumor Cell Lines

### 1.1 Development and characterization of resistant cells

$MTX^R$ human breast cancer (MCF7 cells) have been selected which are 5000 fold less sensitive to this drug than the parental cell line. $MTX^R$ MCF7 cells contain 50 fold higher levels of DHFR than drug sensitive cells. The DHFR in the $MTX^R$ cells is apparently unaltered compared to the W.T. enzyme as it displays the same Ki and Kd for MTX as the enzyme from the parental cells. No alteration in the transport of MTX is protected in the resistant cells.

Dr. Neil Clendennin in collaboration with Dr. R. Kaufman has purified human DHFR from these $MTX^R$ MCF7 cells. The purified enzyme has an apparent M.W. of 19,500 and is homogeneous on polyacrylamide gels. We are currently studying the biochemical parameters of this enzyme including the KM for DHF and NADPH and the Ki and Kd for $MTX_{G1}$ and various Km polyglutamate forms of ($MTX_{Gn}$). We are now beginning to purify the enzyme from the parental cell line in order to compare with the DHFR isolated from the resistant cells.

### 1.2 Genetic analysis

Concomitant with the development of resistance in these $MTX^R$ cells there has been the appearance of several elongated marker chromosomes (HSRs). In collaboration with Dr. Pete Douglas, Dr. Shan Chen and Dr. J. Whang Peng cytogenetic analysis of the $MTX^R$ MCF cells (and other drug resistant cells) have been completed. In the $MTX^R$ cells HSRs have appeared on chromosomes 7, 9, 12 and 21. With increasing resistance additional HSRs are observed on chromosomes 6 and 10. Purification of polyadenylated RNA from the W.T. (wild type) and $MTX^R$ MCF7 cells and analysis by Northern blot hybridization demonstrates that there is a vast excess of polyadenylate RNA coding for human DHFR in the $MTX^R$ cells relative to the parental cell line. Restriction enzyme digest of DNA from $MTX^R$ and W.T. MCF7 and analysis by Southern blot by hybridization indicates that the DHFR gene has been amplified in the $MTX^R$ cells. Furthermore, these studies suggest that the amplified DHFR genes have undergone a unique rearrangement in the region of the 5' end or its flanking DNA sequences. DNA mapping studies and gene cloning experiments are currently directed at identifying this apparent gene rearrangement in order to identify the factors involved in the gene amplification associated with drug resistance (see Section 1.3).

1.3 DHFR gene cloning

We have begun experiments designed to clone the human DHFR gene from the gene amplified drug resistant MCF7 cells so that the structure of the gene and the 5' flanking sequences may be compared to the sequences present in the parental cell line. Charon 4A DNA arms were purified, ligated to appropriate size fragments of $MTX^R$ MCF7 DNA, and the recombinant DNA molecules packaged into viable phages. The recombinant phage library was subsequently screened by hybridization to mouse DHFR gene sequences. One clone has been isolated which contain a 17 Kb insert which contains the entire coding sequence for DHFR on 6 Kb subfragment. The small size of this fragment (6 Kb) compared to the mouse DHFR gene suggests that this fragment is a pseudogene for DHFR. In fact two pseudogenes for DHFR have been recently isolated by J. Chen and A.W. Nienhuis from a human DNA library. We are currently in the process of comparing these three DHFR pseudogenes. Pseudogenes have been previously found for alphaglobin (Leder et al) and tubulin and evidence suggests that psuedogenes may arise by reverse transcription of m-RNA and reinsertion back into the host germ line DNA. This hypothesis has led to pseudogenes being referred to as "processed genes" (Leder) and "retrogenes" (P. Sharp).

We will be examining several questions regarding the significance of human DHFR pseudogenes including the following: (1) are DHFR pseudogenes present uniformly in various patient population subgroups; (2) are similar DHFR pseudogenes present in animal cell lines, and if so when and how did these pseudogenes evolve; and most importantly (3) are DHFR pseudogenes amplified in $MTX^R$ cells. We are also continuing to screen the library containing $MTX^R$ MCF7 DNA in order to isolate other DHFR gene clones. Structural analysis of amplified DHFR gene clones will not only provide information regarding the mechanisms involved in gene amplification but may also provide information regarding the structure and function of promotor sites. This is particularly interesting in view of our work on the stimulation of DHFR synthesis in $MTX^R$ MCF7 cells by estrogen and by MTX (see sec. 1.4).

1.4 DHFR gene Regulation

In collaboration with Drs. R. Levine and M. Lippman (MB/DCT) we are examining the hormonal regulation of amplified DHRF genes. $MTX^R$ MCF7 cells contain estrogen reception which are quantitively and qualitatively similar to the reception proteins in the parental MCF7 cells. Following incubation with $E^2$ there is an induction of progesterone receptors, an increase in cell growth, and increases in DNA, RNA and protein synthesis in the $MTX^R$ cells, which are similar to that observed in the MTX sensitive cell line. Furthermore, despite the fact that the $MTX^R$ cells contain over a 50 fold increase in DHFR levels, there is still a two to four fold increase in the level of this enyzme following the addition of $E_2$. The increase begins 15 to 20 hours after incubation with $E^2$ and the rise in enyzme levels parallels the increase in thymidine incorporation induced by $E_2$. However inhibition of cell DNA synthesis by Ara C does not block the increase in DHFR levels by $E_2$. Thus, although the stimulation of DNA synthesis and induction of

DHFR levels by $E_2$ are temporally related, they are not tightly coupled events. Radiolabeling studies followed by MTX affinity column chromatography have demonstrated that the induction in DHFR levels by $E_2$ is the result of an increased synthesis of this enyzme.

Furthermore pulse chase experiments have demonstrated that DHFR has a much shorter half life (12< hrs) in these human cells than was anticipated based on previous data obtained from mouse and hamster cell lines (t 1/2> hrs). Following the addition of $E_2$ there is little if any change in the t/12 of DHFR. Thus the principal effect of $E_2$ on DHFR is primarily on increased synthesis. We have demonstrated that tamoxifen markedly lowers the level of DHFR. Further studies are in progress to determine the mechanisms of this inhibition and may provide some insight into the synergism which has been shown to occur with these two drugs.

In collaboration with Drs. J. Jolivet (CPR) and R. Levine, we have examined the effect of MTX itself on the regulation of DHFR. Within 24 hours MTX induces a 3-5 fold increase in this enyzme in both W.T. and $MTX^R$ cells. This is observed following even subsaturating inhibitor (MTX) concentrations. We plan to examine whether $E_2$ or other factors may act synergistically with MTX on induction of DHFR and whether tamoxifen may block the effect of MTX on DHFR regulation. Further studies including pulse, and pulse chase experiments are planned in order to clarify the mechanism of DHFR regulation by MTX. Given the relatively slow and non-linear kinetics of MTX transport into cells, this relatively rapid induction of intracellular DHFR levels produced by low levels of MTX may have a significant inhibitory effect on the development of cytotoxicity in moderately resistant tumor cells containing a few amplified genes for DHFR.

We have recently synthesized a fluorescent MTX analogue which binds tightly to DHFR. We plan to use this compound in collaborative studies with Dr. S. Shackney using a FACS to study the regulation of DHFR throughout the cell cycle and in fast and slow growing tumor cell populations. This MTX-F will also increase our ability to rapidly screen DHFR level in patient specimens and various cell lines.

1.5 Cross Resistance Studies

In collaboration with Drs. B. Foster, J. Kreutz, and R. Ozols (MB/DCT) we have demonstrated that these $MTX^R$ MCF7 cells are relatively cross resistant to a wide variety of antineoplastic agents. This is of particular interest since these cells have been exposed only to MTX in their selection and the only identified mechanisms of resistance to MTX in these cells is the overproduction of DHFR. No drug transport defect is observed. In particular, these $MTX^R$ cells are cross resistant to adriamycin as determined in both soft agar cloning assay and growth inhibition studies using either short duration or continuous to adriamycin. This finding is in contrast to the collateral sensitivity to adriamycin which has been reported in $MTX^R$ hamster cells.

There is no apparent difference in adriamycin transport into W.T. and MCF7 cells. The mechanism(s) of cross resistance to adriamycin and other antineoplastic agents in these $MTX^R$ MCF7 cells remains obscure. We plan to examine this phenomena in more detail with particular attention to possible changes in drug metabolism. We also plan to examine the relationship of changes in cell cycle kinetics in resistant cell population and its possible effect on cross-resistance to antineoplastic agents. By examining the cross resistance of different clones of resistant cells isolated following selection in one particular agent (MTX, PALA, parazofuran, vincristine, adriamycin) we hope to gain some insight into a problem which is of the utmost clinical importance.

1.6 MTX Resistance in Vivo

In collaboration with Drs. D. Carney, C. Little (Navy MOB/DCT), and G. Curt (CPB), we have been examining cell lines obtained from patients with small cell carcinoma of the lung in order to determine whether DHFR gene amplification is a frequent cause of clinical resistance. Dr. Carney is determining the relative sensitivity to MTX in the various SCLC cell lines he has established in primary cultures. Drs. G. Curt and B. Chabner are studying the transport and metabolism of MTX in these cell lines. In collaboration with Dr. C. Little we are analyzing by Southern blot hybridization the quantity of DHFR genes and their restriction enyzme sites. Drs. Carney and Curt have recently identified one SCLC cell line (249P) which is resistant to MTX and contains elevated levels of DHFR. This cell line contained numerous double minute chromosomes when first studied. During passage in culture the level of DHFR has decreased and concomittantly there has been a loss of double minute chromosomes. When studied after a considerable time in culture (when DHFR levels had diminished) there was no amplification of the genes for DHFR. We are now studying earlier passage elevated in order to determine if the DHFR gene is reduplicated. This tumor developed clinical resistance to MTX following treatment with first low dose conventional therapy with MTX and subsequently with high dose MTX therapy. MTX resistance in this tumor is clealy associated with DHFR over production and presumably DHFR gene amplification (double minute chromosomes). These cytogenetic abnormalities (double minute) are associated with an unstable resistance phenotype in animal cells. Apparently the loss of these extrachromosomal pieces of DNA (double minutes) resulted in the phenotypic reversion to a drug sensitive state in these SCLC cells.

Why some cells developed resistance through gene amplification associated with HSRS (stable phenotype) while others develop double minute chromosome (unstable resistance) and what factors regulate the generation and loss of the extra chromosomal material are important questions. Since we now have two human cell lines which have developed resistance in distinct different genetic pathways ($MTX^R$-HSR, SCCL 249P double minute chromosomes) we are now provided with separate model systems in which to examine these questions.

We also plan to obtain tumor specimens from patients with lymphoma before and after therapy with a combination chemotherapy regimen containing high dose MTX in order to analyze prospectively the incidence of DHFR gene amplification in patients. These studies will be greatly facilitated using the MTX-F compound and a fluorescent cell sorter (see 1.4).

## 2. Additional Studies on Drug Resistance

### 2.1 PALA Resistance

By serial passage in increasing concentration two lines of PALA resistant ($PALA^R$) human breast cancer cells were obtained which contained increase levels of the target enyzme ATCase. These resistant cell lines also contain a parallel increase in CPSase and DHDase which is consistant with the data obtained from animal cells that these three are contained on a single protein (CAD protein; MW>200,00 daltons) multienyzme complex. The Km for aspartate and carbamylphosphate phosphate and Ki for PALA are unaltered in the resistant cells. Thus resistance to PALA in human cells is associated with increased production of the CAD protein (10 to 20 fold). The level of other enyzmes in the de novo pyrimidine biosynthetic pathway are not affected in these $PALA^R$ cells.

We have also shown by Southern blot hybridization that the gene coding for the CAD protein is amplified in the resistant cells and that the restriction enyzme sites do not appear altered in the amplified CAD genes. This is in contrast with the results and obtained in $MTX^R$ DHFR gene amplified cells in which the 5' end of the amplified genes appears to uniformly rearranged (see section 1.3). We are now selecting cells which are resistant to AT 125, which inhibits the enyzme CPSase in order to increase still further the level of the CAD protein.

### 2.2 Azauridine and Pyrazofiran

MCF7 cells resistant to azauridine and pyrazofuran have been selected. These agents inhibit OMP decathoxylase, the last enzyme in the de novo pyrimidine biosynthetic pathway. Similar to the case with the CAD protein, the enyzmes orotate phosphoribosyltranferase and OMP decarboxylase are both present on a single protein molecule (protein U). $Pyr^R$ MCR7 cells are over 1000 fold less sensitive to both Pyr and Aza U than the parental cell line. However, these resistant cells display a collateral sensitivity to 5FU. Since one of the pathways of activation of 5FR is via the enyzme orotate phosphoribsyltransifirane (5FU 5FUMP), this observation is consistent with an over production of protein U in the $Pyr^R$ cells. We are currently analyzing the target enyzme levels in these cells and plan to correlate the amount of incorporation of 5FU into RNA with 5FU cytoxicity in both W.T. and $Pyr^R$ cells.

PUBLICATIONS:

Cowan, K., and Lippman, M.: Recent Progress in Breast Cancer: Early Multimodality (Adjuvant) Chemotherapy. Arch. Int. Med. 141: 1055-1059, 1981.

Cowan, K., and Lippman, M.: Adjuvant Therapy in Breast Cancer. In Jirsch, D. (Ed.): Horizons in General Surgery. Lancaster, England, MTP Press Limited, 1982, pp. 163-180.

Cowan, K., and Lippman, M.: Steroid Receptors in Breast Cancer. Arch. Int. Med. 142: 363-366, 1982.

Cowan, K., Myers, C.E., and Chabner, B.: Drug Monitoring of Antineoplastic Agents. In Richens, A. and Marks, V. (Ed.): Therapeutic Drug Monitoring. London, Churchill Livingstone, 1981, pp. 471-481.

Cowan, K.H., and Lippman, M.: Steroid Hormone Receptors in Cancer. In Rothfeld, B., (Ed.): In Vitro Nuclear Medicine (in press).

Cowen, K.H., Levine, R., Aitken, S.C., Douglass, P., Goldsmith, M.E., Clendeninn, N., Nienhuis, A.W., and Lippman, M.E.: Dihydrofolate Reductase Gene Amplification and Rearrangement in Methotrexate-Resistant Estrogen Responsive Human Breast Cancer Cells. Biological Medicine (in press).

Cowen, K., and Lippman, M.: The Usefullness of Steroid Receptors in the Management of Breast Cancer. Your Patient and Cancer (in press).

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06517-01-CP |
|---|---|---|

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tissue Defenses Against Oxygen Radical Attack

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles E. Myers, M.D., Chief, CPB, COP, DCT, NCI

Asparandi Katki, Ph.D. — CPB, COP, DCT, NCI
Raymond Klecker, Chemist — CPB, COP, DCT, NCI

COOPERATING UNITS (if any)

LAB/BRANCH Clinical Pharmacology Branch, Clinical Oncology Program, DCT

SECTION

INSTITUTE AND LOCATION NCI, National Institutes of Health, Bethesda, Maryland 20205

| TOTAL MANYEARS: 2 | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) In the process of investigating the problem of adriamycin cardiac toxicity, it became necessary to ask whether normal tissues differed in their complement of enzymatic defenses against damage by reactive oxygen species. This led us to compare cardiac tissue with liver and to discover that cardiac tissue lacked catalase. Subsequently, we have been able to enlarge upon this study and show further that cardiac mitochondria lacked matrix glutathione peroxidase while other tissues such as liver has this enzyme in abundance at that site. In the process of performing these studies, we discovered a new class of membrane bound glutathione peroxidase and are now actively trying to purify and characterize these enzymes.

PUBLICATIONS:

Myers, C.E., Katki, A., and Travis, E.: Effect of selenium and vitamin E on radiation induced tissue damage. Annals New York Academy of Science, in press.

Myers, C.E.: Anthracyclines. In Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. Philadel., W.B. Saunders Co., 1982, pp 416-434.

Myers, C.E.: Antitumor Antibiotics I Anthracyclines. In Pinedo, H.M. (Ed.): Cancer Chemotherapy Annals Excerpta Medica. Amsterdam, 1982.

Myers, C.E.: The Role of Free Radical Damage in the Genesis of Doxorubicin Cardiac Toxicity. In Muggia, F. (Ed.): New York University Anthracycline Symposium, in press.

Myers, C.E.: The Biochemical Basis for Selective Free Radical Injury. UCLA Symposium Series, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06518-01-CP |
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PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pharmacokinetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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James Drake, Biologist, CPB, COP, DCT, NCI
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COOPERATING UNITS (if any)

LAB/BRANCH Clinical Pharmacology Branch

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| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary function of this group has been to apply the principles of pharmacokinetics to answer problems or questions which happen to occur on the clinical service. Studies has been completed or are active on each of the clinical services in COP. These include:

1. Liver perfusion with 5FU - Surgery Branch
2. Intraperitoneal 5FU as adjuvant treatment for colon carcinoma - Surgery Branch
3. Intraperitoneal misonidazole plus radiation therapy - Radiation Oncology Branch
4. BUdR radiosensitization - Radiation Oncology Branch
5. Design of the new ovarian cancer treatment protocol (CHIPS) - Medicine Branch
6. CBDCA Phase I - Medicine Branch
7. Adriamycin ip as for ovarian cancer Phase I - Medicine Branch
8. 5FU ip as treatment for ovarian cancer Phase II - Medicine Branch
9. Test dose MTX - Medicine Branch

This group has been productive and has made a substantial contribution to patient care at the Cancer Institute. As a result, we plan to give this group one of the highest priority for funding and resources over the coming year. Jerry Collins will be the Principle Investigator next year.

PUBLICATIONS:

Jenkins, J., Sugarbaker, P., Gianola, F., and Myers, C.E.: Use of intraperitoneal chemotherapy: Technical considerations in the use of intraperitoneal chemotherapy administered by Tenckhoff Catheter. Surg. Gynecol. Obst., in press.

Tester, W.J., Donehower, R.C., Eddy, J.L., Myers, C.E., and Ihde, D.C.: Evaluation of escalating doses of dichloromethotrexate with pharmacokinetic analysis in patients with hepatocellular carcinoma and other solid tumors. Cancer Chemotherapy and Pharmacol., in press, 1982.

Dresdale, A.R., Barr, L.H., Bonow, R.O., Mathisen, D.J., Myers, C.E., Schwartz, D.E., d'Angelo, T., and Rosenberg, S.A.: Prospective randomized study of the role of N-acetyl cysteine in reversing doxorubicin induced cardiomyopathy. Cancer Clinical Trials, in press, 1982.

Ozols, R.F., Myers, C.E., and Young, R.C.: Intraperitoneal Administration of Adriamycin. In Muggia, F. (Ed.): New York University Anthracycline Symposium. In press.

Ozols, R.F., Young, R.C., Speyer, J.L., Sugarbaker, P.H., Green, R., Jenkins, J., and Myers, C.E.: Phase I and pharmacologic studies of adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res., in press.

Myers, C.E., and Collins, J.: Pharmacology of intraperitoneal chemotherapy. Cancer Investigation, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 03403-17 M |
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PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Miscellaneous Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
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| Other: | Bruce Chabner | Director | DCT | NCI |
| | Charles Myers | Chief | CP | NCI |
| | Richard Fisher | Sr Investigator | M | NCI |
| | Marc Lippman | Sr Investigator | M | NCI |
| | Dan Longo | Sr Investigator | M | NCI |
| | Robert Ozols | Sr Investigator | M | NCI |
| | Jacqueline Whang-Peng | Sr Investigator | M | NCI |
| | Vincent T. DeVita, Jr. | Director | | NCI |
| | Richard Simon | Chief | BR | NCI |
| | Steven Rosenberg | Chief | S | NCI |
| | Eli Glatstein | Chief | RO | NCI |
| | Allen Lichter | Sr Investigator | RO | NCI |

COOPERATING UNITS (if any) Radiation Oncology Branch, NCI; Clinical Pharmacology Branch NCI; Biometric Research Branch,NCI; Surgery Branch,NCI; Immunology Branch, NCI; Laboratory of Molecular Pharmacology, Environmental Epidemiology Branch, NCI.

LAB/BRANCH Medicine Branch

SECTION

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 26 | 19.5 | 7.5 |

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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, soft tissue sarcomas, cervical carcinoma, and brain tumors. Phase I-II clinical trials have been completed this year on the following new experimental agents or combinations: AMSA, 13-cis-retinoic acid, Interferon. Phase II trials continue on AZQ, intraperitoneal chemotherapy of adriamycin and Interferon. New Phase I studies include Aclacinomycin A and a new platinum derivative DCBDA. Additional summaries of clinical studies are summarized under reports entitled "Clinical Program in Breast Carcinoma." Laboratory research of the Branch is summarized under reports entitled "Mechanisms of Drug Resistance, Cytogenetic Studies, Immunologic Aspects of Cancer, Mechanisms of Hormone Dependence of Human Malignancy, and Genetic Regulation of the Immune Response."

| | | | | |
|---|---|---|---|---|
| Other (cont'd): | Elaine Jaffe | Sr Investigator | LP | NCI |
| | Gregory Curt | Clincal Associate | CP | NCI |
| | Leonard Zwelling | Cancer Expert | LMP | NCI |
| | Susan Hubbard | Chief | SI | NCI |
| | Ami Ostchega | Chemo Res Nurse | M | NCI |
| | Joan Jacob | Chemo Res Nurse | M | NCI |
| | Jane Cassidy | Chemo Res Nurse | M | NCI |
| | First and Second Year Associates | | M | NCI |

Major Accomplishments in 1981-1982

General:

In 1981-1982 the Medicine Branch staff published 67 papers, articles, or book chapters and has published or has in press 24 publications. This is one of the largest number of scientific publications in the history of the Branch. Details of the clinical laboratory studies will be reviewed in the subsequent sections.

Non-Hodgkin's Lymphoma: Established:

1) The activity of ProMACE-MOPP induction therapy in advanced diffuse large cell lymphoma has been established. 74 patients with advanced disease have been treated. The complete remission after restaging is 74%. Only 18% of the patients have relapsed with follow-up now in excess of 24 months. The median survival of the entire group of patients has not yet been reached but 62% of all patients remain continuously disease-free after therapy. These results are the best yet reported for the treatment of this disease. Continuous disease-free survivals from all previous studies have been approximately 30-35%. ProMACE-MOPP appears to double the cure rate in this disease.

2) Deficient helper T cell activity exists in patients with non-Hodgkin's lymphoma. Peripheral blood T cells from patients with non-Hodgkin's lymphoma are deficient in helper capacity for non-T cell blastogenic responses to plant lectins. Nevertheless, patient non-T cells have a normal proliferative response in the presence of normal helper T cells. This data suggests that abnormal immunoregulatory cell function may be important in the pathogenesis of the non-Hodgkin's lymphomas.

3) Patients with CLL have high EBV titers and in some patients it has been possible to identify the viral genome in the DNA of CLL cells. In about 5% of patients active viral shedding occurs from cells in culture, and these observations have led to the initiation of a clinical trial using Acyclovir to treat such CLL patients. Preliminary evidence suggests that the drug completely eliminates the virus but exerts no appreciable effect on the CLL.

4) Second malignancies in non-Hodgkin's lymphoma after treatment. A comprehensive analysis of 515 patients treated at the NCI has been completed.

Results show a significant increase in second malignancies, specifically acute leukemia in patients requiring long-term therapy to control their disease. The risk appears in the "indolent" histologies (NPDL or DWDL) where continuous therapy is required. Paradoxically, those patients with aggressive lymphomas (DHL & DML) who are cured with short-term chemotherapy are not at increased risk.

Non-Hodgkin's Lymphoma: Published:

1) The importance of histologic conversion on the relapse frequency, response to therapy, and subsequent survival of patients with non-Hodgkin's lymphoma. Nodular lymphomas which convert to diffuse histologies have a worse prognosis and shorter survival unless induced into a complete remission by aggressive chemotherapy. These data have important implications on the designs of new trials in nodular lymphomas.

2) Factors predicting long-term survival in diffuse mixed, histiocytic or undifferentiated lymphoma.

3) The lack of benefit of total parenteral nutrition (TPN) as an adjuvant to the induction chemotherapy of diffuse lymphoma. This prospective randomized study revealed that TPN did not enhance the response rate, nor did it improve the tolerance to chemotherapy. Furthermore, it was associated with significant complications, particularly venous thrombosis and local infections.

4) Peripheral destruction of platelets in chronic lymphocytic leukemia: detection, prognosis and therapeutic implications.

5) Natural history of malignant lymphoma with divergent histologies at initial presentation.

6) The successful use of a lipid soluble contrast material to enhance the resolution of lymphomatous lesions in the liver and spleen using computed tomography.

Testicular Carcinoma: Established:

1) A new 4-drug combination ($PV_EBV$) composed of high dose cis-platinum (40mg/$M^2$ qd x 5), velban, bleomycin and VP-16 appears to have an extremely high (89%) complete remission (CR) rate in patients with poor prognosis advanced non-seminomatous testicular carcinoma. Standard regimens in the past had been able to produce only a 40-60% CR rate in this group of patients. This improved CR rate has been accomplished without significant renal toxicity by using hypertonic saline as a vehicle for the cis-platin infusions. If the extremely high activity of this regimen is confirmed in larger numbers of patients, it will resolve one of the last major questions in testicular cancer: namely, how does one improve the survival in poor prognosis patients.

Testicular Carcinoma: Published:

1) The lack of benefit associated with aggressive surgical cytoreduction prior to the use of combination chemotherapy in advanced testicular tumor. This is the first prospective clinical trial in any malignancy which tests the potential benefit of surgical cytoreduction prior to curative chemotherapy. In this disease, pre-chemotherapy cytoreduction is of no benefit.

2) The successful cloning of human testicular cancer in the soft agar clonogenic assay. Special staining with fluorescent labeled HCG and alfa feto protein antibodies has established the true origin of these clones. Studies screening new agents against non-seminomatous testicular cancer are now underway.

Ovarian Carcinoma: Established:

1) A new clinical trial in advanced disease has been established (CHIPS) in which alternating sequences of chemotherapy and radiation therapy are employed. Initial therapy with cyclophosphamide and hexamethylmelamine is followed by total abdominal irradiation, intraperitoneal misonidazole and systemic cis-platinum therapy. The latter three agents all show synergistic anti-tumor effects when used in animal systems. There are currently 11 patients on trial. Toxicity is minimal compared to our previous therapies for advanced ovarian cancer. There are no deaths in the patients entered on study so far.

2) Intraperitoneal (I.P.) phase I & II trials have been completed and published and will be reviewed under sections of the Clinical Pharmacology Branch Annual Report. Essentially large volume intraperitoneal chemotherapy with several agents is feasible and produces a 25-300 fold excess of drug within the peritoneal space compared to plasma concentrations. In the adjuvant 5-FU study in patients rendered disease-free with intensive induction chemotherapy, 2 patients without intraperitoneal therapy have relapsed at 8 and 17 months. One patient in the I.P. 5-FU group has relapsed at 24 months. The phase II I.P. adriamycin trial continues. Seven patients have been entered at doses from 10-60 mg/2 liters. Two patients have thus far had objective regression of disease.

3) Extensive experience with the human ovarian cancer clonogenic cell assay has been completed. Over 160 patients have been studied. Approximately 80% of samples from ascites, pleural fluid and peritoneal washings can be successfully cloned, and about 40% have >30 colonies per plate to allow some drug testing. However only about 15% have >100 colonies which would allow extensive drug testing. Clinical correlation with assay findings exceeds 90% for inactive agents and exceeds 64% for those deemed active in the assay. A dose response relationship between adriamycin concentrations and ovarian cancer cells has been demonstrated which gives direct rationale to the intraperitoneal use of the drug in selected patients. Further improvement in the assay is required to allow more effective clinical application. However, focus in our laboratories is being redirected toward the

study of mechanisms of drug resistance and the use of human ovarian cancer cell lines (see Section of Mechanisms of Drug Resistance).

4) Approximately 147 patients have now been randomized to the Ovarian Tumor Study Group/GOG study on early ovarian cancer initiated by the Medicine Branch. Preliminary results from the Stage Ia & Ib study suggest that there will be few relapses in carefully staged patients regardless of initial adjuvant therapy.

Ovarian Carcinoma: Published:

One hundred patients with "early" ovarian carcinoma have now had staging laparotomy prior to definitive therapy at Ovarian Cancer Study Group institutions. Prior to referral only 25% of patients had a surgical incision which was adequate to evaluate the entire abdomen. Thirty-two percent of patients referred, apparently free of disease, had residual disease identified by careful staging. This study has established the need for careful surgical evaluation in "early" ovarian cancer and should alter significantly the future management of such patients. Other published papers include the use of peritoneoscopy in the management of ovarian cancer; reviews of the staging and treatment of ovarian cancer; strategies for effective management of early ovarian cancer; comprehensive reviews of the chemotherapy of gynecologic malignancies.

Hodgkin's Disease: Established:

1) A comprehensive review of the remaining challenges in Hodgkin's disease therapy was completed this year in order to highlight new research directions. Important research areas remain and include: a) the optimal management of early stage disease; b) the role of alternating sequence combination chemotherapy for advanced disease; c) the optimal approach to massive mediastinal Hodgkin's disease; d) the minimization of the long-term toxicities including sterility, second malignancies, and prolonged immunosuppression.

Hodgkin's Disease: Published

1) The lack of influence of drug dose or timing on response to MOPP in previously untreated advanced Hodgkin's disease. Previous investigations had suggested that dose or dose rate might be an important influence on ultimate response and/or survival. Review of 156 patients in the MOPP study revealed that nearly all of our patients received over 75% of the projected dose of chemotherapy and there were no effects of nitrogen mustard or procarbazine dose or timing on treatment outcome.

2) The long-term effect of MOPP therapy on ovarian function of women cured of Hodgkin's disease. Twenty-seven women were studied. Persistent amenorrhea occurred in 46% and was age related. Ovarian failure is often gradual in onset, but to date, children born to women treated with MOPP have all been normal.

Breast Carcinoma:

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

Publications:

Non-Hodgkin's Lymphoma:

1) Fisher, R.I., Hubbard, S.M., DeVita, V.T., Berard, C.W., Wesley, R., Cossman, J., and Young, R.C.: Factors predicting long term survival in diffuse mixed, histiocytic or undifferentiated lymphoma. Blood 58: 45-61, 1981.

2) Longo, D.L., Young, R.C., and DeVita, V.T.: What is so good about the 'good prognosis' lymphomas? In Williams, C.J., Whitehouse, J.M.A. (Eds.): Recent Advances in Clinical Oncology. Edinburgh, Churchill-Livingstone, Ltd. 1982, pp. 223-231.

3) Fisher, R.I., Jones, R.B., DeVita, V.T., Simon R.M., Garvin, A.J., Berard, C.W., and Young, R.C.: Natural history of malignant lymphoma with divergent histologies at staging evaluation. Cancer 47: 2022-2025, 1981.

4) Hubbard, S.M., Chabner, B.A., DeVita, V.T., Simon, R., Berard, C.W., Jones, R.B., Garvin, A.J., Canellos, G.P., Osborne, C.K., and Young, R.C.: Histologic progression in non-Hodgkin's lymphoma. Blood (In Press), 1982.

5) Anderson, T., Chabner, B.A., Young, R.C., Berard, C.W., Garvin, A.J., Simon, R.M., and DeVita, V.T.: Malignant lymphoma. I. The histology and staging of 473 patients at the NCI. Cancer (In Press), 1982.

6) Anderson, T., DeVita, V.T., Simon, R.M., Berard, C.W., Canellos, G.P., Garvin, A.J., and Young, R.C.: Malignant lymphoma. II. Prognostic factors and response to treatment of 473 patients at the NCI. Cancer (In Press), 1982.

7) Rubinstein, D.B., and Longo, D.L.: Peripheral destruction of platelets in chronic lymphocytic leukemia: detection, prognosis, and therapeutic implications. Amer. J. Med. 71: 729-732, 1981.

Testicular Carcinoma

1) Ozols, R., and Javadpour, N.: Cytoreductive surgery in advanced testicular cancer. In Anderson, C.K., Jones, W.G., and Ward, A.M. (Eds.): Germ Cell Tumors. London, Taylor & Frances, 1981, pp. 333-338.

2) Ozols, R., Foster, B.J., and Javadpour, N.: Cloning of human testicular cancer in soft agar: Potential diagnostic and therapeutic applications. In Anderson, C.K., Jones, W.G., and Ward, A.M.(Eds.): Germ Cell Tumors. London, Taylor & Frances, 1981, pp. 216-226.

3) Javadpour, N., Ozols, R.F., Anderson, T., Barlock, A.B., Wesley, R., and Young, R.C.: A randomized trial of cytoreductive surgery followed by chemotherapy versus chemotherapy alone in bulky stage III testicular cancer with poor prognosis features. Cancer (In Press), 1982.

4) Ozols, R.F., Deisseroth, A.B., Javadpour, N., Barlock, A., Messerschmidt, G., and Young, R.C.: Treatment of poor prognosis non seminomatous testicular cancer with a high dose platinum combination chemotherapy regimen. Cancer (In Press), 1982.

Ovarian Carcinoma:

1) Longo, D.L., and Young, R.C.: The natural history and treatment of ovarian cancer. Ann. Rev. Med. 32: 475-490, 1981.

2) Hogan, M., and Young, R.C.: Chemotherapy in ovarian carcinoma. In Berchanal J.H., and Oettgen, H.F. (Eds.): Cancer: Achievements, Challenges, and Prospects for the 80's. New York, Grune and Stratton, 1980, pp. 365-378.

3) Perez, C.A., Young, R.C., and Knapp, R.C.: Gynecologic malignancies. In DeVita, V.T., Hellman, S.A., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.P. Lippincott and Co., (In Press), 1980.

4) Young, R.C., Perez, C.A., and Knapp, R.C.: Ovarian Cancer. In DeVita, V.T., Hellman, S.A., and Rosenberg, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.P. Lippincott and Co., (In Press), 1980.

5) Messerschmidt, G.L., Hoover, R., and Young, R.C.: Gynecologic cancer treatment: Risk factors for therapeutically induced neoplasia. Cancer 48: 442-450, 1981.

6) Ozols, R.F., Howser, D.M., and Young, R.C.: Double alkylator therapy (Thiotepa plus chlorambucil) for previously treated advanced ovarian cancer. Cancer Treat. Rep., (In Press), 1981.

7) Young, R.C., Myers, C.E., Ozols, R.F., and Hogan, W.M.: Current concepts in cancer: Ovary. Chemotherapy in advanced disease. Int. J. Radiat. Oncol. Biophys., (In Press), 1981.

8) Ozols, R.F., Fisher, R.I., Anderson, T., Makuch, R., and Young, R.C.: Peritoneoscopy in the management of ovarian cancer. Am. J. Obstet. Gynecol. (In Press), 1981.

9) Ozols, R.F., and Young, R.C.: Radiotherapy has a limited role in the treatment of most patients with ovarian cancer. In Van Scoy-Mosher, M.B. (Ed.): Controversies in Oncology. (In Press), 1981.

10) Willson, J.K.V., Ozols, R.F., Lewis, B.J., and Young, R.C.: Radiographic contribution to diagnosis and treatment of complications from peritoneoscopy. Am. J. Obstet. Gynecol. (In Press), 1981.

Hodgkin's Disease:

1) Vanhaelen, C.P.J., and Fisher, R.I.: The increased sensitivity of T cells to regulation by normal suppressor cells persists in long term survivors with Hodgkin's disease. Am. J. Med. 72: 385-390, 1982.

2) Fisher, R.I.: Implications of persistent T cell abnormalities for the etiology of Hodgkin's disease. Cancer Treat. Rep. (In Press), 1982.

3) Longo, D.L., Young, R.C., and DeVita, Jr. V.T.: The chemotherapy of Hodgkin's disease: the remaining challenges. Cancer Treat. Rep. 66: (In Press), 1982.

4) Blayney, D.W., and Longo, D.L.: Hypothyroidism in Hodgkin's disease. N. Engl. J. Med. (In Press), 1982.

5) Fisher, R.I., Vanhaelen, C., and Bostick, F.: Increased sensitivity to normal adherent suppressor cells in advanced Hodgkin's diease. Blood 57: 830-835, 1981.

6) Schilsky, R.L., Sherins, R.J., Hubbard, S.M., Young, R.C., and DeVita, V.T.: Long term follow-up of ovarian function following therapy for Hodgkin's disease. Am. J. Med. (In Press), 1982.

Breast Carcinoma:

See Section entitled "Clinical Program in Breast Cancer"

Miscellaneous:

1) Young, R.C., Ozols, R.F., and Myers, C.M.: The anthracycline antineoplastic drugs. N. Engl. J. Med. 305: 139-153, 1981.

2) Ihde, D.C., Young, R.C., Dutcher, J.S., Cordes, R.C., Barlock, A.L., Jones, R.B., and Boyd, M.R.: Phase I trial of pentamethylmelamine. Cancer Treat. Rep. 65: 755-762, 1981.

3) Dunnick, N.R., Ozols, R.F., Long, J.A., Jr., and Young, R.C.: Radiographic contribution to diagnosis and treatment of complications from peritoneoscopy. Gastrointest. Radiol. 6: 69, 1981.

4) Seigel, L.J., and Longo, D.L.: Control of chemotherapy-induced emesis. Ann. Int. Med. 95: 352-359, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 03404-11 M |
|---|---|---|

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunologic Aspects of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Richard Fisher | Sr Investigator | M | NCI |
| | Bruce Silver | Clinical Associate | M | NCI |
| | Frieda Bostick-Bruton | Technician | M | NCI |
| | Toby Hecht | Cancer Expert | M | NCI |
| | Dean Mann | Sr Investigator | E | NCI |
| | Elaine Jaffe | Sr Investigator | LP | NCI |
| | Dan Longo | Sr Investigator | M | NCI |

COOPERATING UNITS (if any)

Epidemiology Branch, DCCP, NCI; Laboratory of Pathology, DCBD, NCI

LAB/BRANCH: Medicine Branch

SECTION:

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

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 2.5 | 1 |

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have recently initiated studies to determine the origin and immunologic function of a neoplastic cell line obtained from a patient with Hodgkin's disease. Initial characterization of this cell line demonstrates that it is a potent stimulator of the human primary mixed lymphocyte response. This MLC stimulation is totally blocked by a monoclonal anti-Ia antibody and occurs despite the absence of detectable interleukin 1 production by the cell line. The cell line is also capable of serving as an accessory cell for the proliferative response of purified T cells to mitogens. In this regard and in its cell surface characteristics, these tumor cells resemble the murine dendritic cells. Patients with Hodgkin's disease have certain immunologic abnormalities that return to normal when they are cured such as delayed hypersensitivity skin test reactivity and prostaglandin mediated suppression of proliferative responses. In contrast, other immunologic abnormalities such as the increased sensitivity to monocyte or T cell suppressor cells remains persistently depressed independent of treatment. These abnormalities may be related to a patient's genetic predisposition

to develop Hodgkin's disease. Patients with untreated non-Hodgkin's lymphomas have diminished proliferative responses to pokeweed mitogen due to deficient helper T cell function. Monoclonal antibody studies reveal that the helper T cells are phenotypically present but functionally impaired. In addition, patients with non-Hodgkin's lymphoma have depressed antibody production as well. Of interest, both B and T cell non-Hodgkin's lymphomas can respond clinically to treatment with a nonspecific anti-lymphocyte serum. The role of immunoregulatory abnormalities in the pathogenesis and biology of the non-Hodgkin's lymphoma is being investigated further. A murine model of ovarian cancer can be successfully treated by nonspecific immunotherapy in the absence of a cellular immune response against the tumor. The lack of cellular immune response is explained by the tumor's lack of H-2 antigens. Interperitoneal chemotherapy and immunotherapy are synergistic in the treatment of this tumor.

Publications:

1. Fisher, R.I., Vanhaelen, C., and Bostick, F.: Increased sensitivity to normal adherent suppressor cells in advanced Hodgkin's disease. Blood 57: 830-835, 1981.

2. Vanhaelen, C.P.J., and Fisher, R.I.: Increased sensitivity of lymphocytes from patients with Hodgkin's disease to concanavalin A-induced suppressor cells. J. Immunol. 127: 1216-1220, 1981.

3. Vanhaelen, C.P.J., and Fisher, R.I.: The increased sensitivity of T cells to regulation to normal suppressor cells persists in long term survivors with Hodgkin's disease. Am. J. Med. 72: 385-390, 1982.

4. Fisher, R.I.: Implications of persistant T cell abnormalities for the etiology of Hodgkin's disease. Cancer Treat. Rep. (In Press), 1982.

5. Fisher, R.I., Silver, B.A., Vanhaelen, C.P., Jaffe, E., and Cossman, J.: Objective regressions of T and B cell lymphomas in patients following treatment with antithymocyte globulin. Cancer Res. (In Press), 1982.

6. Vanhaelen, C., and Fisher, R.I.: Requirements for successful immunotherapy and chemo-immunotherapy of a murine model of ovarian cancer. Cancer Res. 41: 980-983, 1981.

7. Vanhaelen, C.P.J., Fisher, R.I., Appella, E.,and Ramanathan, L.: Lack of histocompatability antigens on a murine ovarian teratocarcinoma. Cancer Res. 41: 3186-3191, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06119-13 M |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cytogenetic Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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| PI: | Jacqueline Whang-Peng | Sr Invest. | MB | NCI |
| Other: | Turid Knutsen | Med Technologist | MB | NCI |
| | Elaine Lee | Chemist | MB | NCI |
| | Y.S. Kao | IPA | Louisiana State Univ. | |
| | Chien-Song Kao-Shan | Visiting Fellow | MB | NCI |
| | Paul Bunn | Sr. Investigator | NNMC-MB | NCI |
| | Kenneth Cowan | Sr. Staff Fellow | CPB | NCI |
| | Ian Magrath | Sr. Investigator | POB | NCI |
| | Susan Seiber | Sr. Investigator | LCHP | NCI |
| | Anthony Fauci | Chief | LIR | NIAID |
| | Leonard Zwelling | Sr. Investigator | LMP | NCI |
| | Su-Ming Hsu | Staff Fellow | DCBD | NCI |

COOPERATING UNITS (if any)
Lab Immunol., NCI; Pediatric Oncology Br, NCI;Lab Chem Pharm, ET, NCI; Clin. Pharmacology Br., NCI; Lab. Immunoregulation, NIAID; Clin. Hematol. Br., NHLBI; Environmental Epidem. Br., DCCP, NCI; Clin. Path., ET, NCI; NNMC-MOB

LAB/BRANCH
Medicine Branch

SECTION
Cytogenetic Oncology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5 | 3 | 2 |

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The areas of investigation:

1. Cytogenetic studies of human neoplastic, hematological, and congenital disease.

2. In vitro cytogenetic studies of direct tumor material and tissue culture lines and colony cultures derived or established from patients with Burkitt's lymphoma, small cell carcinoma of the lung, ovarian cancer, and testicular cancer.

3. 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 possible correlations between chromosomal abnormalities and the early detection of secondary leukemia.

| | | | | |
|---|---|---|---|---|
| Other (cont'd): | Douglas Blayney | Clinical Associate | EEB,DCCP | NCI |
| | Neal Young | Sr. Investigator | CHB | NHLBI |

Areas of Investigation (cont'd):

4. 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.

5. Characterization of chromosomal HSRs (homogeneously staining region) and DM (double minutes) in neoplastic tissue and drug-resistant tissue culture cell lines.

6. Simplification of high resolution banding technique.

7. Application of the high resolution banding technique in cytogenetic studies of genetic disorders such as cystic fibrosis and neurofibromatosis.

8. Serial studies of chromosomal clone formation in cutaneous T-cell lymphoma.

9. Study of steroid protection of spermatogenesis in chemotherapeutic trials using a mouse model.

10. Detection of sister chromatid exchanges in vivo and in vitro following exposure to ultrasound.

11. Adaptation of immuno-biotin complex technique to chromosome banding utilizing anti-thymidine and anti-guanidine; procedure will be applied to normal cells and cells containing HSR and DM.

12. Study of the hematopoietic stem cell in CML in remission; utilizing the diffusion chamber technique to determine whether or not patients who are 100% Phl positive retain any normal stem cells.

Projects Completed in the Past Year:

1. Cytogenetic studies in lung cancer: 38 bone marrows from patients with active small cell lung cancer were analyzed: of the 30 successful specimens, 5 had abnormal karyotypes, all of which included a deletion of chromosome 3 involving bands 3p(14-23). The presence of this marker in direct marrow preparations is further evidence of its specificity for this tumor.

2. Cytogenetic studies of ovarian cancer: A total of 72 patients were studied by direct and/or short-term (1-3 day) culture of ascites, pleural fluid, or tumor; 44 patients were successfully analyzed by the chromosome banding technique. All patients were aneuploid and 39 showed structural abnormalities, most frequently involving chromosomes 1,3 , 2, 4, 9, 10, 15, 19, 6, and 11; the least involved chromosomes were #21 and #5. Clone formation and the number of chromosomes involved in structural abnormalities increased with duration of disease and were more extensive in patients treated with

surgery and chemotherapy than in patients treated with surgery alone. Our data did not show a deletion of chromosome 6 (6q-) to be specific for ovarian cancer.

3. Development of a rapid, simple technique for producing high-resolution chromosome banding: The technique presently in use (introduced by Yunis) is both tedious and time consuming (22 1/2 hours). With our new method, the cells are exposed to 1:1 0.075 M 2-mercaptoethanol: 0.075M Kcl for 20 minutes with 0.05 ug/ml colcemid added during the final 10 minutes. The results are equivalent to those obtained with the Yunis technique.

4. Immuno-biotin complex study: This procedure was combined with the use of anti-thymidine and anti-guanidine to produce differential staining of the centromeres of mouse and human chromosomes. This technique will facilitate the identification of the human or mouse cells in hybridized cells.

Publications

1. Third International Workshop on Chromosomes in Leukemia: Chromosomal abnormalities in acute lymphoblastic leukemia: structural and numerical changes in 234 cases. Cancer Genet. Cytogenet. 4: 101-110, 1981.

2. Biggar, R.J., Lee, E.C., Nkrumah, R.K., and Whang-Peng, J.: Direct cytogenetic studies by needle aspiration of Burkitt's lymphoma in Ghana, West Africa. J. Natl. Cancer Inst. 67: 769-776, 1981.

3. Whang-Peng, J., Kao-Shan, C.S., Lee, E.C., Bunn, P.A., Carney, D.N., Gazdar, A.F., and Minna, J.D.: A specific chromosome defect associated with human small cell lung cancer: deletion 3p(14-23). Science 215: 181-182, 1982.

4. Whang-Peng, J., Kao-Shan, C.S., Lee, E.C., Bunn, P.A., Carney, D.N., Gazdar, A.F., Portlock, C., and Minna, J.D.: Deletion 3p(14-23), double minutes, and homogeneously staining regions in human small cell lung cancer. Cold Spring Harbor Symposium, 1981 (In Press).

5. Whang-Peng, J., Bunn, P.A., Kao-Shan, C.S., Lee, E.C., Carney, D.N., Gazdar, A.F., and Minna, J.D.: A non-random chromosomal abnormality, del 3p(14-23), in human small cell cancer (SCLC). Cancer Genet. Cytogenet. (In Press), 1982.

6. Bentley, S.A., Knutsen, T., and Whang-Peng, J.: The origin of the hematopoietic microenvironment in continuous bone marrow culture. Experimental Hematol. (In Press), 1982.

7. Bradley, E.C., Schechter, G.P., Matthews, M.J., Whang-Peng, J., Cohen, M.H., Bunn, P.A., Ihde, D.C., and Minna, J.D.: Erythroleukemia and other hematologic complications of intensive therapy in long-term survivors of small cell lung cancer. Cancer 49:221-223, 1982.

8. Whang-Peng, J., Bunn, P.A., Knutsen, T., Matthews, M.J., Schechter, G., and Minna, J.D.: Clinical implications of cytogenetic studies in cutaneous T-cell lymphoma (CTCL). Cancer (In Press), 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06700-09 M |
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PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Program in Breast Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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| Other: | Jane Cassidy | Nurse | M | NCI |
| | Margaret Wesley | Biostatistician | BR | NCI |
| | Allan Lichter | Senior Investigator | RO | NCI |
| | Ernest DeMoss | Senior Investigator | S | NCI |
| | Sandra Levy | Senior Investigator | DCCR | NCI |
| | David Danforth | Guest Worker | M | NCI |

COOPERATING UNITS (if any)

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

LAB/BRANCH Medicine Branch and Division of Cancer Control and Rehabilitation

SECTION Medical Breast Cancer Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: 3 1/2 | PROFESSIONAL: 2 1/2 | OTHER: 1 |
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Medical Breast Cancer Section is responsible for the development of a clinical and laboratory program directed at breast cancer. Clinical trials in metastatic disease comparing chemotherapeutic, hormonal and chemohormonal regimens are underway. Biochemical and hormonal receptor studies are undertaken and coordinated by the Medical Breast Cancer Section. Clinical studies consist of a major chemotherapy trial aimed at synchronizing human breast cancer cells with hormonal agents for more successful cell cycle phase specific chemotherapy; a hormonal therapy trial aimed at prospectively evaluating the usefulness of steroid receptors for estrogens, androgens and progestins in human breast cancer; an advanced disease hormonal therapy trial comparing tamoxifen plus fluoxymesterone to tamoxifen plus danazol, and several phase II trials including 13 Cis Retinoic acid and Aclacinomycin. A trial for stage IV no evident 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; 82 patients are on study.

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

Project Description:

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

I. Clinical Trials

A. Recurrent disease trials.

1. MB-160, a randomized trial of chemotherapy + hormonal therapy aimed at inducing cell synchrony was initiated in August of 1976. This trial has approximately 101 patients on study, and preliminary analysis suggests ER+ patients may benefit from synchronization. Response duration and survival currently favor the synchronization arm; response rates are identical.

2. A new randomized primary endocrine trial comparing tamoxifen plus fluoxymesterone to tamoxifen plus danazol has recently been initiated to replace a randomized trial in which tamoxifen plus halotestin has been shown to be superior to tamoxifen alone.

3. A Phase II trial of 13 cis retinoic acid in breast, ovary, testicular cancers and melanoma is complete. No responses in 18 breast cancer patients have been seen.

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

5. A protocol for sequential endocrine approaches to male breast cancer with concurrent receptor analyses is ongoing.

6. A randomized trial of radical radiation therapy versus simple mastectomy is underway with 80 patients on study.

II. Ancillary Studies

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

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

III. Extramural Activities

A. National Surgical Adjuvant Breast Project

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

B. Outside Teaching Responsibilities

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

Publications:

1. Cowan, K., and Lippman, M.E.: Recent progress in breast cancer management: Combined modality (adjuvant) therapy. Arch. Int. Med. 141: 1055-1059, 1981.

2. Cowan, K., and Lippman, M.: Steroid receptors in breast cancer. Arch. Int. Med. 142: 363-366, 1982.

3. Lippman, M.E., and Eil, C.: Steroid Therapy of Cancer. In Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. Philadelphia, W.B. Saunders, (In Press), 1982.

4. Lippman, M.E.: Interactions of psychic and endocrine factors with progression of neoplastic disease. In Levy, S.M. (Ed.): Biological Mediators of Behavior and Disease: Neoplasia. New York, Elsevier Biomedical, 1982, pp. 55-82.

5. Eil, C., Lippman, M.E., DeMoss, E.V., and Loriaux, D.L.: Androgen receptor characteristics in skin fibroblasts from men with pubertal macromastia. J. Clin. Endo. Metab. (In Press), 1982.

6. Cassidy, J., Lippman, M., Lacroix, A., and Peck, G.: Phase II trial of 13-cis-retinoic acid in metastatic breast cancer. European J. Cancer. (In Press), 1982.

7. Tamarkin, L., Danforth, D., Lichter, A., DeMoss, E., Chabner, B., and Lippman, M.: Decreased nocturnal plasma melatonin peak in patients with estrogen positive breast cancer. Science. (In Press), 1982.

8. Allegra, J.C., and Lippman, M.E.: Quantitative estrogen receptor and duration of response to endocrine therapy. New Engl. J. Med. 302: 1259, 1980.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06702-07 M |
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PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Hormone Dependence of Human Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Marc E. Lippman | Senior Investigator | M | NCI |
| Other: | Kenneth Cowan | Senior Investigator | CPB | NCI |
| | Kay Seibert | Clinical Associate | M | NCI |
| | David Danforth | Guest Worker | M | NCI |
| | Susan Scholl | Visiting Fellow | M | NCI |
| | Diane Bronzert | Technician | M | NCI |
| | Karen Huff | Technician | M | NCI |
| | Susan Aitken | Technician | M | NCI |
| | Jeffrey Schlom | Senior Investigator | LCM | NCI |
| | Leonard Zwelling | Staff Fellow | LBP | NCI |

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NCI; Clinical Pharmacology Branch, NCI

LAB/BRANCH: Medicine Branch

SECTION: Medical Breast Cancer Section

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

| TOTAL MANYEARS: 10 | PROFESSIONAL: 10 | OTHER: |
|---|---|---|

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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

A. We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous diseases, melanoma, colon carcinoma, ovarian cancer and male breast cancer.

B. Assays for specific gene products - Thymidylate synthetase, aspartate transcarbamylase dihydrofolate reductase have been developed and we are studying the effects of steroid hormones on the activities and synthesis of these proteins.

C. We are investigating glucocorticoid receptors in various subpopulations of normal and leukemic lymphoid cells. In addition we are studying glucocorticoid receptors in Burkitt's Lymphoma, hairy cell leukemia, ANLL and the lymphomas.

D. We are studying intracellular pharmacokinetics of estrogen and anti-estrogen metabolism and efflux from human breast cancer cells using perfusion systems. These studies have led to new insights into hormone receptor interactions with the genome. Specifically, we have discovered that intranuclear estrogen receptors are changed over time to a less easily extractable form associated with the onset of steroid induced effects. This "processed" receptor appears tightly bound to DNA, is extractable by nuclease digestion and may be the proximate receptor form involved in gene regulation.

E. We are studying the detailed regulation of DNA synthesis in human breast cancer cells and as such have developed ways for accurately quantifying total DNA synthesis together with the scavenger and denovo pathways of pyrimidine biosynthesis.

F. We have developed a soft agar cloning technique which has permitted the development of clones of antiestrogen resistant variant (putative mutant) cell lines derived from hormone dependent wild typed cells. These variant cells are currently being analyzed biochemically and via somatic cell hybridization.

G. We have developed methotrexate and PALA resistant cell lines from human breast cancer cells. Pathways of resistance are under investigation. Gene reduplication has been demonstrated by molecular hybridization studies with cloned DNA. Regulation of DHFR and its specific nRNA are in progress.

H. We have developed assays for all of the enzymes in the denovo pyrimidine biosynthetic pathway and their hormonal regulation is under investigation.

I. The effects of pineal function and its secretion melatonin are being explored as modulators of breast cell growth in vivo and in vitro. Melatonin stimulates estrogen receptors in uterine and breast cancer cells. Melatonin induces a two-fold increase in estrogen receptor by 40 minutes in human breast cancer cells in culture.

J. We are studying the interactions of novel ligands with human estrogen receptors. The goal is to develop better assays and evaluate cytotoxics linked to the hormone moiety. We have shown that $^{125}I$ 16 iodoestradiol can be used to selectively kill human breast cancer cells and have used this technique to develop resistant variants.

K. We are developing a new rapid assay for steroid receptors using HPLC. This technique allows resolution of multiple molecular forms of receptor.

L. We are studying alterations in intercalating drug induced DNA damage by hormonal agents. Estrogen receptor complexes can cause a 50% increase in DNA breaks induced by iminodaunomycin and M-AMSA.

Publications

1. Allegra, J.C., Korat, O., Do, H.M.T., and Lippman, M.E.: The regulation of progesterone receptor by 17 estradiol and tamoxifen in the ZR-75-1 human breast cancer cell line in define medium. J. Receptor Res. 2: 17-28, 1981.

2. Aitken, S.C., and Lippman, M.E.: Hormonal regulation of net DNA synethesis in MCF-7 human breast cancer cells in tissue culture. Cancer Res. 42: 1727-1735, 1982.

3. Nawata, H., Chong, M., Bronzert, D., and Lippman, M.E.: Estradiol independent growth of a subline of MCF-7 human breast cancer cell in culture. J. Biol. Chem. 256: 6895-6902, 1981.

4. Nawata, H., Bronzert, D., and Lippman, M.E.: Isolation and characterization of a tamoxifen resistant cell line derived from MCF-7 human breast cells. J. Biol. Chem. 256: 5016-5021, 1981.

5. Lippman, M.E.: Hormonal regulation of human breast cancer cells in vitro. In Pike, M.C., Siiteri, P., and Welsch, C.W. (Eds.): Hormones and Breast Cancer, Cold Spring Harbor Laboratory, 1981, pp. 171-184.

6. Tamarkin, L., Cohen, M., Roselle, D., Reichert, C., Lippman, M., and Chabner, B.: Melatonin inhibition and pinealectomy enhancement of dimethylbenz(a)anthracene-induced mammary tumors in the rat. Cancer Res. 41: 4432-4436, 1981.

7. Monaco, M., Kohn, P.H., Kidwell, W.R., Strobl, J.S., and Lippman, M.E.: Vasopressin: Action on WRK-1 rat mammary tumor cells. J. Natl. Cancer Inst. 68: 267-270, 1982.

8. Chong, M., and Lippman, M.E.: Effects of RNA and ribonuclease on the binding of estrogen and glucocorticoid receptors from MCF-7 cells to DNA cellulose. J. Biol. Chem. 2996-3002, 1982.

9. Monaco, M.E., and Lippman, M.E.: A new model system for studying the phosphatidylinositol effect. J. Cell Physiol. (In Press), 1982.

10. Kasid, A., Strobl, J., Greene, G., and Lippman, M.: Characteristics of a new nuclear form of oestradiol receptor in MCF-7 human breast cancer cells. Science. (In Press), 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER<br>Z01 CM 06708-03 M |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic Regulation of the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Dan L. Longo | Sr. Investigator | M | NCI |
| Other: | Toby T. Hecht | Cancer Expert | M | NCI |
| | Louis A. Matis | Medical Staff Fellow | M | NCI |
| | Ronald Schwartz | Sr. Investigator | LI | NIAID |
| | Laurie Glimcher | Research Assoc. | LI | NIAID |
| | Alfred Singer | Sr. Investigator | I | NCI |
| | Patricia Dobson | Technologist | M | NCI |

COOPERATING UNITS (if any)

Laboratory of Immunology, NIAID
Immunology Branch, NCI

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES) ..

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Examination of the MHC restriction phenotype of thymocytes from thymus-grafted nude mice.
2. Examination of the MHC restriction phenotype of peripheral T lymphocytes from thymus-grafted nude mice.
3. Role of Epstein-Barr virus infection in the pathogenesis of chronic lymphocytic leukemia.
4. Gamma interferon production by antigen-specific T cell clones.
5. Analysis of the fine specificity of T cell antigen recognition and the influence of the MHC gene products on antigen recognition in T cell clones and T cell hybridomas.
6. Studies on the cell in the thymus responsible for the self-recognition specificity of maturing T cells.

Selected Highlights of Work Completed This Year:

I. Acquisition of T cell repertoire in nude mice: Peripheral T cells from thymus-grafted nude mice appear from our work to have acquired the capacity to recognize antigen in association with thymus MHC gene products but not nude host MHC gene products. Analysis of the thymus itself in such animals reveals that the cells are not responding to host type MHC gene products in the autologous MLR but acquire the phenotype of recognizing thymus type MHC gene products as self.

II. EB virus infection in CLL: We have identified 5 patients (out of a total of 15 patients with CLL that we have studied) who demonstrate serological evidence for chronic EB virus infection, and in 3 of the 5, EB virus has been isolated from their peripheral blood lymphocytes. Because EB virus can transform lymphocytes we sought to examine whether EB virus might be playing a role in the malignancy in these patients. We have treated 3 patients with acyclovir and have been able to eliminate all EBNA positive cells from the peripheral blood and to eliminate EB virus shedding in those patients. At the same time there have been only minor tumor responses to such treatment. Thus, EB virus infection appears to play no role in the pathogenesis of CLL but is more likely present in our patients as a result of their general immune deficiency.

III. Gamma interferon production by T cells: Antigen-specific lyt 1+ T cell clones make high titers of gamma interferon upon exposure to the antigen for which they are specific on the appropriate antigen presenting cell. This interferon production begins about 4 hours after exposure to antigen and peaks at 48 hours after stimulation. While the kinetics of the response differ from the kinetics of antigen-specific proliferation, the amount of interferon produced parallels proliferation and the affinity of the receptor for the antigen controls both the amount of proliferation and the amount of interferon production. These results demonstrate that a single antigen-specific helper T cell is capable providing interferon helper factors as well as to the cells with which it interacts.

IV. Fine specificity of T cell recognition: We have analyzed the fine specificity of antigen and MHC recognition by pigeon cytochrome c-specific T cell clones from two responder strains. The response of T cells from both strains is mapped to the same region of cytochrome c and antibodies directed at shared MHC determinants block the response in both strains. Since clones from both strains recognize similar antigen and MHC determinants, they were analyzed for the ability to recognize antigen on the other responder haplotype antigen presenting cell (APC). About 90% of T cell clones from each strain could recognize antigen only on self APC, but 10% of the clones responded to antigen on either responder MHC. Some of the clones manifested both antigen specificity and allogeneic reactivity against the other responder haplotype. These results tend to support the notion that the T cell receptor is a single (rather than a dual) receptor with antigen specificity derived from recognizing antigen as an altered self structure.

Publications:

1. Longo, D.L., Matis, L.A., and Schwartz, R.H.: Insights into immune response gene function from experiments with chimeric animals. CRC Crit. Rev. Immunol. 2: 83-132, 1981.

2. Seigel, L.J., and Longo, D.L.: Control of chemotherapy-induced emesis. Ann. Int. Med. 95: 352-359, 1981.

3. Rubinstein, D.B., and Longo, D.L.: Peripheral destruction of platelets in chronic lymphocytic leukemia: detection, prognosis, and therapeutic implications. Amer. J. Med. 71: 729-732, 1981.

4. Glimcher, L.H., Longo, D.L., Green, I., and Schwartz, R.H.: The murine syngeneic mixed lymphocyte response. I. The target antigens are self Ia molecules. J. Exp. Med. 154: 1652-1670, 1981.

5. Longo, D.L., and Paul, W.E.: Immune response genes and Ia antigens: the relationships between them and their role in lymphocyte interactions. In Parham, P., and Strominger, J.L. (Eds.): Transplantation Antigens. London, Chapman & Hill, Ltd. 1982, pp. 161-185.

6. Tse, H.Y., Mond, J.J., and Longo, D.L.: B lymphocyte immune response gene phenotype is genetically determined. J. Exp. Med. 155: 1239-1244, 1982.

7. Schwartz, R.H., Glimcher, L.H., Hedrick, S.M., Singer, A., and Longo, D.L.: Restriction to thymic major histocompatibility complex (MHC) and immune response (Ir) phenotype of T cells from allogeneic chimeras. Behring Institute Research Communications. (In Press) (meeting summarized in Science 214: 893-896, 1981).

8. Glimcher, L.H., Schwartz, R.H., Longo, D.L., and Singer, A.: The restriction specificity of the syngeneic mixed lymphocyte reaction, a primary anti-I region T cell proliferative response, is imparted by the thymus. Eur. J. Immunol. (In Press), 1982.

9. Longo, D.L., and DeVita, Jr., V.T.: Lymphomas. In Pinedo, H. M. (Ed.): Cancer Chemotherapy Annual 1982. Amsterdam, Excerpta Medica, (In Press), 1982.

10. Thomas, J.L., Bernstein, M.E., Vermess, M., Barnes, P.A., Fuller, L., Hagemeister, F.B., Doppman, J., Fisher, R.I., and Longo, D.L.: The use of EOE-13 in the detection of hepatosplenic lymphoma. Radiology (In Press), 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06709-02 M |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Drug Resistance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Robert F. Ozols | Sr. Investigator | M | NCI |
| Other: | Robert C. Young | Chief | M | NCI |
| | Charles E. Myers | Sr. Investigator | CP | NCI |
| | W. Michael Hogan | Visiting Assoc. | M | NCI |
| | Jan Keizer | Visiting Fellow | M | NCI |
| | Brenda Foster | Clinical Assoc. | M | NCI |
| | Karen Grotzinger | Med Technologist | M | NCI |
| | Wilma McCoy | Med Technologist | M | NCI |
| | Julian Hill | Clinical Assoc. | M | NCI |

COOPERATING UNITS (If any)

Clinical Pharmacology Branch, NCI

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the in vitro patterns of sensitivity to antineoplastic agents and the mechanisms of resistance of human tumors to anticancer drugs. In particular, these studies are being performed on human ovarian cancer cells using both fresh human ovarian cancer cells and established human tumor cell lines. Since few established human ovarian cancer cell lines are available, we are in the process of establishing human ovarian cancer cell lines and defining the essential growth requirements for these cells. Drug sensitivity studies are performed using a clonogenic assay. The mechanisms of resistance are being examined at a cellular level and are examining drug transport and metabolism.

Ongoing Studies:

1. Ovarian Cancer Cell Lines. We have grown ovarian cancer colonies from malignant effusions from 150 patients. We have used the dose response relationships in these cells to adriamycin to provide a rationale for a phase I trial of intraperitoneal adriamycin in patients with ovarian cancer.

We have grown xenografts of human ovarian cancer cells in nude mice in 40% of attempts. We have placed 75 specimens of fresh ovarian cancer cells in tissue culture and are in the process of characterizing 3 established cell lines. We are also in the process of defining the serum free conditions for ovarian cancer cell growth.

2. Testicular Cancer. We have grown testicular cancer colonies in soft agar from fresh tumor cell suspensions of human testicular cancer. These colonies are able to produce human chorionic gonadotropin and alpha feto protein.

3. Drug Resistance Studies. We have established human ovarian cancer cell lines with differing degrees of resistance to various antineoplastic drugs. Likewise, we have compared the patterns of in vitro resistance in human breast cancer cells. We are in the process of comparing the roles of drug transport and drug metabolism in these drug resistant cell lines. We have demonstrated that amphotericin B cannot restore the sensitivity of human ovarian cancer cells (either fresh specimens or established cell lines) to adriamycin or melphalan.

References:

1. Ozols, R.F., Willson, J.K.V., and Young, R.C.: Human ovarian cancer colony formation. Growth from malignant washings and pharmacologic application. In Salmon, S.E. (Ed.): Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, Inc., 1980, pp. 247-257.

2. Ozols, R.F., Willson, J.K.V., Grotzinger, K.R., and Young, R.C.: Cloning of human ovarian cancer cells in soft agar from malignant effusions and peritoneal washings. Cancer Res. 40: 2743, 1980.

3. Ozols, R.F., Willson, J.K.V., Weltz, M., Grotzinger, K.R., Myers, C.E., and Young, R.C.: Inhibition of human ovarian cancer colony formation by adriamycin and its major metabolites. Cancer Res. 40: 4109-4112, 1980.

4. Ozols, R., Foster, B.J., and Javadpour, N.: Cloning of human testicular cancer in soft agar: Potential diagnostic and therapeutic applications. In Anderson, C.K., Jones, W.G., and Ward, A.M. (Eds.): Germ Cell Tumors. London, Taylor & Frances, 1981, pp 216-226.

5. Young, R.C., Ozols, R.F., and Myers, C.E.: The anthracycline antineoplastic drugs. N. Engl. J. Med. 305: 139-152, 1981.

6. Ozols, R.F., Young, R.C., Speyer, J.L., Sugarbaker, P.H., Green, R., Jenkins, J., and Myers, C.E.: Phase I and pharmacologic studies of adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res. (In Press), 1982.

7. Wu, P-C., Ozols, R.F., Hatanoka, M., and Boone, C.W.: Anticancer drugs: effect on the cloning of Raji lymphoma cells in soft agar. J. Natl. Cancer Inst. 68: 115-121, 1982.

October 1, 1981 to September 31, 1982

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

## 1.0 Summary

### 1.1 General Information

The NCI-Navy Medical Oncology Branch (NNMOB) is an intramural adult oncology program of the Clinical Oncology Program, Division of Cancer Treatment, NCI. It originated from the former NCI-VA Medical Oncology Branch and was created following the signing of a General Memorandum of Understanding (MOU) in April 1979 between the National Naval Medical Center (NNMC), Bethesda, MD, and the National Cancer Institute (NCI). This MOU was ratified by the Director NCI, Director NIH, Surgeon General USPHS, Commanding Officer NNMC, and the Surgeon General of the U.S. Navy Medical Corps.

The MOU provided for a joint NNMC program in medical oncology-hematology to be conducted by the NCI-Navy MOB and the NNMC Hematology-Oncology Branch.

The guidelines and specifics for running the combined program are set down in an interagency agreement (IAG). This agreement has been through several revisions and has been approved by the NCI and NNMC and is being considered for final approval by the Navy Bureau of Medicine (BUMED) policy review board.

The mission of the NCI-Navy MOB is threefold: (1) the conduct of clinical investigation into the diagnosis, treatment, and management of human malignancy; (2) participation with the NNMC Hematology-Oncology Branch in the delivery of medical care to patients with malignancy at the NNMC both through clinical investigation and the delivery of standard care; (3) conduct of laboratory investigation into human tumor cell biology, general cell biology, and molecular genetics. The joining of these three missions under one program provides a unique opportunity to forward the missions of the NCI and the NNMC.

### 1.2 Administrative

The change from the NCI-VA to NCI-Navy Branch has necessitated a large expenditure of staff effort and time. Thus, the administrative section has considerable detail.

#### 1.21 Move

In July 1981, the clinical portion of the former NCI-VA Branch moved from the Veterans Administration Medical Center (VAMC), Washington, DC, to the NNMC. The Laboratory Research Division moved in April 1982. During the interim the Laboratory Division continued to function at the VAMC under a local interim agreement.

## 1.22 Renovation

Renovation of the clinical and laboratory space in Building 1, NNMC, was ongoing during 1981 and 1982, and will continue during 1983. The inpatient clinical unit has and will be located in the new NNMC hospital (Building 10), Ward 6W. The outpatient clinic and offices were located in Building 240 from July 1981 through March 1982; they then moved to Building 1 (floors 4, 5) Tower NNMC, along with the laboratory portion, in April 1982 into the NNMC "Living Space." The renovation of the "permanent" outpatient clinic, offices, and laboratory space in NNMC Building 8 should be completed by October 1984. All of this renovation is being conducted under interagency agreement (YO-1-CM-20200). The renovation time table has been dictated by the NNMC overall retrofit plans and has been under the supervision of the NNMC renovation officers. To insure optimal benefit to the NIH, the NCI-Navy Branch and Administrative Officer DCT have obtained the aid of the NCI's office of renovation/construction (Dr. Fox, Mr. Dolan). This office has reviewed the plans, and will participate in final lab layout design and ascertain the proper completion of the project. In addition to the renovation, the move entailed the procurement of much new equipment particularly for the laboratory, including installation and certification.

## 1.23 Personnel

Because of the move from the Washington VAMC to the NNMC there were a series of complex personnel changes involving shifting some personnel from VA to NIH roles, hiring new office and nursing personnel, and integration and orientation of the NCI with NNMC personnel. Ms. M. A. Anerino, NCI-Navy Administrator, has coordinated these complex operations. The senior staff personnel that moved to the NCI-Navy Branch include: Drs. Minna, Ihde, Bunn, Carney, Matthews and Gazdar, while Drs. Cohen and Fossieck remained at the VAMC. Of the nursing staff Ms. Eddy, Ms. Brooks and Ms. Li came. The remainder of the nursing staff stayed at the Washington VAMC and a series of outpatient-research and inpatient nurses were or are in the process of being hired and trained. Almost an entirely new clinical care support staff and office staff have been hired and are being trained. The laboratory research staff fortunately moved almost intact.

## 1.24 Interagency Agreement

The interagency agreement detailed guidelines have been undergoing multiple drafts and revisions by NNMC and NCI personnel. These are a complex series of negotiations which involve administration, department of medicine, nursing service, patient affairs, comptroller, supply, public works, personnel, pharmacy, laboratory service, radiology, security, radiation safety, and occupational health. These have taken considerable time and effort and should develop a document that will be beneficial and acceptable to both the NNMC and the NCI.

## 1.25 Integration of NCI-Navy Branch with NNMC Staff

To insure successful function of the NCI-Navy Oncology Branch it had to integrate its function with the professional and supporting staff of the NNMC. CDR Stephen Veach, Military Coordinator and chief of the NNMC Oncology-Hematology Branch, has supervised this. This has occurred so far on three levels. First,

the senior physicians and medical oncology fellows have developed an integrated attending schedule (inpatient, outpatient, and consult) and patient care program. This program integrates civilian and military patients. The outpatient-research nurses and supporting staff have formed a joint outpatient clinic with CDR John Phares (head of NNMC Hematology), the administrative head, responsible for outpatient affairs, quality care assurance documentation, and chemotherapy nursing certification. Thus, both NCI and NNMC programs work together and oncology is integrated with hematology as well. Second, inpatient ward coverage and supervision had to change from the NCI-VA system based heavily on the NCI medical staff associates to the NNMC system based on medical residents and interns with medical staff fellow supervision. Third, the inpatient nursing service integrated under the NNMC nursing service. This, however, will require training NNMC nursing personnel in the needs of the 6W service. Fourth, joint staging and treatment planning conferences of the NCI-NNMC total oncology/hematology program with NNMC and NCI radiotherapy and with NNMC radiology. In addition, a joint NNMC pathology/surgery/radiotherapy/oncology tumor board has been strengthened. In this regard an NCI Radiation Oncology Branch fellow in training is assigned full time to the NNMC Radiation Therapy Branch.

1.26 Integration with Clinical Center Medicine Branch (MB) and Radiation Oncology Branch (ROB)

With the geographical proximity of the NCI-Navy and Clinical Center Medical Oncology Branches as well as a directive from the Director NCI, the NCI-Navy Branch is moving to integrate its clinical treatment protocol program. This is primarily because of the large number of Department of Defense (DOD) patients coming to the NNMC who would be eligible to enter intramural NCI protocols. In addition, some civilian patients entering into NCI-Navy protocols may need to receive this treatment at the Clinical Center-NIH. The NCI-Navy (and the former NCI-VA) Branch had already integrated treatment protocols with the NCI-Radiation Oncology Branch for several years. The plan for clinical protocol integration is: (1) integrate the medical staff fellow program under the Clinical Oncology Program (COP) so there are two equal 6-month rotations; (2) conduct joint NCI-Navy-MB protocol planning and review sessions including NNMC and Biometry Research Branch staff; (3) submission of appropriate protocols through NNMC channels for approval and upon obtainment, implementation of the protocols. The Biometry Research Branch will serve as a central data collation and statistical evaluation source. (4) Joint staging-treatment conferences and clinics should eventually develop.

1.27 Clinical Protocol Approvals

In addition to NCI/NIH review, all NCI clinical protocols at NNMC have to be approved through appropriate NNMC review processes. This effort has included a large number of NCI-Navy, Eastern Cooperative Oncology Group (ECOG), and some high priority MB protocols. Most of these protocols have been approved and will be returned to the NCINavy Branch upon final approval of the Interagency Agreement Guidelines by the Navy BUMED.

1.28 USUHS Interaction

The senior staff physicians and fellows are all receiving appointments on the faculty of the USUHS Department of Medicine. The degree of interaction with the USUHS is being determined at present.

1.29 Establishment of a Molecular Genetics Group

Because of the recent developments in molecular genetics and recombinant DNA techniques, a major effort is ongoing to establish a molecular genetics group within the NCI-Navy Branch. These techniques can then be applied to the study of human tumor cell biology, genetics, and drug and radiation resistance. In addition, the area is developing so rapidly that all new techniques for manipulating genes should have relevance in the short-term future to human tumor biology problems and thus the development of new molecular genetic approaches will be encouraged.

The core of this group has been recruited and includes Dr. I. Kirsch, molecular cytogenetics-pediatric oncology; Dr. G. Hollis, movable genetic elements and processed genes; Ms. M. Nau, very experienced in all fundamental molecular genetic techniques; Dr. M. Kuehl, molecular biology of B and T lymphocytes and lymphomas. This group will be completely together by July 1983. In addition, two other young investigators (Dr. P. Hieter and Dr. J. Balbey) currently at Stanford and Harvard, respectively, have offers to join the group in July 1984. All of these investigators will work on independent as well as collaborative projects. They will each have staff fellows, clinical associates, and visiting fellows working under their supervision. The collaborative projects include the DCT as a whole as well as the rest of the NCI-Navy Branch. The group will be under Dr. J. Minna's Section of Genetics, Immunology, and Molecular Biology. Major first efforts will include isolation, characterization, and chromosomal localization of transforming genes from human lung cancer and lymphomas, and the isolation and characterization of genes coding for drug and radiation resistance. To facilitate this Dr. Minna will spend a year working almost exclusively in the laboratory from July 1983 until July 1984.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 03024-13 NMOB |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Clinical Trials and Other Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Senior Staff Oncologists, PI:

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

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

CDR Stephen R. Veach, M.D.
CDR John C. Phares, M.D.
(Continued)

COOPERATING UNITS (if any)
See attached sheets

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
None

INSTITUTE AND LOCATION
National Naval Medical Center, Bethesda, Maryland

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 55 | 15 | 40 |

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The NCI-Navy Medical Oncology Branch studies new methods of evaluating and treating patients with malignant disease and provides general medical oncology consultations for the National Naval Medical Center. Clinical investigations are carried out in patients with small cell lung cancer and other types of lung cancer (epidermoid, large cell, and adenocarcinoma), mycosis fungoides and the Sezary syndrome, carcinoma of the prostate, and multiple myeloma and other plasma cell dyscrasias. New Phase I agents, both chemotherapeutic and immunotherapeutic, are studied. Other interests involve general medical oncology and miscellaneous cancers. Within each disease category, investigations are centered in one or more of the following areas: 1) therapeutic trials and complications of treatment; 2) staging procedures, prognostic factors, and natural history; 3) clinical-cell biologic correlations; 4) review articles. Some 30 oncology consultations per month are seen in the NNMC and outpatient care (150 visits/week) provided for patients requiring chemotherapy who are not eligible for any protocol studies. Medical Staff Fellows are trained in medical oncology and clinical investigation.

Senior Staff Other, PI:

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

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| James Commers, M.D. | NCI-Navy MOB NCI/NNMC |
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Second Year

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| James Mulshine, M.D. | USPHS |
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First Year

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| Gerald Batist, M.D. | NCI-Navy MOB NCI/NNMC |
| Austin Doyle, M.D. | NCI-Navy MOB NCI/NNMC |
| Richard Knop, M.D. | NCI-Navy MOB NCI/NNMC |
| George Morstyn, M.D. | NCI-Navy MOB NCI/NNMC |
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| Mercedes Gilliom, RN | Clinical Research Nurse | NCI-Navy MOB NCI/NNMC |
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Cooperating Units

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| LT Richard Whittington, M.D. | Radiation Oncology Service |
| CAPT Kevin O'Connell, M.D. | Chief, Urology Service |
| CDR Fred Worsham, M.D. | Pathology Service |
| CAPT David Garvin, M.D. | Pathology Service |

Cooperating Units

National Institutes of Health

| | | |
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| E. Jaffe, M.D. | Senior Pathologist | LP/NCI |
| N. Javadpour, M.D. | Senior Staff Surgeon | SB/DCT/NCI |
| T. Kinsella, M.D. | Senior Investigator | ROB/DCT/NCI |
| A. Lichter, M.D. | Senior Investigator | ROB/DCT/NCI |
| M. Lippman, M.D. | Senior Investigator | MB/DCT/NCI |
| D. Longo, M.D. | Senior Investigator | MB/DCT/NCI |
| R. Makuch, M.D. | Statistician | BRB/DCT/NCI |
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| T. Waldman, M.D. | Chief | MET/DCBD/NCI |
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| R. Young, M.D. | Chief | MB/DCT/NCI |
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| L. Napoli, M.D. | Radiologist, Providence Hospital |
| I. Royston, M.D. | University California, San Diego |
| R. Yesner, M.D. | Yale University, New Haven, Connecticut |
| R. Donehower, M.D. | Johns Hopkins Oncology Center, Baltimore, MD |
| J. Aisner, M.D. | University of Maryland Cancer Center, Baltimore, Maryland |

## 2.0 Clinical Research

The major research projects are discussed in their order of priority as determined by the NCI-Navy Branch. Despite difficulties in fully establishing the clinical research operation at NNMC, 41 patients (24 civilian and 17 DOD beneficiaries) have been entered on NCI clinical protocols from July 1982 to May 1982. Another 14 patients have entered Eastern Cooperative Oncology Group (ECOG) protocols.

## 2.1 Small Cell Lung Cancer Limited Stage Disease

This is a unique randomized trial comparing combination chemotherapy with or without simultaneous chest radiotherapy conducted in collaboration with the ROB. Preliminary results suggest benefit of combined modality therapy in the fraction of long-term disease-free survivors.

## 2.2 Small Cell Lung Cancer Extensive Stage Disease

This is a unique single arm trial of combination chemotherapy followed by "intensification" with new high dose chemo-radiotherapy at week 15 and autologous bone marrow support. It is conducted in collaboration with the ROB and MB and preliminary results suggest no striking benefit from intensification therapy.

## 2.3 Non-Small Cell Lung Cancer (NSCLC) All Stages

The protocol for this trial is in the developmental stages. Fundamentally, the trial will include NSCLC patients of all stages and patients will be staged, their tumors biopsied and tumor cell lines initiated for drug and radiation sensitivity testing. Patients will receive "standard" initial treatment and then be randomized on the basis of their in vitro sensitivity testing to receive standard or assay "selected" therapy. This will require collaboration with thoracic surgery, pulmonary medicine, and radiation oncology as well as the research laboratory.

## 2.4 Mycosis Fungoides - Sezary Syndrome

This protocol is a unique randomized trial comparing conservative, topical "watch and wait" therapy to aggressive combined modality chemotherapy and total body electron beam therapy. It is conducted in collaboration with the ROB/Clinical Center.

## 2.5 Multiple Advanced Malignancies: In Vitro Therapy Selection

Because of the large number of patients with a variety of advanced malignancies seen at the NNMC, we are planning to develop a protocol to biopsy and culture tumors from individual patients and to allocate therapy based on the results of the in vitro assays. This would be another direct integration of the clinical with the laboratory research.

2.6 Nodular Lymphoma Favorable Histologies

This randomized trial of "watch and wait" vs. aggressive therapy developed by the MB and ROB at the Clinical Center is being implemented.

2.7 Advanced Stage Non-seminomatous Testicular Cancer

This randomized trial of different new chemotherapy regimens developed by the MB/Clinical Center is being implemented. Because of the NNMC patient population the NCI-Navy group should be able to contribute significant numbers of patients to this protocol.

2.8 Diffuse Histiocytic Lymphoma (DHL)

This randomized trial developed by the Medicine Branch/Clinical Center (MB/CC) comparing two new combination chemotherapies for DHL is under review. The NCI-Navy Branch should be able to enter significant patient numbers.

2.9 Advanced Breast Cancer Rendered Free of Disease

This unique trial developed by the MB/CC is under review.

2.10 Primary Breast Cancer

This unique randomized trial comparing standard surgical therapy vs. lumpectomy and primary treatment with radiation therapy is being reviewed with NNMC surgical and radiation therapy staffs. Because of the potential of large patient numbers, considerable effort will be given to getting this protocol approved and operational.

2.11 Advanced Breast Cancer

The old MB protocol is concluding and we plan to participate with the MB in developing a new trial.

2.12 Prostate Cancer, Stage D

The current single arm but unique trial employs combination chemotherapy before hormonal manipulation. The results show separate sensitivities and resistance to chemotherapy vs. hormonal manipulation. We plan to develop a new randomized trial comparing sequential hormonal manipulation followed by combination chemotherapy vs. combined hormonal-chemotherapy.

2.13 Clinical Applications of Monoclonal Antibodies

Protocols for the clinical application of monoclonal antibodies are under development. The first protocol will be a Phase I-II trial of an anti- T lymphocyte monoclonal antibody (Anti-T101) in mycosis fungoides/Sezary syndrome and CLL in collaboration with the BRMP. Subsequent studies planned include use of anti-breast cancer monoclonals developed by Dr. Schlom's group and anti-lung cancer monoclonals developed by our group in nuclear medicine scanning and ultimately in targeting of radiotherapy, drugs, and toxins.

2.14 Hodgkin's Disease

The trials developed by the MB and ROB/CC for early and late stage Hodgkin's disease as well as bulky mediastinal disease are being reviewed.

2.15 Colon Cancer

The protocol for resecting colon cancer liver metastases and giving subsequent anatomically directed chemotherapy developed by the Surgery Branch/Clinical Center (SB/CC) is under review. If the NNMC surgeons will accept this protocol, we would implement it.

2.16 Multiple Myeloma

The current single arm trial of alternating combination chemotherapy regimens is being closed. The results indicate possible benefit from this regimen. The possibility of a cooperative trial between the NNMC, VAMC, George Washington, and Walter Reed Army Medical Center (WRAMC) is being discussed.

2.17 Multiple Diseases

A variety of ECOG protocols exist, and when these do not compete with intramural NCI protocols, NNMC patients are entered.

2.18 Phase I-II Trials

Until the standard protocols are implemented we are not pressing forward with Phase I-II trials of investigational new drugs. Following successful implementation of the major protocols we will explore with the Navy's central NIDRB to see if these are possible. At present any patient desiring treatment on an NCI Phase I-II trial that cannot be done at the NNMC can be referred for this treatment to the CC/NIH and then returned to the NNMC at the completion of his/her protocol participation.

PUBLICATIONS (Clinical)

1. Anderson, T., Makuch, R., Bunn, P.A., Ritch, P., Radice, P., Huberman, M., and Young, R.: Peritoneoscopy Utilization in Evaluation of Hepatic Metastases. In Bleiberg, H., and Fruhlin, J. (Eds.): EORTC Monograph on the Diagnosis of Hepatic Metastases. (in press).

2. Bleehen, N.M., Bunn, P.A., Cos, J.D., Dombernowsky, P., Fox, R.M., Host, H., Joss, R., White, J.E., and Wittes, R.E.: Role of radiation therapy in small cell anaplastic carcinoma of the lung. Cancer Treat. Rep. (in press).

3. Bradley, E.C., Schecter, G.P., Matthews, M.J., Whang-Peng, J., Cohen, M.H., Ihde, D.C., and Minna, J.D.: Erythroleukemia and other hematologic complications of intensive therapy in long-term survivors of small cell carcinoma of the lung. Cancer 49: 221 - 223, 1982.

4. Brigham, B.A., Bunn, P.A., Horton, J.E., Schechter, F.P., Wahl, L.M., Bradley, E.C., Dunnick, N.R., and Matthews, M.J.: Skeletal manifestations in cutaneous T-cell lymphomas. Arch. Dermatol. (in press).

5. Bunn, P.A.: Nitrosourea Containing Combinations in Small Cell Lung Cancer. In Schein, P., Baker, H., and Carter, S. (Eds.): Nitrosoureas - Current Status and New Developments. New York, Academic Press, 1981, pp. 233-244.

6. Bunn, P.A., Jr., Fischmann, A.B., and Glatstein, E.: Staging and treatment of the Cutaneous T-Cell Lymphomas (Mycosis Fungoides and the Sezary Syndrome) at the National Cancer Institute (USA). In Christophers, E.A., and Goos, M. (Eds.): Cutaneous Lymphoma. Berlin, Springer-Verlag, (in press).

7. Bunn, P.A., Jr., Lichter, A., Glatstein, E.J., and Minna, J.D.: Further Prospects for the Cure of Small Cell Lung Cancer. In Greco, A., Bunn, P.A., Jr., and Oldam, R. (Eds.): Small Cell Lung Cancer. New York, Grune & Stratton, 1981, pp. 413-466.

8. Bunn, P.A., and Poiesz, B.J.: Mycosis Fungoides and the Sezary Syndrome. In Williams, W.J., Beutler, E., Erslev, A.J., and Lichtman, M.A. (Eds.): Hematology, ed. 3. New York, McGraw-Hill Co., (in press).

9. Carney, D.N., Fossieck, B.J., Parker, R.H., and Minna, J.D.: Bacteremia due to Staphylococcus aureus in cancer patients: report of 45 cases in adults and review of the literature. Rev. Infect. Dis. 4: 1 - 12, 1982.

10. Carney, D.N., Marangos, P.J., Ihde, D.C., Bunn, P.A., Cohen, M.H., Minna, J.D., and Gazdar, A.F.: Serum neuron specific enolase: a marker for disease extent and response to therapy in patients with small cell lung cancer. Lancet 1: 583-585, 1982.

11. Carney, D.N., and Minna, J.D.: Small cell lung cancer. Clinics in Chest Medicine 3: 389-398, 1982.

12. Carney, D.N., Parker, R.H., and Fossieck, B.E.: Staphyloccocal bacteremia in cancer patients: intravenous and oral antimicrobial therapy. South Med. Journal 75: 143-146, 1982.

13. Cohen, M.H., Cretien, P.B., Johnston-Early, A., Ihde, D.C., Bunn, P.A., Fossieck, B.E., Makuch, R., Matthews, M.J., Shackney, S.E., and Minna, J.D.: Thymosin Fraction V Prolongs Survival of Intensively Treated Small Cell Lung Cancer Patients. In Terry, W.E., and Rosenberg, S.A. (Eds.): Immunotherapy of Human Cancer. New York, Elsevier North Holland, 1982, pp. 141 - 145.

14. Cohen, M.H., Makuch, R., Johnston-Early, A., Ihde, D.C., Bunn, P.A., Fossieck, B.E., and Minna, J.D.: Laboratory parameters as an alternative to performance status in prognostic stratification of small cell lung cancer patients. Cancer Treat. Rep. 65: 187-195, 1981.

15. Earle, M.F., Fossieck, B.E., Cohen, M.H., Ihde, D.C., Bunn, P.A., and Minna, J.D.: Perirectal infections in patients with small cell lung cancer. JAMA 246: 2464-2466, 1981.

16. Fer, M., Sherwin, S.A., Oldham, R., Greco, F.A., and Matthews, M.J.: Poorly Differentiated Lung Cancer. In Greco, F.A. (Ed.): Lung Cancer, Seminars in Oncology. New York, Grune & Stratton, (in press).

17. Fuks, J.Z., Aisner, J., Carney, D.N., Van Echo, D.A., Ostrow, S.S., Ihde, D.C., and Wiernik, P.H.: A phase II trial of vindesine in patients with refractory small-cell carcinoma of the lung. Am. J. Clin. Oncol. (CCT) 5: 49-52, 1982.

18. Fuks, J.Z., Aisner, J., Van Echo, D.A., Levitt, M., Ihde, D.C., and Wiernik, P.H.: Phase II trial of aziridinylbenzoquinone (AZQ) in patients with refractory small cell carcinoma of the lung. Cancer Clin. Trials (in press)

19. Haynes, B.F., Bunn, P.A., Jr., Mann, D., Thomas, C., Eisenbarth, G.S., Minna, J.D., and Fauci, A.S.: Cell surface differentiation antigens of the malignant T-cell in Sezary Syndrome and mycosis fungoides. J. Clin. Invest. 67: 523-530, 1981.

20. Haynes, B.I., Metzgar, R.S., Minna, J.D., and Bunn, P.A. Jr.: Phenotypic characterization of cutaneous T-cell lymphoma: comparison with other malignant T-cells with the use of monoclonal antibodies. New Engl. J. Med. 304: 1319-1323, 1981.

21. Ihde, D.C.: Optimal Use of Chemotherapy in Small Cell Carcinoma of the Lung. In Periman, P. (Ed.): Management of Advanced Cancer. New York, Masson (in press).

22. Ihde, D.C.: Staging Evaluation and Prognostic Factors in Small Cell Lung Cancer. In Aisner, J., (Ed.): Contemporary Issues in Clinical Oncology: Lung Cancer. New York, Churchill Livingstone (in press).

23. Ihde, D.C., Belville, W.D., Mahan, D.E., Gemski, M.J., and Eddy, J.L.: Serum acid phosphatase in the assessment of response to systemic therapy in metastatic prostate cancer: comparison of radioimmune and enzymatic assays. Military Med. (in press).

24. Ihde, D.C., and Bunn, P.A.: Chemotherapy of Small Cell Bronchogenic Carcinoma. In Williams, C.J., and Whitehouse, J.M.A. (Eds.): Recent Advances in Clinical Oncology: Number One. Edinburgh, Churchill Livingstone, 1982, pp. 305-323.

25. Ihde, D.C., Dunnick, N.R., Johnston-Early, A., Bunn, P.A., Cohen, M.H., and Minna, J.D.: Abdominal computed tomography in small cell lung cancer: assessment of extent of disease and response to therapy. Cancer 49: 1485-1490, 1982.

26. Ihde, D.C., Dutcher, J.S., Young R.C., Cordes, R.S., Barlock, A.L., Hubbard, S.M., Jones, R.B., and Boyd, M.R.: Phase I trial of pentamethylmelamine: a clinical and pharmacologic study. Cancer Treat. Rep. 65: 755-762, 1981.

27. Ihde, D.C., and Hansen, H.H.: Staging Procedures and Prognostic Factors in Small Cell Carcinoma of the Lung. In Greco, F.A., Oldham, R.K., and Bunn, P.A. (Eds.): Small Cell Lung Cancer. New York, Grune & Stratton, 1981, pp. 261-283.

28. Ihde, D.C., Johnston-Early, A., Carney, D.N., Cohen, M.H., Bunn, P.A., Pelsor, F.R., and Minna, J.D.: Lack of efficacy of high dose methotrexate by 30-hour infusion in patients with progressive small cell carcinoma of the lung. Cancer Treat. Rep. 66: 1223 - 1225, 1982.

29. Johnston-Early, A., Cohen, M.H., and White, K.: Venipuncture--the problem veins of cancer patients. Am. J. Nursing (in press).

30. Krasnow, S., Bunn, P.A. Jr., Ihde, D.C., Matthews, M.J., Cohen, M.H., Eddy, J.N., and Minna, J.D.: ICFR-159 in advanced gastric cancer: absence of activity. Cancer Clin. Trials (in press).

31. Levenson, R.M., Ihde, D.C., Huberman, M.S., Cohen, M.H., Bunn, P.A., and Minna, J.D.: Phase II trial of cisplatin in small cell carcinoma of the lung. Cancer Treat. Rep. 65: 905-907, 1981.

32. Levenson, R.M., Ihde, D.C., Matthews, M.J., Cohen, M.H., Gazdar, A.F., Bunn, P.A., and Minna, J.D.: Small cell carcinoma presenting as an extrapulmonary neoplasm. Sites of origin and response to therapy. J. Natl. Cancer Inst. 67: 607-612, 1981.

33. Levenson, R.M., Sauerbrunn, B.J.L., Ihde, D.C., Bunn, P.A., Cohen, M.H., and Minna, J.D.: Small cell lung cancer: radionuclide bone scans for assessment of tumor extent and response. Am. J. Roentgenol. 137: 31-35, 1981.

34. Lichter, A.S., and Bunn, P.A.: The Management of Small Cell Lung Cancer. In Fishman, A.P. (Ed.): Update of Pulmonary Diseases and Disorders. New York, Sanders Book Co., (in press).

35. Matthews, M. J., and Gazdar, A.F.: Large Cell Carcinoma. Is It an Entity? In Yesner, R. (Ed.): Symposium on Lung Cancer. New Haven, Yale Press, (in press).

36. Matthews, M.J., and Gazdar, A.F.: The Pathology of Small Cell Carcinoma of the Lung. In Shimosato, Y., Melamud, M., and Nettesheim, P. (Eds.): Morphogenesis of Lung Cancer. Boca Raton, Florida, CRC Press, Inc., (in press).

37. Matthews, M.J., and Gordon, P.: Pathology of Pulmonary and Pleural Neoplasms. In Straus, M. (Ed.): Lung Cancer Clinical Diagnosis and Treatment, ed. 2. New York, Grune & Stratton, (in press).

38. Matthews, M.J., and Hirsch, F.R.: Problems in Diagnosis of Small Cell Carcinoma of the Lung. In Greco, F.A., Oldham, R., and Bunn, P.A. (Eds.): Small Cell Lung Carcinoma. New York, Grune & Stratton, 1981, pp. 34 - 50.

39. Matthews, M.J., McKay, B., and Lukeman, J.: Pathology of Non-Small Cell Lung Cancer. In Golomb, H.M. (Ed.): Non-Small Cell Carcinoma of the Lung. New York, Grune & Stratton, (in press).

40. Minna, J.D., and Bunn, P.A.: Paraneoplastic Syndromes. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): The Principles and Practice of Oncology. Philadelphia, J.B. Lippincott, 1982, pp. 1476 - 1517.

41. Minna, J.D., Bunn, P.A., Jr., Carney, D.N., Cohen, M.H., Cuttitta, F., Fossieck, B.E., Gazdar, A.F., Ihde, D.C., Johnston-Early, A., Matthews, M.J., Makuch, R., Oie, H., Rosen, S., Lichter, A., and Glatstein, E.: Experience of the National Cancer Institute (USA) in the treatment and biology of small cell lung cancer. Bulletin du Cancer 69: 83-93, 1982.

42. Minna, J.D., Carney, D.N., Alvarez, R., Bunn, P.A., Cuttitta, F., Ihde, D.C., Matthews, M.J., Oie, H., Rosen, S.T., Whang-Peng, J., and Gazdar, A.F.: Heterogeneity and Homogeneity of Human Small Cell Lung Cancer. In Owens, A.H. (Ed.): Tumor Cell Heterogeneity: Origins and Implications. New York, Academic Press, (in press).

43. Minna, J.D., Higgins, G., and Glatstein, E.J.: Lung Cancer. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): The Principles and Practice of Oncology. Philadelphia, J.B. Lippincott, 1982, pp. 396 - 474.

44. Morstyn, G., Ihde, D.C., Eddy, J.L., Bunn, P.A., Cohen, M.H., and Minna, J.D.: Combination chemotherapy of hepatocellular carcinoma with doxorubicin and streptozotocin. Cancer Clin. Trials (in press).

45. Posner, L.E., Fossieck, B.E., Jr., Eddy, J.L., and Bunn, P.A., Jr.: Septicemic complications of the cutaneous T-cell lymphomas. Am. J. Med. 71: 210-216, 1981.

46. Radice, P.A., Matthews, M.J., Ihde, D.C., Gazdar, A.F., Carney, D.N., Bunn, P.A., Cohen, M.H., Fossieck, B.E., Makuch, R.W., and Minna, J.D.: The clinical behavior of "mixed" small cell/large cell bronchogenic carcinoma compared to "pure" small cell subtypes. Cancer (in press).

47. Rosen, S.T., Aisne, J., Makuch, R.W., Matthews, M.J., Ihde, D.C., Whitacre, M., Glatstein, E., Wiernik, P., Lichter, A.S., and Bunn, P.A., Jr.: Carcinomatous leptomeningitis in small cell lung cancer: a clinicopathologic review of the National Cancer Institute experience. Medicine 61: 45 - 53, 1982.

48. Rosen, S.T., and Bunn, P.A., Jr.: CNS Metastases in Small Cell Lung Cancer. In Aisner, J. (Ed.): Lung Cancer. New York, Churchill Livingstone, (in press).

49. Shackney, S.E., Straus, M., and Bunn, P.A.: Cell Kinetic Studies in Small Cell Lung Cancer. In Greco, F.A., Bunn, P.A., and Oldham, R. (Eds.): Small Cell Lung Cancer. New York, Grune & Stratton, 1981, pp. 225 - 234.

50. Schilsky, R.L., Kelley, J.A., Ihde, D.C., Howser, D.M., Cordes, R.S., and Young, R.C.: Phase I trial and pharmacokinetics of aziridinylbenzoquinone (NSC 182986) in humans. Cancer Res. 42: 1582-1586, 1982.

51. Stanley, K.E., and Matthews, M.J.: Analysis of a pathologic review of patients with tumors of the lung. J. Nat. Cancer Inst. (in press).

52. Tester, W.J., Donehower, R.C., Eddy, J.L., Myers, C.E., and Ihde, D.C.: Evaluation of weekly escalating doses of dichloromethotrexate in patients with hepatocellular carcinoma and other solid tumors. Cancer Chemother. Pharmacol. (in press).

53. Thant, M., Hawley, R.J., Smith, M.T., Cohen, M.H., Minna, J.D., Bunn, P.A., Ihde, D.C., West, W., and Matthews, M.J.: Possible enhancement of vincristine neuropathy by VP-16. Cancer 49: 859-864, 1982.

54. Von Hoff, D.D., Weisenthal, L.M., Ihde, D.C., Matthews, M.J., Layard, M., and Makuch, R.: Growth of lung cancer colonies from bronchoscopy washings. Cancer 48: 400-403, 1981.

55. Whang-Peng, J., Bunn, P.A., Knutsen, T., Matthews, M.J., Schechter, G.P., and Minna, J.D.: Clinical implications of cytogenetic studies in cutaneous T-cell lymphoma. Cancer (in press).

56. Winkler, C.F., and Bunn, P.A., Jr.: Cutaneous T-cell lymphomas. CRC Critical Reviews in Oncology/Hematology (in press).

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06575-07 NMOB |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Laboratory Investigation of Tumor Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT


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COOPERATING UNITS (if any)

See Attached Sheet

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Cell Biology Laboratory

INSTITUTE AND LOCATION

Bethesda NNMC Bethesda, MD 20814

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 17.5 | 8 | 9.5 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)


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.

Other Professionals

H. Oie, Ph.D.
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## 3.0 Laboratory Investigation

The laboratory investigation projects are presented in the order of priority.

### 3.1 Dr Minna, Dr. Cuttitta: Production and Characterization of Monoclonal Antibodies with Specificity for Human Lung Cancer or Their Products

These include studies of heterogeneity and homogeneity of antigen expression; antigen biochemical characterization; distribution on normal tissues and immune histochemical stains of normal and tumor tissue. A panel of 81 selective antibodies is under investigation. These antibodies promise to be of potentially great importance in the diagnosis, staging and treatment of lung cancer. These studies include preliminary step necessary to bring the Ab's to clinical application using in vitro and nude mouse heterotransplant models. They include collaboration with cooperative groups for performing immune histochemical retrospective studies for clinical correlations, to collaborations with ROB, chemists and radiation biologists, radiochemists to develop immune vehicles to deliver radiotherapy. In addition to lung cancer cells per se, we are also preparing monoclonal antibodies against defined antigens such as peptide hormones produced by lung cancer cells, particularly hormones we can show are required for the growth of lung cancer cells.

### 3.2 Dr. Carney: Drug and Radiation Sensitivity Testing of Human Tumor Cell Lines

We are conducting detailed studies of chemo-radiotherapy sensitivity of human tumor cell lines, short-term cultures, and direct tumor samples, particularly lung cancer, and correlating this with the clinical response in patients. At present the in vitro and in vivo results seem quite well correlated. The tumor cell lines provide excellent material to characterize the biochemical pharmacology of drug and radiation resistance and serve as DNA donors for isolating drug resistance genes. We are trying to directly test their utility in prospective clinical trials by: (1) screening for new drugs active against the tumor lines in vitro and then testing them in patients; (2) selecting therapy for individual patients based on the in vitro assays (see clinical research section).

### 3.3 Dr. Gazdar: Study of the Biology of Lung Cancer

Following clues from normal bronchial epithelial tissues and normal fetal development, we have been identifying biochemical and immunologic markers associated with human lung cancer. In particular we have found a series of APUD markers and peptide hormones specific for small cell lung cancer (SCLC). We are now developing similar markers for NSCLC. These "normal" markers are of potential great use for tumor typing, staging and following response to therapy. They also provide clues for how to better grow and regulate the lung cancer cells. In addition, we are establishing tumor cell lines representing different steps in differentiation with the ultimate aim of isolating and characterizing the tumor stem cells. We have found that some of the cells blocked early in differentiation are more malignant. The understanding of the clinical biology in patients will depend on studying these stem cells. Clonal heterogeneity of lung cancer is also being investigated.

3.4 Drs. Carney, Oie, Gazdar, Cuttitta, Minna: Identification of Growth Factors for Human Tumor Cells

Lung cancers grow well in patients but are hard to clone or establish as cell lines in vitro. The ability to do this is at present prerequisite of many of the clinical applications such as therapy sensitivity testing. We have developed the strategy of working out the serum-free growth factor supplemented media requirements of human tumor cell lines and then testing these on fresh clinical tumor specimens. This works and selectively allows the growth of human tumor cells considerably better than in serum supplemented media. We are extending the results we have in SCLC and adenocarcinoma of the lung to other lung cancer types and human breast cancer. In addition, by cloning in serum-free medium it is possible to identify more growth factors. In this way we have shown that human lung cancer cells produce peptide hormones such as AVP and bombesin (as well as novel peptides) required for their own growth in an autocrine fashion. This also provides new approaches to hormonally manipulate lung cancer cells.

3.5 Drs. Minna, Gazdar, Carney: Isolation, Characterization, and Chromosomal Location of Genes Responsible for Malignancy and Drug Resistance in Human Lung Cancer Cells

We are using a variety of approaches to study the genetics of lung cancer and drug resistance. These include cytogenetic studies with Dr. J. Whang-Peng of the MB/CC which have identified a specific acquired chromosomal defect (deletion 3p(14-23)) associated with SCLC. We continue to study this defect as well as to identify others associated with NSCLC. Another approach is to transfect via isolated DNA or hybrid cells the malignant phenotype to non-malignant cells and subsequently isolate specific genes by recombinant DNA techniques. In addition, the recent findings by others of the potential role of viral Sarc genes (Kirstin virus) with NSCLC have prompted us to look at expression of p21 Sarc protein in collaboration with Dr. Scolnick, and the status of the viral Sarc genes in lung cancer DNA using existing probes. A central question is: Are similar or different transforming genes active in SCLC as in some NSCLC, and do all NSCLC tumors have the same active genes? Using the available recombinant DNA probes related to Sarc genes the molecular cytogenetics in lung cancer cells is accessible. These studies are of fundamental importance to understanding the the biology of lung cancer.

The same transfection technique will be used to isolate and characterize genes for chemotherapy resistance by transfecting DNA from lung cancer lines selected for drug resistance in patients into sensitive rodent cells. These transfectants should allow isolation of DNA probes specific for drug resistance and the preparation of antibodies to their products. Such reagents will provide entirely new ways to type tumors for their chemotherapy sensitivity or resistance.

3.6 Dr. Bunn: Growth and Characterization of Malignant Human Lymphoma Cells

Using the same serum-free growth factor techniques our laboratory has developed for lung cancer, we are trying to work out conditions for reproducibly growing and cloning in tissue culture human T and B cell derived malignant lymphomas.

Because of recent advances in growing normal T and B cells as well as the panel of monoclonal antibodies for typing the prospective tumor cells and newly recognized cytogenetic abnormalities there is considerable expectation that such an approach will work. The importance of such cell lines is seen in examples like the T cell lymphoma line (NCI-H102) isolated by our laboratory which was the major source for the newly described human T cell lymphoma virus (HTLV).

PUBLICATIONS (Laboratory)

1. Baylin, S.B., Gazdar, A.F., Minna, J.D., and Shaper, H.H.: Cell surface protein phenotype of human lung cancer in culture. Identification of common and distinguishing cell surface proteins on the membranes of different human lung cancer cell types. Proc. Natl. Acad. Sci. USA, 1982 (in press).

2. Baylin, S.B., Jackson, R.D., Goodwin, G., and Gazdar, A.F.: Neuroendocrine related biochemistry in the spectrum of human lung cancers. J. Exp. Lung Res. 1982 (in press).

3. Becker, K.L., and Gazdar, A.F.: The pulmonary endocrine cell and the tumors to which it gives rise. In Reznik-Schuller, H. (Ed.): Comparative Respiratory Tract Carcinogenesis. New York, CRC Press, 1982 (in press).

4. Bhathena, S.J., Oie, H.K., Gazdar, A.F., Voyles, N.R., Wilkins, S.D., and Recant, L.: Insulin, glucagon and somatostatin receptors on cultured cells and clones from rat islet cell tumor. Diabetes, 1982 (in press).

5. Blatt, J., Bunn, P.A., Carney, D.N., Deaman, A., Soprey, P., and Poplack, D.G.: Purine pathway enzymes in the circulating malignant cells of patients with cutaneous T-cell lymphoma. Brit. J. Hematol. (in press).

6. Bunn, P.A., Krasnow, S., Makuch, R.W., Schlar, M.L., and Schechter, G.P.: Flow cytometric analysis of DNA content of bone marrow cells in patients with plasma cell myeloma: Clinical Implications. Blood 59: 528-535, 1982.

7. Carney, D.N., Bunn, P.A., Gazdar, A.F., Pagan, J.A., and Minna, J.D.: Selective growth in serum-free hormone supplemented medium of tumor cells obtained by biopsy from patients with small cell carcinoma of the lung. Proc. Natl. Acad. Sci. USA. 78: 3185-3189, 1981.

8. Carney, D.N., Gazdar, A.F., and Minna, J.D.: The use of experimental systems in the clinical research of small cell lung cancer. Pathobiology Annual. 1982 (in press).

9. Carney, D.N., Gazdar, A.F., Bunn, P.A., and Guccion, J.G.: Demonstration of the stem cell nature of clonogenic cells in lung cancer specimens. Stem Cells 1: 149-164, 1981.

10. Carney, D.N., Gazdar, A.F., Oie, H., Cuttitta, F., and Minna, J.D.: The in vitro growth and characterization of small cell lung cancer. In Greco, F.A., et al (Eds.): Lung Cancer II. New York, Grune and Stratton, 1982 (in press).

11. Carney, D.N., Marangos, P.J., Ihde, D.C., Bunn, P.A., Jr., Cohen, M.H., Minna, J.D., and Gazdar, A.F.: Serum neuron specific enolase: a marker for disease extent and response to therapy in patients with small cell lung cancer. Lancet 1: 583-585, 1982.

12. Cuttitta, F., Rosen, S., Carney, D.N., Gazdar, A.F., Bunn, P.A., and Minna, J.D.: Monoclonal antibodies to lung tumor antigens. In Wright, G.L., Jr. (Ed.) (in press).

13. Cuttitta, F., Rosen, S., Carney, D.N., Gazdar, A.F., Bunn, P.A., and Minna, J.D.: Human tumor associated antigens. In Kennett, R. (Ed.): Monoclonal Antibodies: Progress and Reviews (in press).

14. Cuttitta, F., Rosen, S., Carney, D.N., Gazdar, A.F., and Minna, J.D.: Monoclonal antibodies against human lung cancer: Potential diagnostic and therapeutic use. In Greco, F.A., Oldham, R., and Bunn, P.A., Jr. (Eds.): Small Cell Lung Cancer (in press).

15. Cuttitta, F., Rosen, S., Gazdar, A., and Minna, J.: Monoclonal antibodies that demonstrate specificity for several types of human lung cancer. Proc. Natl. Acad. Sci. USA 78: 4591-4595, 1981.

16. Cuttitta, F., Rosen, S., Moody, T.W., Carney, D.N., Gazdar, A.F., and Minna, J.D.: Monoclonal antibodies against endocrine tumors of the lung. In Becker, K., and Gazdar, A.F. (Eds.) (in press).

17. Gazdar, A.F., Carney, D.N., and Minna, J.D.: In vitro study of the biology of small cell carcinoma of the lung. Yale J. Biol. Med. 54: 187-193, 1981.

18. Gazdar, A.F., Carney, D.N., and Minna, J.D.: The biology of lung cancer. In Osolaw, S., Hayata, Y., and Suemasu, K. (Eds.): Lung Cancer 1982. Princeton, Excerpta Medica, 1982, pp. 14-30.

19. Gazdar, A.F., Carney, D.N., and Minna, J.D.: The biology of non-small-cell lung cancer. Seminars Oncol. (in press).

20. Gazdar, A.F., Carney, D.N., Sims, H.L., and Simmons, A.: Heterotransplantation of small-cell carcinoma of the lung into nude mide: Comparison of intracranial and subcutaneous routes. Int. J. Cancer 28: 777-783, 1981.

21. Gazdar, A.F., Zweig, M.H., Carney, D.N., Van Stirteghen, A.C., Baylin, S.B., and Minna, J.D.: Levels of creatine rinase and its BB isoenzyme in lung cancer tumors and cultures. Cancer Res. 41: 2773-2777, 1981.

22. Gootenberg, J.E., Ruscetti, F.W., Mier, J.W., Gazdar, A., and Gallo, R.C.: Human cutaneous T cell lymphoma and leukemia lines produce and respond to T-cell growth factor. J. Exp. Med. 154: 1403-1418, 1981.

23. Kalyanaraman, V.S., Sarngadharan, M.G., Bunn, P.A., Jr., Minna, J.D., and Gallo, R.C.: Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus. Nature 294: 271-273, 1981.

24. Kohno, Y., Berkower, I., Minna, J., and Berzofsky, J.A.: Idiotypes of antimyoglobin antibodies: shared idiotypes among monoclonal antibodies to distinct determinants of sperm whale myoglobin. J. Immunology 128, 1742-1748, 1982.

25. Marangos, P.J., Gazdar, A.F., and Carney, D.N.: Neuron-specific enolase in human small cell carcinoma cultures. Cancer Letters 15: 67-71, 1982.

26. Minna, J.D., Carney, D.N., Alvarez, R., Bunn, P.A., Jr., Cuttitta, F., Ihde, D.C., Matthews, M.J., Oie, H., Rosen, S., Whang-Peng, J., and Gazdar, A.F.: Heterogeneity and homogeneity of human small cell lung cancer. Bristol-Myers Seminar at Johns Hopkins University. Academic Press, 1982. (in press)

27. Minna, J.D., Carney, D.N., Oie, H., Bunn, P.A., Jr., and Gazdar, A.F.: Growth of human small cell lung cancer in defined medium. Cold Spring Harbor Symposium, Cold Spring Harbor, New York. (in press)

28. Minna, J.D., Cuttitta, F., Rosen, S., Bunn, P.A., Jr., Carney, D.N., Gazdar, A.F., and Krasnow, S.: Methods for production of monoclonal antibodies with specificity for human lung cancer cells. In Vitro, 17, 1058-1070, 1981.

29. Minna, J.D., Gazdar, A.F., Carney, D.N., Radice, P.A., and Simms, E.: In Vitro and In Vivo models for the study of small cell carcinoma of the lung. In Fidler, I.J., and White, R.J. (Eds.): Design of Models for Testing Therapeutic Agents. New York, Von Nostrand Reinhold Company, 1981, pp. 148-157.

30. Moody, T.W., Pert, C.B., Gazdar, A.F., Carney, D.N., and Minna, J.D.: High levels of intracellular bombesin characterize human small-cell lung carcinoma. Science 214: 1245-1248, 1981.

31. Munson, D., Bunn, P.A., and Schecter, G.P.: T-lymphocyte subpopulations in patients with cutaneous T-cell lymphoma. Invest. Dermatol. (in press)

32. Oie, H.K., Gazdar, A.F., Minna, J.D., Weir, G.C., and Baylin, S.B.: Clonal analysis of insulin and somatostatin and L-DOPA decarboxylase expression by a rat islet cell tumor. Endocrinology. (in press)

33. Posner, L.E., Guroff, M., Kalyanaraman, V.S., Poiesz, B.J., Ruscetti, F.W., Bunn, P.A., Minna, J.D., and Gallo, R.C.: Natural antibodies to the retrovirus HTLV in patients with cutaneous T-cell lymphomas. J. Exp. Med. 154: 333-346, 1981.

34. Sherwin, S.A., Minna, J.D., Gazdar, A.F., and Todaro, G.J.: Expression of epidermal and nerve growth factor receptors and soft agar growth factor production by human lung cancer cells. Cancer Res. 41: 3538-3542, 1982.

35. Whang-Peng, J., Bunn, P.A., Jr., Kao-Shan, C.S., Lee, E.C., Carney, D.N., Gazdar, A., and Minna, J.D.: A non-random chromosomal abnormality, del 3p (1423) in human small cell lung cancer. Cancer Genetics and Cytogenetics. (in press)

36. Whang-Peng, J., Ko-Shan, C.S., Lee, E.C., Bunn, P.A., Carney, D.N., Gazdar, A.F., Portlock, C., and Minna, J.D.: Deletion 3p(14-23), double minute chromosomes, and homogeneously straining regions in human small-cell lung cancer. In Schimke, R.T. (Ed.): Gene Amplification. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982, pp. 107-113.

37. Whang-Peng, J., Kao-Shan, C.S., Lee, E.C., Bunn, P.A., Carney, D.N., Gazdar, A.F., and Minna, J.D.: A specific chromosome defect associated with human small cell lung cancer. Deletion 3p(14-23). Science 215: 181-182, 1982.

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PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study and Treatment of Cancer in Pediatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
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| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 35.0 | 20.0 | 15.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute leukemia, Non-Hodgkin's lymphomas (e.g., Burkitt's), neuroblastoma, rhabdomyosarcoma, osteosarcoma, and Ewing's sarcoma are studied. In leukemia, we have devised therapeutic regimens wich may allow maximum tumor cell kill while minimizing sequellae. Emphasis is on tailoring treatment to individual prognostic variables, and improving the therapeutic ratio in CNS prophylaxis. In the solid tumors, we have developed combined modality approaches to primary disease. In refractory tumors, we are studying the utility of high-dose therapy and the role of supportive care (laminar-flow protection, autologous bone marrow rescue) in permitting such therapy. Phase I-II trials are also conducted in refractory tumors; agents include ICRF-187, iphosphamide, dihydroxyanthracenedione, and 2'-deoxycoformycin. We are exploring the biology of selected tumors, including kinetics, immunology, virology, tumor markers, genetics, biochemistry, and pharmacology. Also studied are the effects of cancer and its treatment on growth, development, and organ function.

Objectives:

1. To devise effective therapeutic regimens in acute leukemia which allow maximum tumor cell kill, while leaving intact effective elements of the immune response and minimizing therapeutic sequellae -- especially in the CNS.

2. To develop effective combined modality treatment approaches to previously untreated childhood solid tumors, including rhabdomyosarcoma, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, and Ewing's sarcoma.

3. To study the possible role of high-dose ("ablative") chemotherapy in advanced solid tumors, and the role of supportive care (laminar-flow protection, bone marrow rescue) in permitting such ablative therapy.

4. To study the biology of selected tumors, including kinetics, immunology, virology, prognostic markers, karyotypes, biochemistry, and drug sensitivity.

5. To assess the pharmacology of selected new agents, e.g., high-dose methotrexate, dihydroxyanthracenedione, ICRF-187, 2'-deoxycoformycin, and Iphosphamide in humans.

6. To study the short- and long-term effects of malignant disease and cancer treatment on growth, development, and organ function.

7. To study the psychosocial concomitants of cancer in young patients, their families, and their therapists.

Methods and Major Findings:

A. Acute Lymphoblastic Leukemia (ALL)

1. Current Protocol (POB 77-02) - The current ALL treatment protocol addresses the two major therapeutic problems which emerged from previous NCI studies: 1) The need for improved treatment for patients possessing certain poor prognostic factors, and 2) improvement in the method of delivering CNS preventive therapy with less morbidity. The main objective of this protocol is to investigate the efficacy of high-dose, protracted intravenous methotrexate infusions as central nervous system preventive therapy. Patients are randomized to receive CNS prophylactic therapy with cranial radiation (2400 rads) plus intrathecal methotrexate or with high-dose 24 hour, systemic methotrexate infusions. The hypothesis to be tested in the present protocol is that CNS preventive therapy using high-dose protracted systemic methotrexate infusions alone is equally effective and less toxic than the current standard form of CNS prophylaxis (cranial irradiation and intrathecal methotrexate). An additional aim of this study is to assess the utility of an intensified systemic maintenance schedule which alternates standard maintenance treatment with periodic "induction-type" chemotherapy regimens. Pharmacologically, this 24-hour high-dose methotrexate regimen provides more prolonged therapeutic CSF antifolate concentrations than does a standard dose of intrathecal methotrexate both in ventricular and lumbar cerebrospinal fluid. The current

protocol also attempts to provide patients with optimal exposure to those chemotherapeutic agents considered most active against ALL by employing an escalated, intensified maintenance schedule which alternates standard maintenance treatment with frequent, periodic, "induction-type" chemotherapy utilizing varying reinduction regimens. It is hoped that the application of this intensified maintenance schedule will improve the prognosis for "high-risk" ALL patients. Experience to date has shown this intensified approach can be accomplished without a significant increase in drug related morbidity. To date, 139 patients have been randomized on this study. Forty-six of these individuals were randomized to receive cranial irradiation plus intrathecal methotrexate; ninety-three have been treated with the high-dose, protracted intravenous methotrexate infusion. (The randomization is weighted on a 2 to 1 basis.) There have been 7 CNS relapses (2 and 5 in each treatment group, respectively) and 10 bone marrow relapses (6 in the cranial radiation + intrathecal methotrexate arm). Although it is too early to draw statistically significant conclusions regarding the utility of the methotrexate infusion arm, the data thus far appear very encouraging. Freedom from systemic relapse in the infusion arm is an unanticipated dividend, as is the overall survival improvement amongst poor prognosis patients. It is notable that toxicity associated with the intensive maintenance schedule has been relatively minimal. This collaborative study should provide sufficient patient accrual to complete this study within 1-1/2 years.

2. Studies on the Treatment of Overt CNS Leukemia: Treatment with Systemic (IV) Methotrexate Infusions - Our recent studies in patients and in a sub-human primate model have revealed that therapeutic concentrations of MTX can be achieved in cerebrospinal fluid following intravenous infusion. We have evaluated the utility of 24-hour continuous systemic MTX infusions. A relatively constant plasma drug level is maintained for 24 hours; 12 hours later (36 hours after the start of the infusion) citrovorum factor rescue is initiated. Our studies have demonstrated that this technique is feasible and that IV doses which maintain a CSF MTX level of $1 \times 10^{-5}$M can be given without apparent systemic or neurological toxicity. We have also determined that the ventricular and lumbar CSF: plasma MTX ratios are similar, suggesting that systemic MTX administration has the advantage of providing consistent drug concentrations throughout the CSF space. This contrasts with intralumbar administration, which does not provide optimal drug penetration into the ventricular CSF.

3. Relapse ALL: Phase I Study of 2'-Deoxycoformycin - As part of an evaluation of promising new agents in relapsed patients with ALL, we recently conducted a Phase I study of 2'-deoxycoformycin in a cooperative effort with two other institutions (St. Jude Children's Research Hospital and the Sidney Farber Cancer Research Institute). This agent (2'-dCF) is a known inhibitor of the enzyme adenosine deaminase (ADA), an enzyme of known importance in lymphocyte metabolism. Our interest in 2'-dCF as an anti-leukemia agent was stimulated by our observation that the leukemic blast cells of ALL patients with "T Cell disease" have considerably higher ADA levels than "Non-T, Non-B" lymphoblasts, prompting speculation that, should it be effective against leukemia, 2'-dCF may have "specificity"

for poor prognosis patients with T lymphoblasts. Twenty-six patients with refractory ALL were treated with 2'-dCF. Mild toxicities noted with the drug include nausea, vomiting, diarrhea and conjunctivitis. Dose-limiting toxicity includes effects on the central nervous system (confusion, seizures, encephalopathy), the kidney (acute renal failure), and the liver. The 0.5 mg/kg dose was associated with only mild toxicity and has been demonstrated to inhibit the adenosine deaminase of lymphoblasts. Of the 26 patients treated, 2 achieved complete remission and partial responses were noted in 4 individuals. This study confirmed the activity of 2'-deoxycoformycin in acute lymphoblastic leukemia; a second Phase I study is underway in which dCF is combined with adenosine arabinoside (ara-A). Since dCF will prevent the deamination of ara-A, the known anti-leukemic effect of the latter drug should be potentiated.

B. Non-Hodgkin's Lymphoma

Three clinical protocols are presently open for patient entry. These are the primary protocol (77-04) for untreated patients with undifferentiated lymphomas (including Burkitt's lymphoma) and lymphoblastic lymphomas; an intensive protocol utilizing high-dose cyclophosphamide, ara-C and (where cryopreserved autologous bone marrow is available for re-infusion) total body irradiation; and a protocol examining the activity of Ifosphamide as a single agent after failure of the intensive protocol. Sixty-six patients have been admitted to the primary protocol (77-04) and an analysis of the first 46 patients has recently been completed. The protocol employs the CHOP regimen followed in 10 days (without delay for neutropenia) by a high-dose, protracted methotrexate infusion. Overall survival is 66% at 3 years. Excellent results have been obtained in lymphoblastic lymphoma and patients with completely resected undifferentiated lymphomas. Although a markedly different relapse pattern compared to previous protocols has been observed suggesting more effective control of systemic tumor, patients with widely disseminated disease still have a poor prognosis (30-40% predicted disease-free survival at 3 years) and a future protocol will be directed at these patients.

Very poor results have been obtained with salvage protocols, although Ifosphamide is active even after failure of high dose cyclophosphamide. This drug will probably be incorporated into combination regimens for poor risk patients at presentation and for patients who relapse. A new phase II study of interferon is shortly to be commenced.

C. Ewing's Sarcoma

Based on the analysis of prior protocols for treatment of patients with Ewing's sarcoma at the NCI, current protocols divided patients into those presenting with metastatic or central axis disease and those presenting with lesions of the extremities. Patients presenting with humeral lesions were treated with the more aggressive protocol. The less aggressive treatment protocol consisted of 4-drug chemotherapy plus prophylactic pulmonary irradiation. Results obtained with the less aggressive protocol in patients presenting with extremity lesions have not been significantly different from the best available prior treatment protocols used at the National Cancer Institute or the best treatment arm of the Intergroup Ewing's Sarcoma Study. Results obtained using the more

aggressive treatment protocol which consisted of radiation to known sites of disease, initial chemotherapy, bone marrow storage, total body irradiation with high-dose chemotherapy followed by reconstitution with the harvested marrow, demonstrated important differences from results previously obtained. Although the numbers are small, patients presenting with metastatic lesions demonstrated a 30% actuarial disease-free survival rate. If one considers results only for patients who achieved complete remission, this projection is even higher.

The other result of interest was obtained in patients with primary lesions of the ribs. Of 8 patients with such lesions, all of whom were treated with split course radiotherapy to a total dose of 6000 rads, with surgical resection of the primary lesions following the initial 3000 rads of radiation therapy, there have been no relapses. This is in contrast to previous experience at other centers where long-term disease-free survival rates for patients presenting with such lesions was extremely poor (under 10%). Results of the Intergroup Ewing's Sarcoma Study for similar patients indicate long-term disease-free survival rates on the order of 25%. Results obtained for other patients presenting with central axis lesions, primarily patients with vertebral lesions or lesions of the pelvis, have not been significantly different from the best available previous results of National Cancer Institute protocols and are slightly but not significantly improved, compared to similar patients treated on the Intergroup Ewing's Sarcoma study.

Based upon these results and in vitro results with putative Ewing's sarcoma cell lines indicating a shoulder in the x-ray dose response curve, a new treatment protocol has been devised which utilizes much higher dose total body irradiation and combination chemotherapy. This protocol also decreases the period of maintenance chemotherapy. Pilot patients treated with this regimen indicate that it is in fact tolerable. We are also incorporating in the present treatment more intensive x-ray treatment of the primary lesion using either an intra-operative approach or an external beam boost to the primary site. These are in an attempt to improve local control rates.

We have also analyzed the results obtained in a small group of patients with so-called extraskeletal Ewing's sarcoma which is histologically identical to Ewing's sarcoma but occurs outside the bone, and found that results in this group of patients is favorable with disease-free survival rates on the order of 65%. In this small group of patients, the prognosis for patients presenting with extremity lesions appears to be more favorable than that for patients presenting with central lesions. Likewise, patients who have complete excision of the primary appear to have a more favorable outcome than those who have incisional biopsies.

Ancillary studies carried out in patients with Ewing's sarcoma relate to the optimum frequency of follow-up diagnostic studies. Analysis of the timing of x-ray studies as they relate to hazard rate (the chance of a patient relapsing in a given period of time), indicate that more frequent examinations of patients presenting with central axis lesions are indicated earlier in their course, whereas a more uniform rate of periodic examinations for patients presenting with extremity lesions is indicated. A similar analysis of the utility of radionuclide bone scans has indicated that the lead time or

predictive value of such scans for tumor recurrence is sufficiently short that it is questionable whether periodic radionuclide bone scans are indicated in asymptomatic patients. Bone scans are, however, extremely useful in detecting otherwise unsuspected sites of metastatic disease in patients who have recurrence.

D. Osteogenic Sarcoma

To assess the utility of adjuvant multi-agent intensive chemotherapy following total resection of pulmonary metastases in osteosarcoma, 24 consecutive patients (pts) with pulmonary metastases were enrolled in a clinical trial (1977-1981). Of the 24 pts, 19 had received prior high-dose methotrexate (HD-MTX) as an adjuvant to amputation, and 5/19 had 2-3 discrete episodes of pulmonary metastases treated surgically prior to the episode which made them eligible for the present study. An additional 5 pts presented with synchronous metastases and had received no prior chemotherapy. Of the 24 pts, 13 had multiple bilateral and 11 had solitary metastases. Chemotherapy was initiated within two weeks following metastatectomy, consisting of one course of HD-MTX, cytoxan (40 mg/kg/day x 3), adriamycin (35 mg/$M^2$/day x 6), L-PAM (0.4 mg/kg/day x 3), DTIC (250 mg/$M^2$/day x 5), and cisplatinum (20 mg/$M^2$/day x 5). Maintenance cycles (24) were planned every three weeks consisting of HD-MTX and adriamycin (70 mg/$M^2$/cycle to 560 mg/$M^2$). Minimum and maximum follow-up after metastatectomy are 6 and 60 months, and actuarial continuous disease-free survival at 3 years is 44% ± 20% (95% confidence interval). These results were compared with those of Martini, et al. (Ann. Thor. Surg. 12: 271, 1971), Burgers, et al. (Cancer 45: 1664, 1980), and Telander, et al. (Surgery 84: 335, 1978), who performed metastatectomy without chemotherapy. Actuarial continuous disease-free survival at 3 years was approximately 25% in these series. While our data suggests that intensive multi-agent chemotherapy may offer a small benefit in addition to metastatectomy, it is likely that pulmonary metastases were sought and resected more aggressively in our trial than in historical series, favoring the present result. Thus, randomized studies of resection ± chemotherapy should be undertaken in metastatic osteosarcoma. A pilot study randomizing newly diagnosed patients to chemotherapy or surgery is being initiated.

E. Rhabdomyosarcoma (RMS)

1. Protocol 76-4: Because of the extensive surgery often necessary to achieve local control of RMS at certain body sites, chemotherapy and radiotherapy has been investigated as both an alternative to extirpative surgery as well as to provide initial tumor reduction to permit less radical surgery. We treated 24 patients who had locally extensive (13 patients) and metastatic (11 patients) RMS with chemotherapy and radiotherapy alone. Primary tumor site included head/neck (11), trunk (2) GU (3) and extremity (8). Fourteen patients were treated with VAC (Vincristine, Adriamycin, Cyclophosphamide) while 10 received VAC alternating with VC + Adriamycin. Six patients received escalating doses of Cyclophosphamide up to 180 mg/Kg. RT (median 5500 rads) to primary site began with chemotherapy. Fifteen patients achieved CR, 7 PR and 2 had progressive disease. Local recurrence was noted in 5/15 patients while local regrowth occurred in 8/9 patients not achieving CR. Thus, of 16 patients with relapse or progression, 13 (81%)

had residual tumor at the primary site, either at the time of relapse or subsequently. Local failure was the sole site of relapse or regrowth in 4 patients. While chemotherapy and radiotherapy given in this fashion result in a high initial response rate in non-resectable RMS, long-term local control is suboptimal, suggesting that alternative radiotherapy dose fractionation, radiosensitizers, interstitial treatment, intraoperative RT or integration of surgery into primary therapy may be necessary to provide adequate therapy for the primary tumor.

2. Protocol 75-5 (relapse treatment): We evaluated an intensive chemotherapy regimen using escalating doses of cyclophosphamide in relapsed patients. In some patients, surgery and/or radiation were also utilized. Twenty-two patients have been entered into this trial of whom 5 achieved CR, 9 PR, 5 stabilized and 3 had progressive disease. To date 18 of these patients have relapsed again.

F. Miscellaneous Phase I and II Studies:
With the observation that pediatric patients appear to tolerate higher doses of chemotherapeutic agents than adult patients on similar schedules, we have begun to initiate Phase I and Phase II trials at approximately 80% of the maximally tolerated adult dose on a similar schedule. Current Phase II trials include anthracenedicarboxaldehyde or bisantrene, and iphosphamide both of which are thought to have potential activity in solid tumors in children. Initial experience has indicated activity in osteosarcoma for the drug iphosphamide. Phase I trials in pediatric patients generally require a larger patient accrual than is available at the National Cancer Institute as a single institution, and we plan to pursue such trials with selected other independent institutions for agents of interest.

Significance to Biomedical Research and the Program of the Institute:

Significant progress has been made in delineating the cell of origin in acute leukemia and in identifying factors which may be useful in predicting response to therapy. By further defining the acute leukemias vis-a-vis these prognostic factors, we hope to refine therapy to the end that remission rate and duration will further improve. In this Branch 43% of patients with ALL entered in our most recently completed study are free of disease after having been off all treatment for 4 - 7 years. We have learned that the acute leukemia cell has extraordinary antigenic and biochemical complexity. However, the effects of both specific and nonspecific immunotherapy in this disease have been discouraging, but our understanding of immunological and biochemical mechanisms involved in leukemia has increased. In this regard, we have identified a new agent (2'-deoxycoformycin, an inhibitor of lymphocyte adenosine deaminase) which may be effective in relapsed acute lymphocytic leukemia.

Our studies on the route and scheduling of CNS chemotherapy promise an effective and relatively nontoxic approach to this important problem. It is further evident that the most important contribution that can now be made to the treatment of "good risk" patients with acute lymphocytic leukemia is to provide effective CNS prophylaxis without the significant toxicity we have detected in association with cranial irradiation (CT scan abnormalities; neurologic, intellectual, and psychologic dysfunction; growth hormone deficits). We hope

to develop an effective CNS prophylaxis which can be administered systemically and without significant sequellae.

We are beginning to make progress in the treatment of advanced solid tumors that have been refractory to conventional doses of combination chemotherapy. The response in non-Hodgkin's lymphoma has been particularly gratifying in that it now appears that we will achieve durable survival in about 70% of patients. We further believe that the concept of high-dose combination chemotherapy together with maximal supportive care (including, for example, autologous marrow rescue) may be useful in metastatic rhabdomyosarcoma and Ewing's sarcoma, although our evidence is disappointing in the case of metastatic neuroblastoma. Early intensive treatment of newly diagnosed patients with high-risk (but low bulk) disease may be of particular value. With regard to new drug development, our finding that children can tolerate 2 - 3 times the adult maximum tolerable dose of ICRF-187 indicates the necessity for independent Phase I trials in children.

Proposed Course:

All of the premier studies enumerated previously will be continued, but emphasis is shifting to poor-prognosis patients (those presenting with unresectable and/or metastatic disease, or other known high-risk factors). Aggressive protocols have been developed for acute leukemia patients who relapse from primary induction protocols but remain sensitive to extant chemotherapy. Increased emphasis will be given to Phase I-II trials, in particular studies of molecularly cloned interferon, dihydroxyanthracenedione, deoxycoformycin + ara-A, Iphosphamide, anthracenedicarboxaldehyde and ICRF-187. We shall continue to explore the pharmacokinetics of methotrexate so as to improve the therapeutic index of this drug as it is used in the treatment and prophylaxis of meningeal leukemia. Experiments on the use of high-dose chemoradiotherapy in newly diagnosed metastatic solid tumors will be expanded; in these studies, chemotherapy is assessed in a situation whereby maximum supportive care is possible, including autologous bone marrow rescue. Our interest in laminar-flow-room prophylaxis, and empiric antibiosis will continue with studies of high-dose chemotherapy. We plan to expand studies in clinical and molecular pharmacology and to utilize the results of such studies in planning further clinical chemotherapy trials. Both our clinical and basic studies in the kinetics of hematopoietic reconstitution and differentiation will be used to guide further experiments on clinical marrow reconstitution, using cryopreserved marrow (or stem cells obtainable from the peripheral blood). We also plan to initiate a new comprehensive program in neuroblastoma, utilizing the current techniques of molecular biology and hybridoma research to generate new approaches to therapy.

Publications:

1. Bode, U., and Levine, A.S.: The Biology and Management of Osteosarcoma. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 575-602.

2. Cohen, L.F., and Reaman, G.H.: The use of Biological Markers and Other Physiological or Biochemical Assays in the Diagnosis of Cancer in the Young. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing, USA, Inc., 1982, pp. 181-210.

3. Csako, G., Magrath, I.T., and Elin, R.: Serum total and isoenzyme lactate dehydrogenase activity in American Burkitt's lymphoma patients. Amer. J. Clin. Path., in press.

4. Dwyer, A., Glaubiger, D., Ecker, J., Doppman, J., Pruitt, J., and Plunkett, J.: The radiographic follow-up of patients with Ewing's sarcoma. A demonstration of a general method. Radiology, in press.

5. Glaubiger, D.L.: Chemotherapy: Biologic Basis, Molecular Mechanisms and Clinical Considerations. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 213-236.

6. Glaubiger, D., Makuch, R., and Schwarz, J.: Influence of prognostic factors on survival in Ewing's sarcoma. Proceedings of the Symposium on Sarcomas of Soft Tissue and Bone in Childhood. J. Natl. Cancer Inst., Monograph 56: 285-288, 1981.

7. Glaubiger, D.L., Tepper, J., and Makuch, R.: Ewing's Sarcoma. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 603-614.

8. Leventhal, B., Kashima, H., Levine, A.S., and Levy, H.: Treatment of recurrent laryngeal papillomatosis with an artificial interferon inducer (Poly ICLC). J. Pediatr. 99: 614-617, 1981.

9. Levine, A.S.: A Design for Training Programs. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 751-754.

10. Levine, A.S.: Interferon. NIH Medicine for the Layman Series. NIH Publication, 1981.

11. Levine, A.S.: Perspectives on the Biology and Treatment of Cancer in the Young: The Evolution of our Understanding. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 13-34.

12. Levine, A.S.: The Influence of Social and Cultural Evolution on the Relation Between Professional and Patient. In: Change: A Conference on the Future of Nursing Care. NIH Clinical Center Monograph Series, NIH Publication No. 81-2312, 1981, pp. 13-20.

13. Levine, A.S.: Walking on water wasn't built in a day: Interferon as a treatment for cancer. (Guest Editorial). J. Natl. Cancer Inst., in press.

14. Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, 767 pp.

15. Levine, A.S., Brennan, M.F., Ramu, A., Fisher, R.I., Pizzo, P.A., and Glaubiger, D.L.: Controlled clinical trials of nutritional intervention as an adjunct to chemotherapy, with a comment on nutrition and drug resistance. Cancer Res. 42 (Suppl.): 774s-781s, 1982.

16. Levine, A.S., Durie, B., Lampkin, B., Leventhal, B.G., and Levy, H.B.: Interferon Induction, Toxicity, and Clinical Efficacy of Poly (ICLC) in Hematologic Malignancies and Other Tumors. In Terry, W., and Rosenberg, S. (Eds.): Immunotherapy of Human Cancer. New York, Elsevier North-Holland, 1982, pp. 411-418.

17. Levine, A.S., and Hersh, S.P.: The Psychosocial Concomitants of Cancer in Young Patients. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 367-387.

18. Levine, P.H., Kamarajn, L.S., Conelly, R.R., Berard, C.W., Dorfman, R.F., Magrath, I.T., and Easton, J.M.: The American Burkitt's lymphoma registry: Eight years experience. Cancer, in press.

19. Levy, H.B., and Levine, A.S.: Antitumor effects of interferon and poly ICLC, and their possible utility as antineoplastic agents in man. Tex. Rep. Biol. Med., in press.

20. Magrath, I.T.: Infectious Mononucleosis -- Its Relationship to Malignant Neoplasia. In Schlossberg, D. (Ed.): Infectious Mononucleosis. New York, Springer-Verlag, in press.

21. Magrath, I.: Malignant Lymphomas (Including Hodgkin's Disease). In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 473-574.

22. Moss, H.A., Nannis, E.D., and Poplack, D.G.: The effects of prophylactic treatment of the central nervous system on the intellectual functioning of children with acute lymphocytic leukemia. Am. J. Med. 71: 47-52, 1981.

23. Oliff, A., and Levine, A.S.: Late Effects of Anti-Neoplastic Therapy. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishsing USA, Inc., 1982, pp. 731-750.

24. Pizzo, P.A.: Rhabdomyosarcoma and the Soft-tissue Sarcomas. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 615-632.

25. Poplack, D.G.: Acute Lymphoblastic Leukemia and Less Frequently Occurring Leukemias in the Young. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 405-460.

26. Poplack, D.G., and Blatt, J.: Neuroblastoma. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 663-682.

27. Poplack, D.G., and Bleyer, W.A.: Meningeal leukemia: New approaches to treatment. Eur. J. Cancer, in press.

28. Poplack, D.G., Reaman, G., and Wesley, R.: Treatment of acute lymphoblastic leukemia in relapse: Efficacy of a four-drug reinduction regimen. Cancer Res., in press.

29. Poplack, D.G., Sallan, S.E., Rivera, G., Holcenberg, J., Murphy, S.B., Blatt, J., Lipton, J.M., Venner, P., Glaubiger, D.L., Ungerleider, R., and Johns, D.: Phase I Study of 2'-Deoxycoformycin in acute lymphoblastic leukemia. Cancer Res. 41: 3343-3346, 1981.

30. Ramot, B., and Magrath, I.T.: Hypothesis: The environment is a major determinant of the immunological sub-type of lymphoma and acute lymphoblastic leukemia in children. Br. J. of Haem., in press.

31. Reaman, G.H., and Cohen, L.F.: Less Frequently Encountered Malignant Neoplasms in the Young. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 707-727.

32. Sariban, E., and Magrath, I.T.: Dysphagia -- an unusual presentation of Burkitt's lymphoma. Am. J. Dis. Child., in press.

33. Sariban, E., Magrath, I.T., and Shawker, T.: Abdominal lymphoma mimicking pancreatic pseudo cyst. Am. J. Gastroent., in press.

34. Spiegel, R.J., and Pizzo, P.A.: Symptomatic and Supportive Care of the Cancer Patient. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 339-365.

35. Spiegel, R.J., Schaefer, E.J., Magrath, I.T., and Edwards, B.K.: Plasma lipid alterations in leukemia and lymphoma. Am. J. Med. 72: 775-782, 1982.

36. Srinivasan, U., Reaman, G.H., Poplack, D.G., Glaubiger, D.L., and Levine, A.S.: Phase II study of 5-Azacytidine in sarcomas of bone. Cancer Clin. Trials, in press.

37. Susman, E.J., Hollenbeck, A., Nannis, E.D., Strope, B.E., Hersh, S.P., Levine, A.S., and Pizzo, P.A.: A prospective naturalistic study of the impact of an intensive medical treatment on the social behavior of child and adolescent cancer patients. Journal of Applied Developmental Psychology 2: 29-74, 1981.

38. Susman, E., Pizzo, P.A., and Poplack, D.G.: Adolescent Cancer: Getting Through the Aftermath. In Ahmed, P. (Ed.): Coping with Cancer. New York, Elsevier, North-Holland, 1981, pp. 99-117.

39. Thomas, W.J., North, R.B., Poplack, D.G, Slease, R.B., and Duval-Arnould, B.: Chronic myelomonocytic leukemia in childhood. Am. J. Hematol. 10: 181-194, 1981.

40. Trigg, M., Glaubiger, D., and Nesbit, M.: Frequency of central nervous system involvement in Ewing's sarcoma. Cancer, in press.

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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Infectious Complications of Malignancy: Prevention, Diagnosis and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
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| OTHER: | K. Robichaud | Research Nurse | PO C |
| | J. Commers, J. Hiemenz | Medical Staff Fellows | PO C |
| | J. Gress/ I. Ioannou | Guest Worker/ Visit. Fellow | PO C |
| | F. Hoffman | Senior Investigator | CTEP C |
| | A. Macher/ V. Gill/ F. Witebsky | S. Fellow/S'visor/Asst. Chief | CP CC |
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COOPERATING UNITS (if any)

Medicine Branch, Biometric Research Branch, NCI; LCI; NIAID; Clinical Pathology, Epidemiology, CC; WRAIR; Univ. Florida; Johns Hopkins.
(and Metabolism Branch, NCI)

LAB/BRANCH

Pediatric Oncology Branch

SECTION

Infectious Disease Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 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 (200 words or less - underline keywords)

Infection is the major cause of morbidity and mortality in patients with malignant disease. We are developing and evaluating the utility of various techniques and procedures to reduce the incidence of severe infection in cancer patients. The role of oral absorbable antibiotic prophylaxis is being evaluated in patients receiving standard doses of chemotherapy, and the role of protected environments (laminar air-flow room isolators) is being studied in patients receiving intensive chemotherapy. We are also evaluating new methods for detecting occult infections in neutropenic patients. Empiric and specific antibiotic treatment of granulocytopenic patients with fever of unknown origin or proven infections is being prospectively studied, and the roles of newer antimicrobial agents are also being investigated. The role of chemically-defined immunoregulatory agents is being evaluated in in vitro and in vivo models to develop methods of improving host defenses following immunosuppressive chemotherapy, and to help reduce the incidence and severity of infectious complications.

Objectives:

1. To assess the current etiologies of febrile episodes in patients with malignancy, and the relationship of these episodes to cancer treatment, prior infection, degree of host compromise, underlying disease, and granulocytopenia.

2. To determine the most appropriate means for the evaluation of fever in the compromised host by assessing invasive and noninvasive diagnostic techniques.

3. To improve methods by which occult infections may be detected and treated.

4. To assess the possibility of reducing the incidence of infectious complications in granulocytopenic cancer patients by the use of prophylactic antibiotics.

5. To assess the efficacy and toxicity of empiric and specific antibiotic regimens for patients who become febrile and/or infected during periods of granulocytopenia. The role of antifungal therapy in high-risk patients will also be determined.

6. To improve the utility of the "total protected environment" in reducing the incidence of severe infection in patients with various lymphomas and solid tumors who are undergoing very intensive chemotherapy (with or without autologous marrow rescue), and to learn whether the intensive treatment possible in this circumstance results in improved tumor response and prolongation of survival.

7. To evaluate methods for bolstering host defenses and stimulating granulocyte recovery following chemotherapy-induced immunosuppression as an adjunct to the treatment and prevention of infections.

Methods Employed:

Since 1976, 302 Pediatric Branch patients who became febrile have been entered onto study. A total of 1259 febrile episodes have been evaluated in these patients, 940 (75%) occurring in association with granulocytopenia. Beginning in July, 1981, patients from the Medicine Branch have also been entered into our studies. These febrile-granulocytopenic episodes have been entered into trials related to the diagnosis and management of infections. Additionally, trials to prevent infectious complications in granulocytopenic cancer patients have also been conducted.

A. Studies related to diagnosis:

1. The utility of surveillance cultures in predicting and guiding management of infection and modification of therapy was surveyed in 652 patients. Body surveillance cultures from noninfected sites were expensive and not useful in predicting infection and are not recommended for routine management. Blood cultures in patients receiving antibiotics were positive in only 0.9%, although helpful in selected cases where modification of therapy was undertaken.

2. The role of viruses as co-factors or antecedent agents causing fever and/or secondary bacterial infection is being prospectively evaluated with R. Yolken at Johns Hopkins using ELISA assays on throat washings and stool samples from patients who become febrile. Since this study began in December, 1982, 36 samples have been collected and the study is in progress.

3. The prevalence of C. difficile as a cause of diarrhea in patients receiving chemotherapy or antibiotics is being prospectively investigated. The study began in March, 1982, and 9 samples have been analyzed so far, 1 containing a toxinogenic C. difficile.

4. The ability to detect antigens to Candida and Aspergillus in either serum or urine is being analyzed with Dr. J. Bennett using antibody-coated latex beads or double sandwich ELISA samples from patients with documented fungal infections have been categorized and the analysis is in progress.

5. The association of the indwelling right arterial silastic catheters of the Hickman-Broviac type with bacteremia has been evaluated in 43 patients for whom 51 catheters were placed. The incidence of bacteremia is 39%, in both granulocytopenic and nongranulocytopenic patients, predominately with gram-positive organisms. Infectious morbidity related to the catheters is negligible and the majority can be treated with parenteral antibiotics without the need for catheter removal.

6. Approach to the management of pulmonary infiltrates appearing in patients already receiving broad spectrum antibiotics was retrospectively evaluated in the 34 patients (11.7%) who developed such infiltrates over a 4-year period. The most common etiology was fungal and outcome depended significantly on whether the infiltrate occurred in association with granulocyte recovery. If infiltrates occurred and progressed without granulocyte recovery, the possibility of a fungal pneumonia was highest and such patients required early antifungal therapy with amphotericin B.

7. The initial management of patients with diffuse pulmonary infiltrates with empiric antibiotics vs. an attempt to make a diagnosis using open lung biopsy represents an area of current controversy. In March of 1982,we initiated a prospective study in which patients will be randomly assigned to initial empiric antibiotic therapy (without biopsy) vs. initial biopsy and specific therapy. Five patients have been randomized to date.

8. A protocol for studying chemotherapy-induced changes in leukocyte subpopulations and their influence on the risk for developing infectious complication has been approved. To date no patients have been entered.

B. Studies related to management:

1. In December, 1981, a trial comparing the new third-generation cephalosporin ceftazidime to our conventional broad-spectrum antibiotic regimen (KGC) in the initial management of fever in granulocytopenic patients was instituted. To date 38 adults and 13 pediatric patients (children

<12 years of age have not been eligible so far) have been entered of whom 26 have been randomized to ceftazidime and 25 to KGC. Patients with unexplained fever have had comparable outcomes regardless of which antibiotic they were treated. The study is still in progress.

2. If patients have a microbiologically proven infection, it remains unclear as to whether appropriate treatment should be a specific antibiotic vs. a broad-spectrum combination, especially if they remain granulocytopenic. Our prior retrospective study showed that broad spectrum antibiotics were optimal if the period of granulocytopenia exceeded one week. In a prospective study we are randomizing patients to specific therapy vs. broad-spectrum. To date 6 patients have been randomized, 1 to specific therapy and 5 to broad spectrum.

3. For patients with unexplained fever (FUO), our previous studies have shown that it is optimal to continue antibiotics if patients remain granulocytopenic for more than one week. In present studies, patients with FUO who defervesce after initiation of antibiotics are treated for a full course of therapy as if they had an undiagnosed infection and are then randomized on day 14 to either discontinue antibiotics or to continue until resolution of granulocytopenia. To date, 9 patients have been randomized to discontinue therapy and 10 to continue. The study is still in progress.

4. Our prior study has shown that patients with persistent fever and granulocytopenia benefit from the continuation of antibiotics and the addition of empiric antifungal therapy after one week of fever and granulocytopenia. We are presently comparing the efficacy and toxicity of a new antifungal drug, ketoconazole, with our standard, amphotericin-B. Since initiating this trial in July, 1981, 15 patients have been randomized, 7 to receive empiric amphotericin-B, 8 to receive empiric ketoconazole.

C. Studies related to prevention:

1. Patients undergoing intensive chemotherapy may be treated in protected isolation; if the patient is receiving an autologous bone marrow infusion, the patient may be randomized to receive intensive therapy either in or out of a protected isolation. Since 1975, 85 patients have received intensive therapy in a protected environment. Results show a significant reduction in infections and infection-related mortality, although the efficacy of this program is adversely influenced by patient compliance with the regimen. At best, only 48% of patients are fully compliant.

2. We recently completed a double blind randomized trial comparing trimethoprim-sulfamethoxazole plus erythromycin to placebo for preventing fever and infection in granulocytopenic patients. In this study, 150 patients were randomized to the antibiotics or placebos. A significant reduction in the incidence of fever and/or infection was observed for the antibiotic treated patients, but only if they totally complied in taking the medication. Compliance with the prophylactic regimen also turned out to be an independent variable.

3. In order to further extend our observations related to antibiotic prophylaxis and assess whether erythromycin might have adversely affected the prophylaxis by impeding "colonization-resistance" is being explored in a prospective study begun in December, 1981, in both adult and pediatric patients. Patients are randomized to receive trimethoprim-sulfa alone, T-S plus erythromycin or to serve as a control. To date, 77 pediatric episodes and 41 adults have been randomized: 40 to B+E, 37 to Bactrim alone and 41 to control.

4. The role of lithium carbonate in enhancing granulocyte recovery in patients who have severe prolongation in bone marrow recovery is to be explored and a protocol approved. Studies will measure the leukocyte and bone marrow response as well as changes in CFUc, CFUe, $CFU_{MEG}$, CFU-GEMM. The function of the leukocytes will be studied for their chemotaxis, bactericidal activity and superoxide generation.

D. Preclinical studies:

1. The role of chemically-defined immunoregulatory agents on granulopoiesis has been measured in in vitro long-term bone marrow (Dexter) cultures of both murine and human derivation. The assays used have been cell count, CFUc and CFUs. In vivo murine studies have also been performed.

   The cyanoaziridine, azimexon, produces a dramatic dose-response increment in WBC, granulocyte count and CFUc. The utility of this as an adjunct to infection modulation and prevention is being explored.

2. Lithium carbonate has also been explored in both in vivo and in vitro experiments of the enhancement of granulopoiesis. While effective in vitro in increasing Dexter culture cell counts, CFUc and CFUs, it fails to have this effect when administered in vivo to the same mouse strain. The mechanism of action for this is being explored.

3. The mechanism of action of the cyanoaziridines and lithium is being explored in vitro by assessing its effect on the generation of IL-2. Lithium and azimexon produce a significant increase in IL-2 production in both murine and human systems.

4. Studies to explore the role of committed granulocytes grown from in vitro bone marrow cultures as a source for therapeutic and prophylactic leukocyte transfusions in a murine model are in progress. The effect of immunoregulatory agents on the generation and function of the leukocytes for transfusion are part of this study.

5. The possibility of improving human leukocyte transfusions by the attachment of antibody to their surface is being explored. In initial experiments, monoclonal antibody against P. aeruginosa is being bound to leukocytes by co-passage through thalate oils, saturating the human leukocyte membrane beyond simply Fc receptor sites. These "armed" polys are being studied in an in vitro ADCC assay against P. aeruginosa. Animal studies using a Pseudomonas pneumonia model are planned as part of this preclinical study.

Significance to Biomedical Research and the Program of the Institute:

Infection remains the leading cause of morbidity and mortality in the cancer patient. The majority of infections occur as a consequence of disease and/or treatment-induced alteration of host defenses (especially granulocytopenia), and they are the major impediment to the delivery of cancer chemotherapy. Consequently, effective supportive management of the patient is essential if the potential benefits of chemotherapy are to be achieved. This includes an understanding of the natural history of infectious complications in the compromised host, especially their early recognition and diagnosis. Our studies to date have helped to define the appropriate evaluation of the febrile, neutropenic cancer patient, as well as the specific management of particular infections and fevers of undetermined origin. These changes in management have resulted in a significant reduction in the morbidity and mortality related to infection.

Moreover, our studies of infection prevention (both the protected environment and empirically administered antibiotics) suggest that the frequency and morbidity of infectious complications can be significantly reduced, thus permitting the optimal delivery of cancer chemotherapy.

Proposed Course:

We shall continue our studies of the natural history of infectious complications in cancer patients as outlined in the progress report, since this evaluation will help further to define high-risk patients and assist in their diagnosis and management. Rapid diagnostic assays which do not depend on culture of the organisms will be studied in order to provide the most rational basis for immediate antibiotic and anti-fungal management. Our studies on the optimal empiric use of antibiotics in febrile, neutropenic patients will be continued, as will our clinical trials related to the specific management of septicemia, local bacterial infections, and viral, protozoan, and fungal complications. Our 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. We will seek more effective methods for suppressing and/or eliminating the host's endogenous microbial flora with absorbable and nonabsorbable antimicrobial agents. Our major research target will be to develop methods for immunostimulation of the host's defenses and during chemotherapy-induced immunosuppression. Methods to activate cellular and humoral immunity, the macrophage-monocyte system, and mechanisms which expand and/or protect the neutrophil mass following chemotherapy will be sought through the use of chemically-defined immunoregulatory agents. While combining these hosts bolstering techniques with prophylactic antibiotics, we will also explore chemotherapeutic schedules which may have a more selective effect on tumor cells.

Publications:

1. Commers, J.R., and Pizzo, P.A.: Infectious Syndromes in Acute Lymphoblastic Leukemia. In Pochedly, C., Miller, D., and Wolfe, J. (Eds.): Childhood Lymphoblastic Leukemia. Masson Publishing USA, Inc., in press.

2. Commers, J.R., and Pizzo, P.A.: The Role of Empiric Antifungal Therapy for Granulocytopenic Cancer Patients with Persistent Fever. In Klastersky, J. (Ed.): Infections in Cancer Patients. New York, Raven Press, 1982, pp. 157-170.

3. Commers, J.R., and Pizzo, P.A.: Therapy and Prevention of Infection in Acute Lymphoblastic Leukemia. In Pochedly, D., Miller, D., and Wolfe, J. (Eds.): Childhood Lymphoblastic Leukemia. New York, Masson Publishing USA, Inc., in press.

4. Hoffman, F.A., and Pizzo, P.A.: Infections in the Compromised Host. In Eichenwald, H., and Stroder, J. (Eds.): Practical Pediatric Therapy. Chemie, Inc., in press.

5. Johnston, M.R., Pizzo, P.A., and Fauci, A.S.: Thoracic mass lesions in immuno-incompetent patients. Chest, in press.

6. Kramer, B.K., Pizzo, P.A., Robichaud, K.J., and Wesley, R.: The role of serial microbiologic surveillance and clinical evaluation in the management of cancer patients with fever and granulocytopenia. Am. J. Med. 172: 561-568, 1982.

7. Pizzo, P.A.: Antibiotic Management of the Pediatric Cancer Patient. In Freeman, A., and Pochedly, C. (Eds): Controversies in Pediatric Hematology and Oncology. New York, Masson Publishing USA, Inc., in press.

8. Pizzo, P.A.: Antibiotic Prophylaxis in the Immunosuppressed Patients with Cancer. In Remington, J.S., and Swartz, M.N. (Eds.): Current Clinical Topics in Infectious Disease, 4th Edition, in press.

9. Pizzo, P.A.: Do Results Justify the Expense of Protected Environments? In Wiernick, P. (Ed.): Controversies in Oncology. New York, John Wiley and Sons, Inc., 1982, pp. 267-277.

10. Pizzo, P.A.: Infectious Complications in the Young Patient with Cancer: Etiology Pathogenesis, Diagnosis, Management and Prevention. In Levine, A.S. (Ed.): Cancer in the Young. New York, Masson Publishing USA, Inc., 1982, pp. 299-337.

11. Pizzo, P.A., and Levine, A.S.: Infectious Complications in Children with Cancer: Principles of Management. In Bozek, J. (Ed.): Progress in Diagnosis and Treatment of Tumors in Children. Warsaw, PZWL Publishers, 1981, pp. 241-254.

12. Pizzo, P.A., and Robichaud, K.J.: Bacteremia in children with cancer: The impact of infection-control studies. Infect. Dis. Rev., in press.

13. Pizzo, P.A., Robichaud, K.J. Gill, F.A., and Witebsky, M.D.: Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. Am. J. Med. 72: 101-110, 1982.

14. Pizzo, P.A., Robichaud, K.J., Wesley, R., and Commers, J.: Fever in the pediatric and young adult patient with cancer: A prospective study of 1001 episodes. Medicine 61: 153-165, 1982.

15. Pizzo, P.A., Waters, C., Purvis, D., and Ford, D.: Microbiological evaluation of food items for patients undergoing gastrointestinal decontamination and protected isolation. J. Am. Diet. Assoc., in press.

16. Pizzo, P.A., and Young, R.C.: Management of Infections of the Cancer Patient. In De Vita, V.T., Hellman, S.I., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott Co., 1982, pp. 1677-1702.

17. Shamberger, R.C., Pizzo, P.A., Goodgame, J.T., Jr., Lowry, S.F., Maher, M.M., Wesley, R.A., and Brennan, M.F.: The effect of total parenteral nutrition on chemotherapy induced myelosuppression: A randomized study. Am. J. Med., in press.

18. Straus, S., Pizzo, P.A., and Fialk, M.: Infectious Complications in Patients with Lung Cancer. In Straus M. (Ed.): Lung Cancer. New York, Grune and Stratton, 1982, pp. 799.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06840-07 PO |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biology and Immunology of Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| | A. Muchmore/S. Broder | Senior Investigators | MET C |
| | S. Korsmeyer | Clinical Associate | MET C |
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COOPERATING UNITS (if any)

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LAB/BRANCH

Pediatric Oncology Branch

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| 5.0 | 3.0 | 2.0 |

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project the biology and immunology of acute leukemia are studied, with particular emphasis on investigation of acute lymphocytic leukemia. Leukemic lymphoblasts are being characterized on the basis of cell surface markers and specific antigens detectable by various monoclonal antibodies and heteroantisera. Such characterization permits comparison of leukemic and normal lymphoid cells in terms of cellular differentiation and malignant transformation. Biochemical studies, such as determination of the role of the purine pathway enzymes in lymphoid leukemia and lymphoma, permit insight into the relationship between malignant and normal lymphoid cells. Certain immunologic functions of leukemic lymphoblasts are studied such as their immunoregulatory capacity. Study of the role of the monocyte-macrophage system in the lymphoid malignancies is also in progress. In addition to these biological studies, examination of the pharmacology of those agents currently utilized to treat acute leukemia is also undertaken.

Objectives:

1. To develop methods of distinguishing between immunological subtypes of leukemic lymphoblasts and further to characterize such subtypes.

2. To define possible correlations between immunological cell surface markers and various biochemical characteristics of leukemic lymphoblasts.

3. To study the biochemical status of the purine enzyme pathways in malignant lymphoid conditions in an attempt to assess their role in lymphoid differentiation.

4. To evaluate heterologous anti-leukemia antisera and hybridoma-produced antibodies as potential diagnostic and therapeutic tools in the treatment of acute lymphoblastic leukemia.

5. To evaluate the status of immunoglobulin genes in acute leukemic lymphoblasts.

6. To explore the role of the monocyte-macrophage in disease states (including leukemia) and to assess the functional heterogeneity of this cell system during normal maturation.

7. To study the pharmacology of antineoplastic agents used to treat acute leukemia.

Methods and Major Findings:

A. Immunological and Biochemical Correlations Involving Leukemic Lymphoblasts

Our recent research efforts have been aimed at elucidating biochemical markers of potential diagnostic and therapeutic value. Five enzymes have been studied in this regard, including adenosine deaminase, 5'nucleotidase, purine nucleoside phosphorylase, terminal deoxynucleotidyl transferase and acid phosphatase. Our results suggest that a "biochemical profile" of acute lymphoblastic leukemia cells provides the mechanism for further defining subsets of ALL and offers an avenue for new diagnostic and therapeutic approaches. Our studies have been extended to other lymphoid malignancies, including the lymphomas and the Sezary syndrome.

B. Study of Hybridoma Antibodies and Heterologous Antisera in Acute Lymphoblastic Leukemia

We have studied the reactivity of leukemic lymphoblasts with a variety of recently developed hybridoma antibodies both as an aid to classification and as a means of understanding the biology of leukemic cell differentiation. Our results demonstrate the presence of a wide variation in the expression of antigenic determinants on acute lymphoblastic and acute myelogenous leukemia cells. In collaboration with Drs. Ronald Billing and Paul Terasaki, we have been developing a variety of hybridoma antibodies to antigens on the leukemic lymphoblasts of our patients. The antibodies developed are being evaluated: 1) to determine their usefulness as an aid in the sub-classification of ALL,

2) to assess their potential role in increasing diagnostic sensitivity in the assessment of bone marrow relapse, and 3) as a possible means of in vitro immunotherapy for this disease. A murine model has been developed to study this latter approach.

C. Studies of Immunoglobulin Genes in Acute Leukemic Lymphoblasts

Under the direction of Dr. Stanley Korsmeyer, and in collaboration with Dr. Thomas Waldmann, studies initiated to examine the status of immunoglobulin genes in ALL lymphoblasts have demonstrated that the majority of patients with null cell ALL have evidence of immunoglobulin gene rearrangement which indicates they are of B-cell lineage.

D. Role of Acute Lymphocytic Leukemia Cells in Suppression of the Immune Response

We have studied the ability of leukemic lymphoblasts to effect suppression of antigen-induced lymphocyte responses. Our studies have shown that the interaction of HLA-DR sera with antigens on the leukemic cell surface induces a strong signal for suppression of antigen-induced lymphocyte responses.

E. Role of the Macrophage in Leukemogenesis

We have studied the role of the mononuclear phagocyte in the development of Friend-virus induced leukemia. We have determined that the susceptibility of the newborn animal to viral infection and subsequent leukemia is based on an immunologic mechanism specifically involving a defect in macrophage function.

F. Study of Pharmacology of Antileukemic Agents

We are studying the pharmacology of those antileukemic agents being used during maintenance treatment. Our studies of oral 6-MP, using a new HPLC assay demonstrate that the bioavailability of this agent is poor. These findings have profound implications for the way we give maintenance therapy.

Significance to Biomedical Research and the Program of the Institute:

Our studies on the characterization of leukemia cells using immunological and biochemical markers have revealed the presence of distinct biochemical differences among leukemic lymphoblasts of different immunologic subclasses. In addition, extension of these studies into other lymphoid malignancies has revealed that development of a biochemical profile may provide a useful mechanism for classifying lymphoid malignancies in terms of their state of lymphoid differentiation. These findings may be of both prognostic and therapeutic significance. In addition, evaluation of the immunological functions of these cells, e.g., immune suppression and Fc receptor status, is providing valuable insight into the degree of differentiation of the various leukemic lymphoblast populations. Our studies of the utility of specific anti-leukemic antisera and hybridoma antibodies may yield information helpful in the diagnosis and treatment of acute lymphoblastic leukemia.

Our studies on the role of the mononuclear phagocyte in leukemogenesis in the Friend leukemia virus system, although preliminary, may confirm a major role for this cell type in the leukemogenic process, underscoring the need for further definition of the role of the mononuclear phagocytic system in patients with leukemia.

The findings that the bioavailability of oral 6-MP is low have stimulated us to pursue clinical and laboratory studies evaluating the effectiveness of this agent as a maintainence drug in the treatment of ALL.

Proposed Course:

We are planning to expand our efforts to characterize acute leukemic lymphoblasts both biochemically and immunologically. In particular, we are extending our evaluation of the role of the purine pathway enzymes not only in acute lymphoblastic leukemia but also in other lymphoid malignancies. Preliminary data suggest that determination of a biochemical profile by assessment of these enzymes may yield important information as to the state of maturation and differentiation of both normal and malignant lymphoid conditions. We are also planning to pursue the prognostic and therapeutic relevance of our findings. Our interest in heteroantisera to acute leukemic lymphoblasts has been expanded. Specific antileukemic hybridoma antibodies are being developed which may be of use in the diagnosis and the treatment of acute lymphoblastic leukemia. We will pursue our interests in the pharmacology of antileukemic agents with particular reference to examining maintenance therapy in ALL.

Publications:

1. Blatt, J., Spiegel, R.J., Papadopoulos, N.M., Lazarou, S.A., Magrath, I.T., and Poplack, D.G.: Lactic dehydrogenase isoenzymes in normal and malignant human lymphoid cells. Blood, in press.

2. Korsmeyer, S.J., Hieter, P.A., Ravetch, J.V., Poplack, D.G., Leder, P., and Waldmann, T.A.: Patterns of immunoglobulin gene arrangement in human lymphocytic leukemias. In Knapp, W. (Ed.): Leukemia Markers. London, Academic Press, Inc., 1981, pp. 85-97.

3. Ladisch, S., Poplack, D.G., and Blaese, R.M.: Inhibition of human lymphoproliferation by intravenous lipid emulsion. Immunol. Immunopath., in press.

4. Poplack, D.G., Blatt, J., and Reaman, G.: Purine pathway enzyme abnormalities in acute lymphoblastic leukemia. Cancer Res. 41: 4821-4823, 1981.

5. Reaman, G.H., Blatt, J., and Poplack, D.G.: Purine pathway enzymes in a patient with acute lymphoblastic leukemia and increased B cell markers. Blood 58: 330-332, 1981.

6. Riccardi, R., Vigersky, R.A., Barnes, S., Bleyer, W.A., and Poplack, D.G.: Methotrexate levels in the interstitial space and seminiferous tubule of rat testis. Cancer Res. 42: 1617-1619, 1982.

7. Trigg, M.E., and Poplack, D.G.: Transplantation of Leukemia Bone Marrow Incubated with Cytotoxic Antibodies. In Knapp, W. (Ed.): Leukemia Markers. London, Academic Press, Inc., 1981, pp. 425-428.

8. Trigg, M.E., and Poplack, D.G.: Transplantation of leukemic bone marrow pretreated with anti-leukemic cytotoxic antibodies and complement. Science, in press.

9. Trigg, M.E., Wesley, R.A., Holiman, B.J., Cole, D.E., and Poplack, D.G.: A rapid, single step technique for determination of B and T cell surface markers in malignant lymphoid cells. J. Exper. Hematol., in press.

10. Venner, P.M., Glazer, R.I., Blatt, J., Sallan, S., Rivera, T., Holcenberg, J.S., Lipton, J., Murphy, S.B., and Poplack, D.G.: Clinical pharmacology of 2'-deoxycoformycin: Level of 2'-deoxycoformycin, adenosine and deoxyadenosine in patients with acute lymphoblastic leukemia. Cancer Res. 41: 4508-4511, 1981.

11. Wortman, R.L., Holcenberg, J., and Poplack, D.G.: Relationship of 5'-nucleotidase activity and antileukemic effect of 2'-deoxycoformycin therapy. Cancer Treat. Rep. 66: 387-390, 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06860-06 PO |
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical and Experimental Pharmacology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| OTHER: | H. M. Friedman | Clinical Associate | PO C |
| | H. Khaled | Visiting Fellow | PO C |
| | J. Hearst | Professor, Chemistry, Univ. of CA, Berkeley | |
| | D. G. Poplack | Head, Leukemia Biol. Sect. | PO C |
| | R. G. Smith | Professor, Med., Texas Southwestern School of Med. | |
| | A. Ramu | Lecturer, Exp. Onc., Dept. of Rad. & Clinic. Onc., Hadassah Univ. Hosp. | |

COOPERATING UNITS (if any)

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LAB/BRANCH

Pediatric Oncology Branch

SECTION

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INSTITUTE AND LOCATION

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project concerns clinical and pre-clinical studies of anti-cancer drugs: Pre-clinical level - we have focused on investigations of resistance and sensitivity of mammalian cell lines to anthracyclines. We have investigated the composition of these cells and their relative sensitivity and resistance to other anti-cancer drugs. Using human cells, we have developed a rapid in vitro screening test for sensitivity and resistance of primary explants to anti-cancer drugs. We are investigating the use of this assay as a measure of sensitivity to combined modality therapy including both x-irradiation and chemotherapy.

Objectives:

1. To investigate mechanisms of resistance and sensitivity of tumor cells to chemotherapeutic agents both in vivo and in vitro.

2. To develop clinically useful assay systems for chemotherapeutic sensitivity of human cells.

3. To investigate the interaction of the modalities of x-irradiation and chemotherapy regarding their cytotoxic effect on cancer cells.

Major Findings and Methods:

A. In vitro testing of human tumor cells - A double layer soft agar technique permitting the preferential growth of human tumor cells from primary explants was developed by Hamburger and Salmon and has become known as the human tumor stem cell assay. The method shows promise in detecting in vitro sensitivity of human tumor cells to chemotherapeutic agents. The method also permits direct isolation of resistant cell clones. Using a variant of this method, we have been able to develop a tritiated thymidine incorporation assay into high molecular weight nucleic acid which permits measurement of sensitivity of human tumor cells from primary explants to prospective chemotherapeutic agents in approximately 5 days. This compares to 2 to 3 weeks required for performance of the normal assay. The radiolabel method is more reproducible, less labor intensive, yet only uses standard scintillation counting techniques. The concordance rate between this assay and the previously described assay is approximately 95%.

B. Assessment of anti-metabolite sensitivity of human tumor cells - Utilizing the method described above and the relative incorporation of tritiated uridine into DNA, we are now able in preliminary experiments, to assess the sensitivity of human tumor cells to methotrexate. Utilizing the time frame and concentration defined by these experiments, we hope to use this method to measure the relative sensitivity of human tumor cells from primary explants to other anti-metabolites such as 5-fluoruracil and cytosine arabinoside.

C. X-ray sensitivity - Utilizing the method described under A, we have measured the x-ray sensitivity of human tumor cells and compared this method to direct cloning techniques. In model systems, results are similar to approximately a four-log decrement in cell survival. We hope to expand these measurements to other model human tumor cell lines which are relatively x-ray resistant to assess the ability of this assay to distinguish x-ray sensitivity in resistance. The method then has potential to measure x-ray sensitivity of primary human tumor explants.

D. Using the method under A, we are measuring chemotherapeutic sensitivity of x-ray sensitive and x-ray resistant primary explants of human tumors from patients with newly diagnosed neuroblastoma, when the tumor cells are likely to be both x-ray and chemotherapeutic sensitive. Studies will be repeated at relapse at which time the cells are chemotherapeutically resistant, and in preliminary experiments are also x-ray resistant.

E. Utilizing automated optical counting of colonies formed in the standard human tumor stem cell assay system, we have been able to measure size distributions of colonies as a function of time and are therefore able to measure the relative progression of size with time in both the presence and the absence of chemotherapeutic drug. Results indicate that this method can detect chemotherapeutic sensitivity and may be able to indicate that there is a spectrum of chemotherapeutic resistance.

F. Utilizing incorporation of tritiated anthracyclines into whole cells, we have been able to demonstrate a difference in uptake of daunomycin between sensitive and resistant murine leukemia cell lines. These cell lines also demonstrate differences in overall lipid composition of the plasma membrane, yielding different physical chemical properties as measured by lipid soluble probes, including 1,6 diphenolhexatriene (DPH) and adriamycin itself. These cells also demonstrate resistance to other drugs of differing mechanisms of action, suggesting that resistance may be due to the relative lipid solubility of these drugs. In addition, the resistant sublines appear to have differing sensitivities to antimetabolites, such as 6-MP. The enzymatic correlations of these sensitivity and resistance patterns are currently under study.

Significance to Biomedical Research and the Program of the Institute:

The development of a reliable, objective and reproducible in vitro assay of the chemosensitivity of human tumor cells obtained from primary explants would be of clear utility in the treatment of patients with cancer. Such a test would minimize unnecessary patient toxicity and maximize potential therapeutic benefit. The demonstration that such an assay could also be used to measure sensitivity to x-irradiation would enhance the ability of investigators to study the interaction between chemotherapeutic drugs and x-irradiation as well as potentially allow in vitro testing of relative synergism or antagonism of the two modalities. Results obtained in the murine leukemia cell line resistant to anthracyclines indicates the possibility that a general mechanism of resistance of tumor cells to chemotherapeutic agents is based on the physical chemical properties of the drug, rather than the mechanism of action.

Proposed Course:

We hope to expand the testing of primary human tumor explants to include a number of antimetabolites as well as to explore the interaction of combined modality therapies in vitro. We also hope to expand our biochemical investigations of resistance and sensitivity utilizing as models mammalian cell lines which have been made resistant to a number of chemotherapeutic agents. Utilizing the materials we have at hand, we hope to examine the relative cross-resistance of such mammalian cell lines as well as to examine these cells biochemically with particular regard to their sensitivity or resistance to antimetabolites.

Publications:

1. Friedman, H.M., and Glaubiger, D.L.: Assessment of in vitro drug sensitivity of human tumor cells using [$^{3}$H]thymidine incorporation in a modified human tumor stem cell assay. Cancer Res., in press.

2. Gangji, D., Ross, W.E., Bleyer, W.A., Poplack, D.G., and Glaubiger, D.L.: The effect of probenecid on methotrexate cytotoxicity in L1210 mouse leukemia cells. Cancer Treat. Rep., in press.

3. Glaubiger, D., and Ramu, A.: Mitomycin C, Actinomycin D, and Mithramycin. In Chabner, B. (Ed.): Clinical Pharmacology of Antineoplastic Agents. Philadelphia, W.B. Saunders Co., in press.

4. Glaubiger, D.L., Von Hoff, D.D., Holcenberg, J.S., Kamen, B., Pratt, C., and Ungerleider, R.S.: The relative tolerance of children and adults to anticancer drugs. Front. Radiat. Ther. Onc. 16: 42-49, 1982.

5. Hamel, E., Johnson, G.J., and Glaubiger, D.: Pharmacokinetics of leucovorin rescue using a new methotrexate independent biochemical assay for leucovorin and $N^5$-methyltetrahydrofolate. Cancer Treat. Rep. 65: 545-553, 1981.

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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Experimental Approaches to the Treatment of CNS Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
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| | J.H. Wood | Assistant Professor | U. of Pennsylvania |
| | S. Cohen | Associate Professor | Johns Hopkins U. |
| | S. Reich | Assistant Professor | U. of Massachusetts |
| | J. Schwade | Senior Investigator | RO C |
| | J.M. Strong | Senior Investigator | LCHPH C |
| | D. Jackson | Assistant Professor | Bowman-Gray Med. Sch. |
| | J. Holcenberg | Professor | Milwaukee Children's Hosp. |
| | P. Gormley | Senior Investigator | LCHPH C |

COOPERATING UNITS (if any)

Rad. Oncol. Br. (NCI); Lab. of Chem. Pharm. (NCI); U. of Washington; U. of Pennsylvania; Johns Hopkins U.; Bowman-Gray Med. Sch.; Milwaukee Children's Hosp.; U. of Massachusetts School of Medicine

LAB/BRANCH

Pediatric Oncology Branch

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| 6.0 | 4.0 | 2.0 |

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental approaches to the treatment of meningeal leukemia and other meningeal and non-meningeal CNS neoplasms are explored. A unique subhuman primate model which allows sterile, repetitive access to the cerebrospinal fluid is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities attendant upon various chemotherapeutic and radiotherapeutic treatments; and to evaluate and screen, in a preclinical setting, newer CNS treatment modalities and drug schedules. Information gained from studies with this model is then applied to the design of clinical protocols used to treat patients with meningeal and non-meningeal malignancies.

Objectives:

1. 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.

2. To study the CNS pharmacokinetics of currently employed and potentially useful CNS antineoplastic agents.

3. To assess the neurotoxicity of chemotherapeutic agents used in the treatment of CNS malignancy.

4. To better understand the physiology of the blood-brain barrier.

5. 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. Pharmacokinetic Studies Using the Subhuman Primate Model

We developed a subhuman primate system which allows for repetitive sterile sampling of CSF over an extended period of time in unanesthesized animals. The model involves the subcutaneous implantation of an Ommaya reservoir in rhesus monkeys. Studies to date have demonstrated that this model provides CNS pharmacokinetic data which are similar to that obtained in man.

B. Experimental Methods of Improving CNS Antifol Therapy

We have investigated potential methods of improving methotrexate therapy to the central nervous system, and have shown that administration of methotrexate by the hyperberic intrathecal technique results in improved cerebrospinal fluid 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 of either the flat or Trendelenberg position for at least one hour following intralumbar administration of methotrexate results in substantially greater drug levels within ventricular CSF. We have evaluated the feasibility of utilizing high-dose intravenous methotrexate infusions to treat CNS leukemia. Clinical studies of this approach, which were piloted in the monkey, are currently underway. We have also studied the CSF pharmacokinetics of Aminopterin in the subhuman primate model.

C. Studies of CSF Pharmacokinetics of Other Antineoplastic Agents

We have studied a variety of antineoplastic agents with respect to their penetration, following IV administration, into the CNS, as well as their CSF pharmacokinetics following intrathecal injection. Agents evaluated include AZQ, dihydroxyanthracenedione, aclacinomycin, cytosine arabinoside, L-asparaginase, m-AMSA and 6-mercaptopurine.

D. Effect of Alteration of Drug Metabolism on Cerebrospinal Fluid Pharmacokinetics of Intrathecally Administered Agents

In these studies we explored the effect of tetrahydrouridine on the pharmacokinetics of intrathecally administered Ara-C following either intrathecal or intravenous tetrahydrouridine administration. Inhibition of Ara-C deamination by THU resulted in a profound effect on Ara-C pharmacokinetics. This approach may have potential therapeutic value in man.

E. Studies on the Neurotoxicity of Methotrexate and/or Cranial Radiation

We have developed a subhuman primate model of methotrexate leukoencephalopathy. Studies in our model confirm the synergistic role of methotrexate plus cranial irradiation in the pathogenesis of this entity.

F. Studies of the Effect of CNS Treatment on the Neuroendocrine System

Abnormalities in hypothalamic-pituitary function have previously been reported in children given cranial irradiation for CNS prophylaxis in ALL. We have been studying the effects of graded doses of cranial irradiation on the hypothalamic-pituitary axis in subhuman primates, and have demonstrated evidence of a marked dose-response curve. These observations will be of considerable aid in planning the treatment of patients with CNS malignancy.

Significance to Biomedical Research and the Program of the Institute:

Rational treatment of central nervous system neoplasms requires knowledge of the physiology of the blood-brain barrier and a clear understanding of the CNS pharmacokinetics of antineoplastic agents. Detailed pharmacologic investigations in humans is limited by the lack of a ready route of access to cerebrospinal fluid. The development of the subhuman primate model facilitates such studies in a setting that approximates the human situation. In addition, the model 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 the development of toxicity. Data obtained in this model have led to a variety of new treatment approaches now being assessed in man.

Proposed Course:

Use of the primate model to screen agents of potential value in treating CNS malignancy will continue. Studies of a variety of agents, including 6-MP, AZQ, the antifols, platinum compounds, nitrosoureas, radiosensitizers, and Phase I agents (e.g., 2'-Deoxycoformycin) are in progress. Particular emphasis will be placed on the intravenous approach to the treatment of CNS malignancy, and studies will be designed to assess the penetration of intravenously administered compounds, e.g., methotrexate, into brain tissue. Exploration of various combination chemotherapeutic approaches to treating the CNS will also be continued. Finally, a comprehensive study of post-therapy leukoencephalopathy is underway in an attempt to learn more about those factors which predispose to the development of this syndrome.

Publications:

1. Blatt, J., Venner, P.M., Riccardi, R., Cohen, L.F., Gangji, D., Glazer, R.I., and Poplack, D.G.: Cerebrospinal fluid levels of 2'-deoxycoformycin after systemic administration in monkeys. J. Natl. Cancer Inst 68: 391-393, 1982.

2. Chrousos, G.P., Poplack, D.G., Kostolich, M., Wiede, C., Oliff, A. Brown, T , and Bercu, B.: Hypothalamic-adenohypophyseal function in the Rhesus monkey: A primate model. J. Med. Primatol. 10: 61-71, 1981.

3. Gormley, P.E., Gangji, D., Wood, J.H., and Poplack, D.G.: Pharmacokinetic study of cerebrospinal fluid penetration of cis-diamminedichloroplatinum (II). Cancer Chemotherapy Pharmacol. 5: 257-260, 1981.

4. McGovren, J.P., Stewart, J.C., Elfring, G.L., Smith, R.B., Soares, N., Wood, J.H., Poplack, D.G., and Von Hoff, D.D.: Plasma and cerebrospinal fluid pharmacokinetics of acivicin in Ommaya reservoir-bearing Rhesus monkeys. Cancer Treat. Rep. 66: 1333-1341, 1982.

5. Riccardi, R., Bleyer, W.A., and Poplack, D.G.: Enhancement of Delivery of Antineoplastic Agents to the Cerebrospinal Fluid. In Wood, J.H. (Ed.): Neurobiology of Cerebrospinal Fluid, Vol II. New York, Plenum Press, in press.

6. Riccardi, R., Chabner, B., Glaubiger, D., Wood, J.H., and Poplack, D.G.: Influence of tetrahydrouridine on cerebrospinal fluid ARA-C levels in the subhuman primate. Eur. J. Cancer, in press.

7. Riccardi, R., Chabner, B., Glaubiger, D.L., Wood, J.H., and Poplack, D.G.: Influence of tetrahydrouridine on the pharmacokinetics of intrathecally administered 1-β-D-arabinofuranosylcytosine. Cancer Res. 42: 1736-1739, 1982.

8. Riccardi, R., Holcenberg, J.S., Glaubiger, D.L., Wood, J.H., and Poplack, D.G.: L-Asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of Rhesus monkeys and humans. Cancer Res. 41: 4554-4558, 1981.

9. Trigg, M., Gangji, D., and Poplack, D.G.: Cerebrospinal Fluid Markers of Central Nervous System Radiation and Chemotherapy Damage. In Wood, J.H. (Ed.): Neurobiology of Cerebrospinal Fluid, Vol. II. New York, Plenum Press, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01-CM-06890-03 PO |
|---|---|---|

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Lymphoma Biology and Epstein-Barr Virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | I. Magrath | Senior Investigator | PO C |
| OTHER: | D. Benjamin | Visiting Fellow | PO C |
| | C. Janus | Clinical Associate | PO C |
| | H. Sieverts | Guest Worker | PO C |
| | R. Parsons | Head, DNA Binding Proteins Section | FCRC |
| | G. Tosato | Investigator | MET C |
| | R. Maguire | Clinical Associate | PO C |
| | M. Blaese | Senior Investigator | MET C |
| | E. Jaffe | Senior Investigator | LP C |
| | M. Frank | Chief | LCI I |
| | T. Gaither | Investigator | LCI I |
| | N. Papadopoulos | Investigator | FCRC |

COOPERATING UNITS (if any)
Metabolism Branch, Laboratory of Pathology, NCI; Laboratory of Clinical Investigation, NIAID; Clinical Chemistry Dept., DNA Binding Proteins Section, FCRC

LAB/BRANCH
Pediatric Oncology Branch

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

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies utilizing fresh lymphoma cells or derived cell lines are carried out with the primary objectives of understanding lymphomas in the context of normal lymphocyte differentiation, and identifying the normal counterpart cells of specific malignant lymphomas. Detailed phenotypic characterization of tumors and cell lines is carried out and studies of immunoglobulin secretion and the characteristics of the secreted molecular species are in process. The development of heteroantisera and hybridoma antibodies with specificity for non-endemic Burkitt's lymphoma is proceeding and has led to the recognition that American tumors differ phenotypically from African tumors. Characterization of these antigenic differences is in process. Studies of the biological effects of various EBV strains on normal lymphocytes continue.

Objectives:

1. To obtain tumor-derived cell lines from lymphoma patients and to use these in the study of lymphoma biology.

2. To induce differentiation in vitro and to determine the correlation between cell surface differences and in vitro behavior, including infectibility with Epstein-Barr virus (EBV) and response to plant lectins.

3. To study the cytogenetics of lymphomas and derived cell lines.

4. To compare isolates of the EBV which have been obtained from different clinical settings (e.g., African Burkitt's lymphoma, American Burkitt's lymphoma, infectious mononucleosis), and to determine whether differences can be discerned which might clarify the diverse biologic and pathologic expressions associated with this virus.

5. To investigate the role of other co-factors (e.g., C-type viruses) in the oncogenic expression of EBV.

6. Since the ability of a virus to remain latent may play a role in oncogenesis, factors which modify virus expression, transcription, and production will be investigated.

7. To investigate the immunostimulatory effects of EBV on human lymphocytes: The classes of immunoglobulins secreted in response to EBV stimulation; the role of T-cells in EBV stimulation (both as helpers and independent responders) and whether the response differs with T-cells obtained from seropositive vs. seronegative individuals. In addition, the manner in which different strains of EBV (i.e., transforming vs. infecting) stimulate immunoglobulin production will also be investigated.

Methods and Major Findings:

A. Studies of Immunoglobulin Synthesis by Tumor Cells

Initial studies of immunoglobulin secretion by undifferentiated lymphoma cells have been completed. Tumor cells (whether fresh, or cell lines) secrete only IgM, and, as a group, American lines secrete greater quantities of IgM than African lines. The IgM has been shown to be polyvalent and to contain J-chain. An ELISA assay for the quantitation of IgM, IgG and IgA down to about 10ng has been developed, and current studies focus on the influence of differentiation inducing agents on Ig synthesis.

The presence of monoclonal IgM in the serum of patients with undifferentiated lymphomas has been confirmed. It has been shown to correlate with stage and clinical course. IgM bands have been documented in about 60% of patients with stage C and D tumors. They disappear on treatment.

B. Development of Specific Antisera

We have been able to demonstrate the presence of antigens on American Burkitt cell lines which are not present on African cell lines by raising heteroantisera in goats and absorbing these with cord blood lymphoblastoid cell lines or African Burkitt's lymphoma cell lines. Data so far has been collected by employing indirect immunofluorescence and flow cytofluorometry. Similar antigens appear to be present on common ALL cells and American undifferentiated lymphomas. These antigens appear also to be expressed on platelets. Preliminary evidence indicates that at least some of the antigens recognized by the polyvalent antisera are not proteins. The possibility that these antigens are glycolipids is currently under investigation.

C. Characterization of Complement Receptors

We have confirmed the existence of two separate receptors for C3b utilizing blocking with highly purified C3b or C4b. One receptor will bind C4b and one will not. The latter also appears to have a lower affinity for C3b than the former. The non-C4b binding receptor is expressed preferentially by the majority of our tumor derived cell lines, the C4b binding receptor by cord blood lymphocyte-derived cell lines and normal lymphocytes. We have further confirmed that, contrary to reports in the literature, Raji cells do have specific C3b receptors and do not bind C3b via C3bi receptors.

D. Identification of Transforming Virus in the P3HR1 Cell Line

We have demonstrated that the P3HR1 cell line which is widely accepted as releasing only so-called "lytic" virus, does in fact release virions capable of transforming human cord blood lymphocytes. The use of monoclonal antibodies specific for transforming strains of EBV has confirmed this. Further studies of the relationship between the ability of EBV to induce immunoglobulin secretion and its ability to transform B lymphocytes are in progress.

E. EBV as a Polyclonal Activator of B Cells

Studies on differences between EBV strains with regard to their ability to induce immunoglobulin secretion and to transform human cord blood lymphocytes continue.

F. Expression of Some Surface Proteins Correlates with Growth Phase

Cytofluorometric studies have demonstrated that B75, IgM, HLA and DR locus antigen expression varies with cell growth phase, being maximal in log phase. This does not apply to β2 microglobulin whose expression appears to be constant throughout the growth cycle.

Significance to Biomedical Research and the Program of the Institute:

Attempts to comprehend lymphomas are doomed to failure if a purely morphological approach is used. As data accumulates, the resemblance of neoplastic lymphoid cells to their normal counterparts becomes more and more striking. Moreover, it is becoming clear that the behavior of lymphomas which have been

identified precisely, e.g., by detailed immunochemical phenotypic characterization, can be predicted on the basis of what is known of the normal cell counterpart. Further, response to therapy and the approach to therapy also differs according to cell type when accurately determined, as opposed to utilizing a purely histological approach. Thus, our objective--to comprehend at a cellular level the biology of lymphomas--should result ultimately in an improved ability to separate different pathological entities, therefore to improve our ability to analyze the results of therapeutic trials and, hopefully, to indicate possible novel approaches to treatment.

EBV is closely associated with at least three human diseases--infectious mononucleosis, African Burkitt's lymphoma, and nasopharyngeal carcinoma. As such, it represents a potential human tumor virus, and clarification of its role in the diseases with which it is associated is of paramount importance. Studies of different strains of EBV will help to determine whether or not differences in virus properties are relevant to the association of EBV with sub-clinical, benign, and malignant diseases. Studies of the modification of normal lymphocyte behavior induced by EBV, and T-cell regulation of these phenomena, will provide insights into the spectrum of EBV-associated diseases, and may also lead to the definition of specific genes related to different biological properties, e.g., transformation versus immunoglobulin production.

Proposed Course:

The studies in lymphoma biology and EBV will be continued. In particular, we would like to attempt to characterize B-cell malignancies in terms of lymphocyte differentiation antigens. This would involve the generation of monoclonal antisera with mouse myeloma hybridomas. Such reagents would potentially provide objective identification of individual lymphoid malignancies, and lead to definitive answers to such questions as the relationship between undifferentiated B-cell lymphomas of various types. Such antisera may also be useful in studying EBV and its influence on B cells, e.g., are there specific B-cell populations which EBV infects? What are the differences in transcription (in terms of immunologically identified proteins) between lymphocytes which produce Ig in response to EBV but are not transformed, and transformed cells? Further studies are planned in conjunction with the Laboratory of Clinical Investigation on the relationship between EBV and complement receptors, and the significance of this to an understanding of the nature and evolution of virus receptors--a concept of considerable importance in the case of latent viruses which can function as horizontal transmitters of genetic information. Collaborative studies to examine oncogene expression on lymphoma cells have been commenced.

Publications:

1. Benjamin, D., Magrath, I.T., Maguire, R., James, D., Todd, H.D., and Parsons, R.G.: Immunoglobulin secretion by cell lines derived from African and American undifferentiated lymphomas of Burkitt's and non-Burkitt's type. J. Immunol., in press.

2. Blatt, J., Spiegel, R.J., Papadopoulos, N.M., Lazarou, S.A., Magrath, I.T., and Poplack, D.G.: Lactic Dehydrogenase Isoenzymes in normal and malignant human lymphoid cells. Blood, in press.

3. Douglass, E.C., Magrath, I.T., and Terebelo, H.: Burkitt cell leukemia without abnormalities of chromosomes No. 8 and 14. Cancer Genet. Cytogenet. 5: 181-185, 1982.

4. Freeman, C.B., Magrath, I.T., Benjamin, D., Douglass, E.C., and Santella, M.L.: Classification of cell lines derived from undifferentiated lymphomas according to their expression of complement and EBV receptors: Implications for the relationship between African and American Burkitt's lymphoma. Clin. Immunol. Immunopath., in press.

5. Gerber, P., Ablashi, D., Magrath, I., Armstrong, G., Anderson, P., and Trach, L.: Persistence of transforming and non-transforming Epstein-Barr virus in high passages of a P3HR-1 cell line. J. Natl. Cancer Inst., in press.

6. Jaffe, E., Smith, S.A., Magrath, I.T., Freeman, C.B., Alabaster, O., and Susman, E.H.: Induction of complement receptors in human cell lines derived from undifferentiated lymphomas. Lab. Invest. 45: 295-301, 1981.

7. Magrath, I.T.: Lymphocyte differentiation pathways - an essential basis for the comprehension of lymphoid neoplasia. (Invited Editorial) J. Natl. Cancer Inst., in press.

8. Magrath, I.T., Freeman, C.B., Novikovs, L.: Induction of complement receptor expression in cell lines derived from human undifferentiated lymphomas. I. Mode of action of theophylline and inhibition by certain purines. J. Immunol. 127: 1034-1038, 1981.

9. Magrath, I.T., Freeman, C.B., Santaella, M., Gadek, J., Frank, M., Spiegel, R.J., and Novikovs, L.: Induction of complement receptor expression in cell lines derived from human undifferentiated lymphomas. II. Characterization of the induced complement receptor and demonstration of the simultaneous induction of EBV receptor. J. Immunol. 127: 1039-1043, 1981.

10. Pizzo, P.A., Chattopadhyay, S.K., Magrath, I.T., Del Giacco, E., Sherrick, D., and Gray, T.: An examination of Epstein-Barr virus and C-type proviral sequences in American and African lymphomas and derivative cell lines. Cancer Res. 41: 3165-3171, 1981.

11. Pizzo, P.A., Magrath, I.T., and Jay, G.: Characterization of the EB virus isolated from a cell line derived from a patient with American Burkitt's lymphoma. Cancer Res. 41: 3161-3164, 1981.

12. Tosato, G., Magrath, I.T., and Blaese, R.M.: T-cell mediated immunoregulation of Epstein-Barr virus-induced B-lymphocyte activation in EBV-sero-positive and EBV-sero-negative individuals. J. Immunol. 128: 575-579, 1982.

# ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

## NATIONAL CANCER INSTITUTE

## OCTOBER 1, 1981 - SEPTEMBER 30, 1982

The Radiation Oncology Branch (ROB) continues in a transition. In the last two years, the turnover has been virtually complete of all staff, physician, technician, biology, and administration.

The three main goals of the Radiation Oncology Branch 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 and clinical questions of relevance.

3. A training program in radiation therapy, equivalent in stature to the present programs in Medical, Surgical, Pediatric Oncology branches within the National Cancer Institute.

At the moment, clinical trials are progressing on a wide variety of fronts. The biology program is progressing, although it has been difficult to optimize because of problems with holding facilities for animals. The present B2 animal facility in Building 10 is not adequate for long-term experimentation, which is the main thrust of the in vivo work for the ROB. Frequency of infections that takes place in the B2 facilities has meant multiple interruptions and sacrifice of ongoing experiments that are designed to last 12-24 months. It is a major limitation of the B2 facility and it has caused the ROB to plan a major proportion of its future renovations on the B3 level to include our own animal house. Frustration with the B2 facility is, in large part, responsible for the departure of Dr. Travis from our Branch.

Concerning the training program, a provisional approval has been obtained from the AMA Residency Review Committee. This program, in conjunction with the Uniformed Services University of the Health Sciences, working through Walter Reed Army Medical Center and the National Naval Medical Center in Bethesda, consists of a three-year training program under the direction of Dr. Eli Glatstein. Essentially 18 months will be within the National Cancer Institute, one year within Walter Reed Army Medical Center and six months within Bethesda Navy Medical Center. The necessity of sharing the training experience with other Medical Centers is required by the fact that the Cancer Institute patient base is limited to specific diseases. The clinical material of the military centers complements the Cancer Institute material nicely, with major emphasis on gynecologic neoplasms, head and neck cancers, and genitourinary cancers. These are areas in which the Cancer Institute patient base is presently lacking. There are four people presently in our program.

The in vitro laboratory program includes work on low dose rate radiation and experimentation of chemotherapeutic agents and hyperthermia as well. In addition, we hope to experiment in the area of photosensitivity.

Much of the present work has been centered on human CFUc and human tumor cell lines, in collaboration with other branches.

The clinical program within the Radiation Oncology Branch is centered around combined modality studies. Most of these are collaborative with other branches. The most important of these revolves around the study of small cell carcinoma of the lung, in collaboration with the NCI-Navy Medical Oncolgy Branch. This study consists of a controlled prospective study of the value of radiation therapy to the chest in patients with limited small cell disease. Preliminary results suggest significant benefit can be achieved with combined modality treatment over what can be obtained by chemotherapy alone. Nonetheless, additional patients are required before a final conclusion can be made. In addition, a collaborative venture is ongoing for advanced oat cell carcinoma of the lung that includes consolidation of a chemotherapeutic response with short-term radiation therapy followed by marrow ablative cytotoxic treatment and bone marrow reconstitution. Another study in collaboration with the NCI-NAVY Medical Oncology Branch revolves around electron beam treatment for mycosis fungoides. This study is well underway, although it was interrupted with mechanical problems on the linear accelerator which have now been corrected. Such treatment of whole skin electron beam treatment is carried out in only a few medical centers in the United States. There are also collaborative ventures with active participation with the Surgery Branch in soft tissue sarcomas and with the Pediatric Oncology Branch in Ewing's sarcoma and rhabdomyosarcoma. In addition, a pilot study has begun with combined modality treatment in ovarian cancer, in collaboration with the Medicine Branch.

Primary ROB studies presently center around intraoperative radiation therapy. Presently, patients are operated on the 10th floor, with massive surgery carried out for carcinoma of the pancreas, stomach, or retroperitoneal sarcomas. Maximum surgery is performed and the patient is then transported through the hallways and elevators to the ROB while under general anesthesia. They are then taken to the treatment room, transposed to the treatment couch, and re-opened under anesthesia so that a large single dose of electron beam treatment can be applied intraoperatively to the tumor bed, with critical normal viscera moved out of the way. This has been done in conjunction with misonidazole, and the enthusiasm runs high for this investigational approach for these extremely difficult management problems. Randomized studies are being carried out in these diseases to delineate the benefit of this approach. At the present time, the important aspects revolve around clear delineation of the safety of this approach. Ultimately, these are seen as first steps to later studies that will incorporate chemotherapy as well.

Another series of studies within the ROB have revolved around radiosensitizing compounds. Intravenous midonidazole has been studied in some detail, and the pharmacology has been delineated. The intravenous compound was studied in carcinoma of the esophagus. Initially, patients were treated with pre-operative radiation, half the patients being randomized to receive the intravenous misonidazole with each fraction of radiation. After the first eight cases, it became apparent that the combination of pre-operative radiation followed by surgery was potentially lethal in terms of acute respiratory failure. As a consequence, the study was altered to consist exclusively of radiotherapy alone, with half the patients receiving a radiosensitizing compound. After 26 patients, the study was discontinued because the data suggested that no major benefit would accompany the use of misonidazole over that which can be obtained with radiotherapy alone.

Another major ROB study revolves around Stage I and II breast cancer, comparing radical surgery to radical irradiation with preservation of the breast. This study, in conjunction with the Surgery Branch, has accrued almost 75 patients in the first 2 1/2 years. This modest number is considered a major accomplishment, in view of the facts that no prior patient base has been recruited for such a study, and the extreme difference of the two arms makes for a difficult randomization. Nonetheless, the study appears to be accruing reasonably well, and we anticipate that the accrual will improve further with time.

A final ROB study is in progress in the treatment of locally unresectable osteogenic sarcoma and chondrosarcoma. This study deals with intravenous misonidazole, the hypoxic cell sensitizer. Despite the fact that these tumors are reported to be "radioresistant," overt tumor shrinkage has been seen in virtually all patients treated on this study, and at least one patient has been followed for three years without any growth of the tumor mass.

Under the direction of Dr. Jan van de Geijn, CT scanning has been fully incorporated into our radiotherapy treatment planning. Virtually all patients who are treated are now scanned in the treatment position, and computerized treatment plans are routinely generated, superimposed on CT sections. Dr. van de Geijn has developed a program which allows for adequate dose calculations, even when blocks are placed in the field. It is possible to also account for tissue inhomogeneity as well. The treatment plans now generated from within the ROB are extraordinarily sophisticated compared to what can be done in other medical centers with commercial units. These treatment plans are employed routinely in all curative treatments. Down time of the CT scanner itself has been the only major limitation, along with the relatively small aperture available on the CT scanner, which restricts kinds of positions that can be scanned. Fairly soon, we will be able to scan in other planes as well, not just simply cross-sectional. We have been able to interface our treatment planning system with ultrasound as well, which has certain advantages, particularly when we are treating the breast.

A major portion of the time has been devoted to plans for the new radiation therapy treatment facility. Extraordinarily long delays in reconstruction, along with what was felt to be an exorbitant cost estimate for completing the facility, led to the discharge of the contractor, who was more than 24 months behind on an 18-month contract. A new contractor has almost completed the building, and two units are in the process of being installed at the time of this dictation. As soon as the facility is completed, the branch will move into its new home, after which full renovation of the present B3 facility will be made to convert it into radiation biology laboratories. Such laboratory space will consist of two major floors; one for radiologic physics and tissue culture radiobiology, and the other committed to an in vivo radiobiology program. In addition, the fourth bay within the new department will be dedicated to an intra-operative facility. At the present time, the intra-operative program can only be carried out once a week because of the disruption that it causes within the department. With one bay fully dedicated to intra-operative radiation in terms of operating space and radiation device, such procedures will be performed on a more frequent basis. The major advantages of the intra-operative program appear to be the precise localization of tumor and the ability to eliminate critical normal tissues, or at least protect them, from typical external

radiation fields. In addition, it offers a unique opportunity in which to combine radiosensitizing drugs and even hyperthermia in the treatment of intra-abdominal neoplasms. It will probably prove to be an ideal approach for retroperitoneal nodes and pelvic neoplasms. One new area of investigation has been begun. A pilot study, in conjunction with the Medicine Branch, has begun for advanced cervix cancer, utilizing radiation therapy and adjuvant chemotherapy. Ultimately this should be a good group of patients in which to study hyperthermia, as soon as a new cervix applicator with a built-in microwave source has arrived.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 00650-27 RO |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Service Radiation Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Allen S. Lichter, M.D. | Medical Officer | ROB | NCI |
| Others: | Tim Kinsella, M.D. | Sr. Investigator | ROB | NCI |
| | Marilyn Glover, R.N. | Clinical Nurse | ROB | NCI |
| | Andrea Zola, R.T. | Medical Radiation Technician | ROB | NCI |
| | Joy Greig, R.T. | " " " | | |
| | Barbara Kelly, R.T. | " " " | | |
| | Kathy Yeakle, R.T. | " " " | | |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose 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 Oncology Branch, NCI/Navy Medical Oncology Branch, Neurosurgical Service, Endocrine Service, and other Federal Hospitals in the area where technical expertise and technical equipment dictate a need for such consultation.

Project Description

Objective: To provide consultation and radiation treatment for Clinical Center patients.

Methods

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 approximately 300 telephone consultations 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 - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 00684-27 R0 |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Nonclinical Irradiation Services

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | J. van de Geijn | Expert | ROB | NCI |
| Others: | F. Harrington | Engin. Tec. | ROB | NCI |
| | B. A. Fraass | Staff Fellow | ROB | NCI |
| | R. W. Miller | Health Physicist | ROB | NCI |
| | J. E. Doolittle | Electronic Tec. | ROB | NCI |

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The physics section provides radiation physics services, equipment, and advice on experiments involving radiobiology. Cells, tissue cultures, mice, rats, and dogs were irradiated for radiobiology experiments.

Project Description

Objective: To provide radiation physics expertise and equipment to researchers involved with radiobiological projects.

Methods Employed

Dosimetric investigations have been made to assist radiobiologists in irradiating cells, tissue cultures, mice, rats, and dogs, using both linear accelerators and the 250 kVp X-ray unit. Many devices have been fabricated to hold animals in specific positions relative to the radiation beams while shielding certain critical organs.

Major findings

Cells, tissue cultures, mice and rats, were irradiated.

Significance to Biomedical Research and the Program of the Institute

Radiation physics support is essential to the Radiobiology Section, Radiation Oncology Branch.

Proposed Course

To be continued. Dosimetry in these difficult cases will be improved. Continuing technical support will be provided.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 00998-04 RO |
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PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Radiation Sensitizers in Carcinoma of the Esophagus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | J. Schwade, M.D. | Senior Investigator | ROB | NCI |
| Other: | E. Glatstein, M.D. | Chief | ROB | NCI |

COOPERATING UNITS (if any)

Surgery Branch, COP, DCT, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study radiation sensitizing compounds and their ability to increase the effect of radiation in sterilizing neoplastic cells. The plan is to take patients who have carcinoma of the esophagus that appears to be clinically confined to the mediastinum and give all patients irradiation. Half the patients will receive misonidazole, a hypoxic cell sensitizer, administered by an intravenous method. Following completion of the radiotherapy, survival, freedom from lapse, and tumor will all be evaluated. In addition, these patients, who are frequently cachectic, will serve as the basis for controlled studies of total parenteral nutrition. In addition, this study utilizes an innovative fractionation schedule for the irradiation (400 rad twice weekly, rather than 2000 rads, 2 days per week).

Project Description

Objectives: By assessing the survival, freedom from relapse, and tumor response, we hope to be able to determine whether or not misonidazole is effective in augmenting radiation effects in patients with carcinoma of the esophagus. If this compound does appear to increase radiation effectiveness, then further work in radiosensitizing compounds is clearly warranted.

Methods Employed

Patients with previously untreated carcinoma of the esophagus confined clinically to the mediastinum will be accessioned and treated with irradiation. Half the patients will receive, on a randomized basis, intravenously administered misonidazole with each fraction of irradiation.

Major Findings

Twenty-six patients completed this study. There was no improvement in complete response rate or local control or long-term survival in patients who received the radiosensitizer. Indeed, the only two survivors that we have after three years are on the control arm. The radiation fractionations appear to be excellent from the standpoint of palliation, but little evidence of radiosensitization was seen.

Significance to Biomedical Research and the Program of the Institute

Radiation sensitizing drugs may represent an avenue to increase effectiveness of therapy by augmenting the effects of an existing modality, i.e., radiation. This study represents the only controlled randomized study to assess the efficacy of this compound in carcinoma of the esophagus. Misonidazole showed no suggestion of benefit, and thus the study was terminated.

Proposed Course - Study terminated.

Publication:

Dunnick, N.R., Schwade, J.G., Martin, S.E., Johnston, M.R., and Glatstein, E.: Interstitial pulmonary infiltrate following combined therapy for esophageal carcinoma. Chest 81: 453-456, 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06310-03 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Surgery versus Radiation Therapy in Treatment of Primary Breast Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Allen S. Lichter, M.D. Medical Officer ROB NCI

Others: Staff within the Radiation Oncology Branch NCI

COOPERATING UNITS (if any)
Surgery Branch, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5 | 2 | 3 |

CHECK APPROPRIATE BOX(ES)

X ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study whether techniques of treatment that preserve the breast (lumpectomy followed by radiation therapy) provide equal survival opportunity when compared to women treated with standard surgical techniques (mastectomy) for primary breast cancer. After a work-up confirms localized disease, the patients are randomized to treatment with either mastectomy or lumpectomy plus radiation therapy. Both groups have axillary node dissections and are treated with chemotherapy should the nodes be positive.

Project Description

Objectives: Survival and recurrence figures are comparable for the two treatments, it should be far more acceptable for women to be treated with less than radical surgical procedures for localized breast cancer. The cosmetic result of localized treatment will be carefully evaluated. The psychological, sexual, and sociological impact of mastectomy versus lumpectomy will be noted. The ability to combine radiation therapy with aggressive chemotherapy in node positive patients will also be assessed.

Methods Employed

Patients with previously untreated carcinoma of the breast clinically and radiographically confined to the breast and axillary lymph nodes will be accessioned into the study. They will be randomized to have treatment with lumpectomy and radiation therapy versus mastectomy. Patients with positive axillary lymph nodes will receive chemotherapy.

Major Findings

This study has been active for 33 months. Currently 81 patients are enrolled and it is far too early to assess results.

Proposed Course - Patient accrual will continue.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER ZO1 CM 06313-03 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Dose to Lung and Opposite Breast vs. Technique for Primary Breast Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | B. A. Fraass | Staff Fellow | ROB | NCI |
| Others: | A. S. Lichter | Senior Investigator | ROB | NCI |
| | H. A. Fredrickson | Comp. Systems Analyst | ROB | NCI |
| | J. van de Geijn | Expert | ROB | NCI |

COOPERATING UNITS (if any)
Computer Systems Laboratory DCRT, NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .1 | .1 | 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Treatment planning techniques for primary breast irradiation are investigated to optimize dose to areas at risk while minimizing dose to critical stuctures. When the high-dose volume is increased to include the internal mammary chain (IMC), dose to lung and opposite breast increase. This effect has been investigated extensively with both treatment planning and dose measurements.

Project Description

Objective: To quantify the dose to critical structures such as lung and opposite breast, as a function of treatment techniques which include the internal mammary chain (IMC) within the high-dose volume.

Methods Employed

Dose distributions for many treatment techniques were simulated using CT scans from 15 patients. Dose to opposite breast was also calculated. Extensive film, thermoluminescent dosimetry (TLD), and ion chamber measurements have been made in water and polystyrene phantoms. These measurements have been used to verify the computer results, and have been related to the surface TLD measurements made on patients under treatment.

Major Findings

Typical radiographic verification and simulation films are misleading with respect to the volume of lung irradiated. No single technique is optimal for all patients. Dose to the opposite breast has been quantified.

Significance to Biomedical Research and the Program of the Institute

An improvement in therapeutic ratio (dose to area at risk/dose to normal tissue) is possible if the treatment technique is determined individually for each patient.

Proposed Course

To investigate tehniques for modifying the dose to the opposite breast, and to investigate the feasibility of the combined photon and electron treatment technique.

Publication

Lichter, A. S., Fraass, B. A., Fredrickson, H. A., and Van de Geijn, J.: The role of computerized tomography in treatment planning of primary breast cancer. In Ling, C. (Ed.): Proceedings of the Conference on Radiotherapy and Computerized Tomography. Arlington, Virginia, Raven Press, in press, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06317-03 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Toxicologic and Pharmacokinetic Studies of Misonidazole

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | S. Hancock, M.D. | Senior Investigator | ROB | NCI |
| Other: | E. Glatstein, M.D. | Chief | ROB | NCI |
| | J. Strong, Ph.D. | Senior Staff Fellow | DTP | NCI |

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to develop more effective radiation sensitizers, as well as more intelligently utilize those currently available, information must be gained regarding the toxicity of current compounds as well as their pharmacology. With regards to the latter point, studies regarding the modification of the pharmacology may lead to the ability to use the currently available compounds, most notably misonidaozle, with less toxicity.

Project Description

Objectives: Pharmacokinetic parameters, in plasma, normal tissues and tumors are to be studied.

Methods Employed

Patients have received twice weekly doses of misonidazole intravenously noted to determine base line pharmacokinetic parameters and toxicity.

Major Findings

Elucidation of the pharmacokinetic parameters of misonidazole in the intravenous form has been determined, as well as maximum tolerated dose in a twice weekly fractionation schedule (1.5 gm/$m^2$, 2X/wk X 5 wks). In addition, analysis of data has shown that the risk of neurotoxicity with this compound relates directly to area under the pharmacokinetic curve, rather than to any other parameter.

Significance to Biomedical Research and the Program of the Institute

Radiosensitizing drugs may allow more effective use of an existing modality of treatment, radiation therapy, by augmenting the effects of radiation killing in a select population of tumor cells, while having little effect on normal tissue. The Radiation Oncology Branch is the only group in the country currently investigating misonidazole in an intravenous formulation.

Proposed Course

The esophageal carcinoma study has been terminated because of lack of suggestion of benefit. For locally unresectable sarcomas, this study will continue. In addition, IV misonidazole is used routinely in the intra-operative program, and will continue for the time being.

Publications

1. Strong, J.M., Schwade, J.G., Shoemaker, D., and Gangji, D.: Misonidazole dose and tumor level relationship: effects of individual variation on rate of misonidazole metabolism and absorption from the gastrointestinal tract. Clinical Trials 1981, in press.

2. Schwade, J.G., Strong, J.M., and Gangji, D.: IV misonidazole (NSC 261037)--report of initial clinical experience. Cancer Clinical Trials 44: 33-39, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06318-03 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Locally Unresectable Osteogenic Sarcomas and Chondrosarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Steven Hancock, M.D. | Senior Investigator | ROB | NCI |
| Other: | Eli Glatstein, M.D. | Chief | ROB | NCI |

COOPERATING UNITS (if any)
Surgery and Pediatric Oncology Branches, DCT, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | 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 (200 words or less - underline keywords)

The purpose of this project is to study the hypoxic cell radiosensitizer misonidazole in conjunction with radiation therapy in the treatment of locally unresectable osteogenic sarcoma and chondrosarcoma. Patients who have a local neoplasm for which no radical surgery can be successfully undertaken will be treated with fractionation radiation therapy in conjunction with intravenously administered misonidazole. Because of the small number of patients who are appropriate for such a study, this will be a one-armed study. Following completion of radiation therapy, combination chemotherapy will be administered to patients with osteogenic sarcoma with high grade neoplasms. Following completion of all treatment, survival, freedom from relapse, and complications of treatment will be carefully assessed.

Project Description

Objectives: By assessing the survival, freedom from relapse, and complications of radiation therapy with intravenously administered misonidazole, we hope to be able to determine whether or not such treatment is appropriate for these neoplasms, which have here before been considered "radioresistant". Should the study prove that such treatment is effective, then such treatment could be contemplated for other osteogenic sarcoma patients.

Methods Employed

Patients with locally unresectable osteogenic sarcoma or chondrosarcoma, without overt metastatic disease, will be treated with radiation therapy in conjunction with intravenously administered misonidazole.

Major Findings

Ten patients have been entered on the study. Regression has been seen in virtually all of these putatively radioresistant neoplasms. Local regrowth of tumor has not been seen in several. The bony matrix increases in density, and one patient appears to be free of all disease for three years.

Significance to Biomedical Research and the Program of the Institute

Radiation sensitizing compounds represent a new avenue of investigation. These compounds are electron affinic and appear to mimic the effects of oxygen, and therefore appear to have the specific ability to make hypoxic cells within a tumor more responsive to radiation killing. Thus, these compounds take a known effect (e.g. radiation killing) and appear to be able to magnify it. If these compounds prove to be effective, then the need to invest large sums of money in research directions such as high L.E.T. radiation may be circumvented.

Proposed Course - Patient accrual is underway.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06319-03 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Use of prematurely condensed chromosomes (PCC) in biological dosimetry of ionizing radiation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | James B. Mitchell, Ph.D. | Expert | ROB | NCI |
| Other: | Scott McPherson | Biologist | ROB | NCI |
| | Janet Gross | Biologist | ROB | NCI |

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiobiology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | 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 (200 words or less - underline keywords)

The purpose of this project is to determine if the use of premature chromosome condensation (PCC) technique will improve the resolution of the lymphocyte biological dosimeter system for low total doses of radiation (<10 rad). With the PCC technique, chromosomal damage (gross breaks in chromosomes) of interphase cells can be studied immediately following radiation exposure. Assays will be made before the cells have had time to repair many of the initial breaks, thereby increasing the number of breaks counted as opposed to counting aberrations conventionally 24-48 hours after exposure in metaphase I and II.

Project Description

Objective: By scoring radiation damage in chromosomes (gross breaks) immediately following the exposure, we will construct radiation dose response curves which may provide greater resolution in the low dose region (1-10 rad). If this technique does provide greater resolution to the lymphocyte biological dosimeter system, then the determination of small radiation doses to accidentally exposed persons could be done with a considerable amount of confidence.

Methods Employed

Blood lymphocytes and bone marrow will be irradiated with graded doses of gamma photons and fused immediately with mitotic inducer cells. Slight modifications of the Rao and Johnson PCC technique will be used. Gross breaks in whole G1 PCC's chromosomes will be scored.

Major Findings

The study is in preliminary stages and the results are presently not available for assessment.

Significance to Biomedical Research and the Program of the Institute

Determination of low doses of radiation received by persons accidently exposed to radiation has been an issue of concern over the past 30 years. More precise methods of accessing low doses of radiation would be of value not only for accidental diagnostic exposure but also for environmental exposures to the general population.

In addition, these studies should provide better understanding as to the nature of chromosome breakage and repair.

Proposed Course - Project is currently underway.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06320-03 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Response of mammalian cells exposed to chemotherapeutic drugs and continuous radiation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | James B. Mitchell, Ph.D. | Expert | ROB | NCI |
| | Angelo Russo, M.D., Ph.D. | Clinical Assoc. | ROB | NCI |
| Other: | Scott McPherson | Biologist | ROB | NCI |
| | Janet Gross | Biologist | ROB | NCI |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiobiology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cell killing and cell cycle kinetics will be studied for combinations of various chemotherapeutic drugs and continuous low dose rate radiation (5-300 rad/h). In addition, the morphological changes of cells exposed to these combinations of treatment will be documented by time lapse photography.

Project Description

Objective: The objective of this project is to determine if there are combinations of continuous low dose rate radiation and chemotherapeutic drugs that will provide more cell-killing to tumor cells (in vitro tumor cell lines) than to normal tissue cell lines.

Methods Employed

In vitro cell cultures will be exposed to the various agents mentioned above and assayed for cellular reproductive integrity using conventional tissue culture techniques.

Major Findings

The project is in the early stages of development. The cobalt 60 unit for continuous irradiation was installed in April 1981. As soon as dosimetry has been completed, this project will start.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of interactions between radiation and drugs, which might be of value to clinical radiotherapy.

Proposed Course

Dose-response curves have been generated for a variety of chemotherapy drugs, the next step will now be to combine the drugs with low dose rate radiation.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06321-03 RO |
|---|---|---|

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Dose-rate effects on aerated and hypoxic cells grown in culture.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | James B. Mitchell, Ph.D. | Expert | ROB | NCI |
| | Angelo Russo M.D., Ph.D. | Clinical Assoc. | ROB | NCI |
| Others: | Scott McPherson | Biologist | ROB | NCI |
| | Janet Gross | Biologist | ROB | NCI |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the effects of ionizing radiation with respect to cell killing and cell cycle perturbations to cells grown in culture. Particular emphasis will be placed on studying the response of cells to varying dose rates of radiation under both aerated and hypoxic culture conditions. Both continuous and fractionated irradiation schedules will be studied.

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 clincal radiotherapy.

Methods Employed

In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to various dose rates of radiation either under aerated or hypoxic conditions. Oxygen enhancement ratios (OER) will be determined.

Major Findings

There appears to be a dependence on cellular glutathione for the hypoxic radiation response. We are depleting cells of glutathione (GSH) by agents which block GSH synthesis and sensitizing these cells under hypoxic conditions to ionizing radiation.

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 levels of GSH and X-rays at low dose-rate.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06328-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Field Configuration in Definitive Radiotherapy of the Intact Breast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | B. A. Fraass | Staff Fellow | ROB | NCI |
| Others: | A. S. Lichter | Senior Investigator | ROB | NCI |
| | J. van de Geijn | Expert | ROB | NCI |
| | F. Harrington | Engin. Tec. | ROB | NCI |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .15 | .05 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work has resulted in the development and implementation of a new irradiation technique to produce in a more reliable fashion a uniform dose distribution in the breast tissue and the supraclavicular area. The necessary numerical data for routine application are obtained by using a specially developed computer program.

Project Description

Objective: To develop and implement a field arrangement for treatment of cancer of the breast. It is necessary to achieve a uniform dose across the entire treatment volume, while minimizing the dose to adjacent critical structures.

Methods Employed

Extensive experimental work has demonstrated that a new method can be applied to generate a uniform matching of the supraclavicular field with the tangential breast fields.

Major Findings

This method is clinically applicable.

Significance to Biomedical Research and the Program of the Institute

This work makes adequate treatment of all breast tissue routinely possible.

Proposed Course

Continued improvement.

Publication

Lichter, A. S., Fraass, B. A., Van de Geijn, J., and Padikal, T. N.: An improved technique for primary breast irradiation. Int. J. Rad. Onc. Biol. Phys., in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06329-02 RO |
|---|---|---|

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Radiation Physics Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | J. van de Geijn | Expert | ROB | NCI |
| Others: | F. Harrington | Engin. Tech. | ROB | NCI |
| | B. A. Fraass | Staff Fellow | ROB | NCI |
| | R. W. Miller | Health Physicist | ROB | NCI |
| | K. Yeakel | Ther. Rad. Tec. (Dos.) | ROB | NCI |
| | J. E. Doolittle | Electronic Tec. | ROB | NCI |
| | J. Caulkins | Health Tec. | ROB | NCI |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.0 | 2.0 | 3.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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.

## Project Description

Objective: To ensure high quality physics support for radiotherapy.

## Methods Employed

A new, efficiently graded system has been developed and implemented for monitoring the performance of the two linear accelerators, the simulator and the CT scanner. Special mechanical supports and measuring devices were developed to quantify the position of patients and to improve the reproducibility of daily patient set-ups. The data acquisition for treatment planning has been simplified and improved. A new method for computer-assisted treatment planning has been introduced. Considerable efforts have been invested in the dosimetry of intra-operative, total-body and total-skin radiotherapy.

## Major Findings

The introduction of beam monitoring jigs enables daily monitoring of output, beam flatness, symmetry and alignment of light field and X-ray fields for both linear accelerators. The method allows simple documentation of performance. The dosimetry of photon beam total-body irradiation as well as that of total-skin electron beam irradiation for mycosis fungoides requires further attention. Much attention had to be spent on total body irradiation and mycosis fungoides dosimetry.

The most important contribution in computer-assisted treatment planning is the availability of routine interactive optimization and routine multi-slice imaging of dose distributions superimposed on CT scans. An important improvement is the capability to image the effects of irregular shielding blocks which is of essential interest in the treatment of soft-tissue sarcomas and cancers of the esophagus.

## Significance to Biomedical Research and the Program of the Institute

The improvements in quality assurance, patient positioning and treatment planning are essential as a basis for optimal patient treatment and for meaningful evaluation of treatment protocol studies.

## Proposed Course

Continuation of further development of means and methods to improve the physical and technological basis of radiotherapy.

## Publications

Van de Geijn, J., Harrington, F., Fraass, B. A., and Glatstein, E.: A graticule for evaluation of megavolt X-ray port films. J. Rad. Onc. Biol. Phys., in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06330-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Extension of a 3-D Dose Field Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PT: | Johannes van de Geijn | Expert | ROB | NCI |
| | Cheng Po Cheng | Visiting Student | ROB | NCI |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.5 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The capability to calculate the distribution of absorbed dose produced by photon beams and electron beams of the most general characteristics is of vital importance in radiotherapy. Conceptually, this model takes as a basis the empirical distributions along three mutually perpendicular reference lines in a "master field" and mathematical expressions to describe the effect of variation effects of field size, depth and focal distance. This concept is applied to the beam-modifying devices as well. The approach is attractive from a theoretical as well as a practical point of view. The current investigations concern the generalization for irregular fields modified by irregular blocks for photon beams and electron beams including the influence of inhomogeneities.

## Project Description

Objective: 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.

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 both photons, electrons and neutrons. Mathematical representations for these variations have been established.

3) Over the present reporting period, special attention has been paid to verification of the model for the local radiation machines and to extension of the model to irregular fields modified by irregular blocks.

## Major Findings

It has been found that the concept applies well to the local facilities for regular rectangular beams including the use of wedges.

It has been established that the concept is applicable to irregular fields as well. Preliminary results for electron beams are most promising. The validity for neutron beams has been confirmed by investigators at Fermilab.

Most of these results have been incorporated in a clinical treatment planning system.

## Significance to Biomedical Research and 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.

Publications

1. Van de Geijn, J., and Po Cheng, C.: The net fractional depth dose: concept, physical properties and computational advantages. Paper Exhibit #R8, AAMP Annual Congress, p. 549, 1981.

2. Van de Geijn, J., and Po Cheng, C.: Computation of Multi-Slice Dose Distributions in Irregular Fields Modified by Irregular Blocks. Poster, AAPM Annual Congress. Medical Physics, 1981.

3. Van de Geijn, J.: The use of the projective beam model for electron beams. In Paliwal, B. (Ed.): Proceedings of the Electron Dosimetry and ARC Therapy Symposium. Wisconsin, American Institute of Physics, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER ZO1 CM 06331-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Computer-assisted 3-D Radiation Treatment Planning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Johannes van de Geijn | Expert | ROB | NCI |
| Other: | Hal A. Fredrickson | Comp. Systems Analyst | DCRT | NCI |
| | Daniel Syed | Head, CSL | ROB | NCI |
| | B. A. Fraass | Staff Fellow | ROB | NCI |
| | R. W. Miller | Health Physicist | ROB | NCI |

COOPERATING UNITS (if any)

Computer Systems Lab

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.8 | 0.2 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this 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 has been completed for the photon and electron fields available from the local 6 MV and 12 MV linear accelerators. The current capabilities include interactive simulation of most irradiation techniques, including the effect of most beam modifying devices. The transverse contours are overlaid on corresponding CT scans.

## Project Description

Objectives: To develop and implement a generalized system for computer-assisted radiation treatment simulation.

## Methods Employed

The dose field mode, developed elsewhere by the present principle investigator, was further developed and experimentally tested for the local radiation facilities. The theoretical model was extended to cover irregularly-shaped beams as well as irregularly-shaped shielding blocks.

The associated computer program for the local PDP-11/70 system, was further extended with expert assistance from the Computer Systems Laboratory. A facility has been developed which enables the computation and display of dose distributions in planes perpendicular to the respective beam axes. The capabilities of the graphical input system, the use of CT images in addition to or instead of mechanically-obtained patient contours, the interactive system for the variation of input parameters, and a DEANZA color display system have been further expanded.

## Major Findings

The system, although continuing to be further expanded, is in routine use for clinical treatment planning. In comparison to other existing systems, it offers high speed computation and display of complete dose distributions in multiple slices, superimposed on CT images, including effects of wedge filters, irregular shielding blocks and diaphragm rotation. Several modes of display are available. The newly developed Beam's Eye View capability is being implemented for routine use and promises to be very useful.

## 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.

## Proposed Course

Implementation of the Beam's Eye View option for regular and irregular electron fields.

Establishment of a "Slave Monitor System" to enable the display and limited modification of treatment plans during the daily Patient Conferences.

Extension of the capabilities to compute and display dose distributions in sagittal coronal and beams eye view sections of the patient, on an interactive basis.

Publications

Van de Geijn, J., Chien, J-chu, Po Cheng, C., and Fredrickson, H.: A Unified 3-D Beam Model for External Beam Dose Distributions. In Umegaki, Yoichiro (Ed.): Proceedings of the VIII Internal Conf. on Computers in Radiotherapy, Tokyo, 1980.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06332-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Use of a Match-line Wedge for Radiation Field Matching

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | B. A. Fraass | Staff Fellow | ROB | NCI |
| Others: | J. van de Geijn | Expert | ROB | NCI |
| | Eli Glatstein | Chief | ROB | NCI |
| | F. Harrington | Engin. Tec. | ROB | NCI |

COOPERATING UNITS (if any)

none

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .15 | .05 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop a clinically useful method of matching adjoining megavoltage radiation fields so that the dose distribution through the match region is uniform. A "match-line wedge" has been developed which satisfies the above requirement. Simplicity of use will assure that the wedge will be effective clinically.

## Project Description

Objective: To find a means of modifying the edges of adjoining radiation fields so that the dose distribution throughout the match region is uniform.

## Method Employed

A match-line wedge has been designed so that a wide pseudo-penumbra is created when the wedge is placed in the edge of the radiation beam. This wide pseudo-penumbra makes the dose distribution in the match region less sensitive to set-up errors and more uniform than is possible with normal matching methods when adjacent fields are matched. The design, mounting, simulation, set-up, and treatment techniques have been developed so that use of the wedge is safe, useful, and simple. The dose distributions which result from variation of different parameters in the system have been studied in detail.

## Major Findings

Use of the match-line wedge results in uniform dose distributions in the match region between adjacent radiation fields.

## Significance to Biomedical Research and the Program of the Institute

Use of this device improves the uniformity of dose received by patients who are treated with matching fields, thereby improving the accuracy of treatment.

## Proposed Course

Implementation of the match-line wedge will continue, until it is in routine clinical use. Use of the wedge with a wider range of treatment techniques will be explored.

## Publications

1. Fraass, B. A., and Tepper, J. F.: Clinical use of a match-line wedge for adjacent megavoltage radiation field matching. Med. Phys. 8:546, 1981.

2. Fraass, B. A., Tepper, J.E., Glatstein, E., and Van de Geijn, J.: Clinical use of a match-line wedge for adjacent megavoltage radiation field matching. Int. J. Rad. Onc. Biol. Phys., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06333-02 RO

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Dosimetry of Total Skin Electron Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | B. A. Fraass | Staff Fellow | ROB | NCI |
| Others: | R. W. Miller | Health Physicist | ROB | NCI |
| | K. Yeakel | Rad. Ther. Tec. (Dos.) | ROB | NCI |
| | J. Caulkins | Health Tec. | ROB | NCI |
| | Eli Glatstein | Chief | ROB | NCI |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .5 | .4 | .1 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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.

Project Description

Objective: To quantify the variation of dose to all parts of the body for patients receiving whole-skin irradiation.

Methods Employed

The dosimetry system of the MeV XII accelerator has been substantially improved, allowing more precise definition of the total skin dose given. The absolute dose has been calibrated using various ionization chambers and thermoluminescent dosimeters (TLD). Extensive TLD measurements have been made on five patients. These measurements have made possible the mapping of the dose distribution over the whole body, and also have shown the daily variations and patient-to-patient variations which are possible with this treatment technique.

Major Findings

The dose to the skin is fairly uniform over the trunk, but the distribution of dose to legs, arms, and head is significantly different. Daily and patient-to-patient variations in dose are not overly significant.

Significance to Biomedical Research and the Program of the Institute

This work makes adequate treatment for mycosis fungoides possible with the whole-skin irradiation technique.

Proposed Course

More work toward improving the absolute dosimetry is contemplated. Also necessary is the improvement of methods to assure uniform dose to the whole skin.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06334-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Dose to Gonads from Radiation Treatment for Lymphomas and Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | B. A. Fraass | Staff Fellow | ROB | NCI |
| Other: | T. Kinsella | Medical Officer | ROB | NCI |
| | K. Yeakel | Ther. Rad. Tec. (Dos.) | ROB | NCI |
| | R. Shering | Medical Officer | Endocrine Branch | NCI |
| | E. Shapiro | Clinical Associate | Surgery Branch | |
| | J. Caulkins | Health Tec. | ROB | NCI |

COOPERATING UNITS (if any)
Endocrine Branch, NCI
Surgery Branch, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .25 | .2 | .05 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Doses to gonads have been measured on patients who are irradiated as part of their treatment for lymphomas or sarcomas. Ion chamber and thermoluminescent dosimetry (TLD) measurements have been made to verify the measurements on patients.

Project Description

Objective: To accurately determine the gonadal doses received by lymphoma and sarcoma patients who are treated with radiation.

Methods Employed

Thermoluminescent dosimetry (TLD) measurements have been made to determine the dose to testes and ovaries of patients treated with mantle, para-aortic, pelvic and leg radiation fields. Extensive ion chamber and TLD measurements have been made to verify the validity of the TLD measurements on patients.

Major Findings

Gonadal doses can now be calculated retrospectively, as long as there is adequate information about radiation field and patient geometry. A gonadal shield useful for the above categories of patients has been developed.

Significance to Biomedical Research and the Program of the Institute

Gonadal doses will be correlated with fertility and hormonal function tests obtained by the Surgery and Endocrine Branches. The results will quantify the effects of radiation on fertility. Shielding which reduces the complications of these radiation treatments is clearly of major importance to the patients.

Proposed Course

The patient TLD and verification measurements will continue. Doses received by previously-treated patients will be calculated. Fertility and hormonal function test results will be correlated with gonadal doses. Improvement of the gonadal shield will continue.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06337-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Real-Time Radiotherapy Treatment Monitor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | B. A. Fraass | Staff Fellow | ROB | NCI |
| Other: | J. F. Doolittle | Electronic Tec. | ROB | NCI |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .05 | .02 | .03 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to develop a real-time monitor for radiation treatments. Although routine quality assurance is the immediate aim, continued development will make feasible many projects which rely on real-time patient dose monitoring.

Project Description

Objective: To design and develop a real-time monitoring device for use with radiotherapy.

Methods Employed

A two-dimensional array of radiation-sensing diodes is used to monitor the radiation which is transmitted through the patient during a treatment. From knowledge of the transmitted intensity, patient dose information is obtained. A microcomputer - based system is used to accumulate and analyze the data from the diode array.

Major Findings

Initial hardware interfacing, computer programming, and diode selection have been accomplished. The system has been tested and found to be promising. Optimization of the many factors affecting system performance is now underway.

Significance to Biomedical Research and the Program of the Institute

This project is expected to improve the quality of radiotherapy in the Radiation Oncology Branch. Further development will lead to innovative and more precise types of treatments.

Proposed Course

To be continued. The present data acquisition system will be developed and refined. Design of the system to be used for automatic monitoring and recording of patient treatments will proceed. Investigation into the use of the diode array system for beam symmetry, quality, and calibration checks, compensating filter design, dynamic radiotherapy treatments, quality control, and real-time treatment analysis will continue.

Publication - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06343-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to Septmeber 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of the Radiosensitizer Bromodeoxyuridine (BUdR) NSC 38297

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Timothy Kinsella, M.D. | Senior Investigator | ROB | NCI |
| Others: | E. Glatstein, M.D. | Chief | ROB | NCI |
| | C. Myers, M.D. | Chief, Clinical Pharmacology Branch | | NCI |
| | P. Kornblith, M.D. | Chief, Surgical Neurology Branch | | NINCDS |

COOPERATING UNITS (if any)
Surgical Neurology Branch NINCDS
Clinical Pharmacology Branch, COP, DCT, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Bromodeoxyuridine is a radiation sensitizing drug which sensitizes rapidly dividing cells. Thymidine is replaced by the uridinedeoxyriboside compound predisposing DNA to single strand breaks, as well as inhibiting single strand repair. BUdR has been used in the past in head and neck lesions, osteosarcoma, and particulary in glioblastoma and other brain tumors, but used to be felt to require intra-arterial delivery to be effective.

Project Description

Objective: To assess the pharmacokinetics and toxicity with BUdR.

Methods Employed

Patients with brain metastases or primary malignant gliomas or glioblastoma are given BUdR intravenously for twelve hours every 24 hours. Patients with brain metastases are treated with 2000 rads in one week with a two week rest, followed by an additional 2000 rads in one week. BUdR is given on each day of treatment. Patients with malignant gliomas and glioblastoma are given BUdR for the first and last ten treatments of their 6000 rad/33 fraction schedule.

Major Findings

In the 11 patients treated thus far, intra-arterial levels of BUdR have been achieved that are comparable to those obtained by intra-arterial infusion. Toxicity has been seen in terms of skin reactions and in terms of fall of blood counts.

Significance to Biomedical Research and the Program of the Institute

The Radiation Oncology Branch has been deeply involved in the evaluation of new radiosensitizing compounds. BUdR represents a mode of radiosenitization different than the nitroimidazoles, a group of radiosensitizing compounds which sensitizes hypoxic cells. BUdR may be particulary useful in tumors which are dividing much more rapidly than the surrounding normal tissue, such as brain tumors and tumors in the lung.

Proposed Course - Patient accrual continues

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06344-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of Radiosensitizer Desmethylmisonidazole (NSC 261036)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Steven Hancock, M.D. | Senior Investigator | ROB | NCI |
| Others: | Eli Glatstein, M.D. | Chief | ROB | NCI |
| | J. Strong, Ph.D. | Senior Staff Fellow | DTP | NCI |

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacy, DTP, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiation Therapy

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3 | 2 | 1 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Radiation Oncology Branch is involved in the evaluation of numerous sensitizing compounds. Misonidazole has been evaluated by us in an intravenous form, and desmethylmisonidazole represents an improved compound. This compound is more hydrophilic, and has a shorter half-life, and will hopefully result in a significant decrease in neurotoxicity, the dose limiting toxicity seen with misonidazole.

Project Description

Objective: To assess the pharmacokinetics and toxicity of desmethylmisonidazole.

Methods Employed

Patients are given escalating doses of desmethylmisonidazole on a schedule prescribed in accordance with a national cooperative study being conducted by the Radiation Therapy Oncology Group.

Major Findings

Pharmacokinetics have been studied on ten patients from the Radiation Oncology Branch. Work on this study stopped when Dr. Schwade left, but it is about to re-open with the coming of Dr. Hancock.

Significance to Biomedical Research and the Program of the Institute

The Radiation Oncology Branch has been involved in the testing of radiosensitizing drugs, and this is a continuation of this work.

Proposed Course - Patient accrual continues.

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06345-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of radiosensitizer, Misonidazole (NSC 261037)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | E. Glatstein, M.D. | Chief | ROB | NCI |
| Others: | J. Strong, Ph.D. | Senior Staff Fellow | DTP | NCI |

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacy, DTP, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiation Therapy

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3 | 2 | 1 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pharmacokinetic and toxicity studies have been performed on patients undergoing treatments with misonidazole, a nitroimidazole radiosensitizing agent. The studies have allowed better delineation of toxicity and pharmacology of these compounds, and was obtainable with the previously evaluated oral compounds.

Project Description

Objective: Assessment of the pharmacokinetics and toxicity of misonidazole.

Methods Employed

Patients receiving radiation therapy were treated with misonidazole in escalating doses twice weekly for five weeks.

Major Findings

Toxicity of misonidazole was found to correlate with area under the curve of concentration versus time. A dose of 1.5 gm/$m^2$ twice a week for five weeks was found to be a well-tolerated dose with only minimal peripheral neuropathy.

Significance to Biomedical Research and the Program of the Institute

Radiation sensitizing drugs are thought to increase the effectiveness of radiation by allowing an increased effect of radiation on hypoxic cells, while not effecting well-oxygenated cells. The Radiation Oncology Branch at NCI has been one of the leading groups involved in evaluating these very promising and innovative compounds.

Proposed Course

The Radiation Oncology Branch is currently evaluating other nitroimidazole radiosensitizers, such as desmethylmisonidazole.

Publications

Schwade, J.G., Strong, J.M., and Gangi, D.: I.V. misonidazole (NSC 261037): Report of initial clinical experience. Cancer Clinical Trials. 44:33-39, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06347-02 RO |
|---|---|---|

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (60 characters or less)

X-ray Sensitivity of Skin Fibroblast Cultures Ataxia Telangiectasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Timothy J. Kinsella, M.D. | Senior Investigator | ROB | NCI |
| | James B. Mitchell, Ph.D. | Expert | ROB | NCI |
| Other: | Nirmolini Soares, M.S. | Biologist | ROB | NCI |

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiobiology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | 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 (200 words or less - underline keywords)

Ataxia telangiectasia is a human autosomal recessive disorder characterised by progressive degenerative changes in skin and the central nervous system. Affected persons have an increased risk of malignancy and demonstrate marked sensitivity to X-rays when used in cancer treatments. Cultures of skin fibroblasts also show an increased sensitivity to X-rays compared to normal controls. This sensitivity is thought to represent a DNA-repair defect which may be related to their increased risk of cancer. Recently, presumed heterozygotes were reported to show increased sensitivity to X-rays under hypoxia. We investigated the findings in homozygotes and heterozygotes of increased X-ray sensitivity. This study has been completed.

Project Description

Objective: To investigate the X-ray sensitivity of cultured skin fibroblasts from ataxia telangiectasia (AT) homozygotes and heterozygotes under oxic and hypoxic conditions.

Methods Employed

In vitro cell proliferation will be assayed by the stage cell plating technique. Cells were exposed to X-ray under oxic and hypoxic conditions and oxygen enhancement ratios (OER) determined.

Major Findings

AT homozygotes and heterozygotes have exhibited the same OER (about 2.5-3.0).

Significance to Biomedical Research and the Program of the Institute

AT heterozygotes were estimated to occur in up to 1% of the population. If the increased sensitivity of cultured skin fibroblasts to X-rays under hypoxia represents a DNA repair defect, then this population may be at a higher risk of induced malignancy by X-ray like carcinogens.

Proposed Course - Completed

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06348-01 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Interactive Linear-source Brachytherapy Dosimetry Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | R. W. Miller | Commissioned Officer | ROB | NCI |
| Other: | J. van de Geijn | Expert | ROB | NCI |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .1 | .1 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop an interactive computer program for calculating dose distributions in an arbitrary plane from arrays of filtered, linear radioactive sources used primarily for intercavitary radiotherapy. The sources used are 137 Cs capsules with stainless steel walls. Dose distributions are calculated using the Sievert Integral with experimentally determined attenuation coefficients.

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 brachy therapy, in order to enable interactive optimization of source strength and geometric distributions.

Methods Employed

1) The algorithm is adopted from an existing model based on a special development of the Sievert integral, elsewhere developed by Van de Geijn.

2) The computer program is in part based on an existing program, developed elsewhere by Van de Geijn. This program is being adapted and extended for interactive operation on the PDP 11/70 system, making use of current manipulative and imaging technology.

3) Coordination of I/O methodology with separate and external beam therapy.

4) Comparison of computed distributions with experimental results.

Major Findings

The developments have now reached a level where they are applied to clinical problems in the treatment of cervical cancer. An especially important asset is found to be the facility to manipulate the relative spatial position of a source configuration and the possibility to simulate the effects of changing, removing or adding sources.

Significance to Biomedical Research and the Program of the Institute

The existence of a versatile program of this kind, enabling interactive adjustment to the individual clinical problem at hand is highly important. The potential for adding together dose distributions from external beam and internally applied sources, which is currently being effectuated, is especially attractive in the context of various clinical research protocols.

Proposed Course - Continuation

Publications - None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER Z01 CM 06349-01 RO |
|---|---|---|

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Relationship of Cellular Redox State and Thermotolerance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | James B. Mitchell, Ph.D. | Expert | ROB | NCI |
| | Angelo Russo, M.D., Ph.D. | Clinical Assoc. | ROB | NCI |
| Other: | Scott McPherson | Biologist | ROB | NCI |
| | Janet Gross | Biologist | ROB | NCI |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiobiology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3 | 1.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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.

Project Description

Objectives: To determine how the cellular redox state is altered during thermal stress.

Methods Employed

In vitro cell cultures will be exposed to heat and assayed for reproductive integrity using conventional tissue culture techniques and assayed for various biochemical compounds important in maintaining the cellular redox state.

Major Findings

There is a relationship in elevated glutathione and the induction of thermotolerance.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of how heat kills cells, which might be of value to future clinical efforts.

Proposed Course

Continue studying the relationship of glutathione (a cellular reducing compound) and thermotolerance.

Publications

Bromer, R. H., Mitchell, J. B., and Soares, N.: Response of human hematopoietic precursor cells (CFUc) to hyperthermia and radiation. Cancer Research 42: 1261-1265, 1982.

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1981 to September 30, 1982

Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Prospective randomized protocols in the treatment of soft tissue sarcomas have explored the role of adjuvant chemotherapy as well as the role of limb-sparing surgery. Active clinical protocols are in progress evaluating the effect of intraperitoneal chemotherapy in the treatment of colon cancer, and the effect of adjuvant chemotherapy and radiation therapy in the treatment of esophageal cancer. The role of intraoperative radiation therapy is being studied for the treatment of patients with pancreatic cancer, gastric cancer, retroperitoneal sarcomas, and bony tumors of the pelvis. The role of hepatic resection, as well as the intrahepatic infusion of chemotherapy, is being explored for treatment of metastatic disease to the liver. Active laboratory research programs in tumor immunology, tumor immunotherapy and host-tumor metabolic interactions are also in progress.

Surgical procedures performed in the Surgery Branch from April 1, 1981 to March 31, 1982 are presented in Tables 1 - 3.

TABLE I
Cases - General Surgery
April 1, 1981 - March 31, 1982

| | | |
|---|---|---|
| General: | Abdominal-perineal resection | 2 |
| | Aortocaval lymph node dissection and biopsy for ovarian cancer staging | 7 |
| | Appendectomy | 2 |
| | Colon/bowel resection | 38 |
| | Cholecystectomy | 12 |
| | Colonoscopy | 13 |
| | Endoscopy, UGI | 4 |
| | Exploratory laparotomy | 57 |
| | Feeding gastrostomy | 2 |
| | Gastrectomy | 3 |
| | Gastroscopy | 2 |
| | Hepatic resection | 10 |
| | Herniorrhaphy/hernia repair | 9 |
| | Laparoscopy | 4 |
| | Major soft tissue or muscle group excision | 59 |
| | Pancreatectomy - partial/total | 7 |
| | Peritoneoscopy | 6 |
| | Plication, duodental | 1 |
| | Polypectomy with sigmoidoscopy/sigmoid bx/EUA | 10 |
| | Revision of colostomy | 4 |
| | *Radiation therapy - intraoperative | 11 |
| | Splenectomy | 5 |
| | Staging laparotomy ± splenectomy for lymphoma | 11 |
| | Total pelvic extenteration | 3 |
| | Wound exploration/secondary closure | 3 |
| Surgery for Melanoma: | Excision solitary nodules/nevi | 7 |
| | Lymph node dissection: Axillary | 5 |
| | Wide excision with/without STSG | 2 |
| Head & Neck: | Radical neck + other resection | 2 |
| | Tracheostomy | 9 |
| Plastic: | Skin grafting | 9 |
| GYN: | Hysterectomy | 1 |
| | Marshall/Marchetti Procedure | 1 |

* Actual operative procedure listed elsewhere

TABLE I (continued)

| | | |
|---|---|---|
| GU: | Aortocaval/retroperitoneal lymph node dissection | 11 |
| | Cystoscopy ± other procedures, biopsy | 70 |
| | Circumcision | 1 |
| | Debulking procedure | 2 |
| | Excision Adrenal Tumor | 1 |
| | Meatoplasty | 1 |
| | Neophrostogram | 3 |
| | Nephrostomy/nephrolithotomy/nephrectomy/pyeloplasty | 8 |
| | Orchiectomy/orchiopexy | 15 |
| | Penile prosthetic implant | 4 |
| | Prostatic biopsy | 6 |
| | Repair varicocele/hydrocele | 2 |
| | Transureteroureterostomy/ureterolithotomy | 2 |
| | Transureteroprostatectomy | 2 |
| | Testicular biopsy | 8 |
| | Ureteropelvic reconstruction | 1 |
| | Urethral dilation | 2 |
| Breast: | Axillary node dissection with biopsy | 19 |
| | Breast biopsy | 94 |
| | Modified radical mastectomy | 19 |
| | Radical mastectomy | 3 |
| | Simple mastectomy | 1 |
| Orthopedics: | Above knee amputation | 11 |
| | Bone biopsy | 12 |
| | Forearm amputation | 2 |
| | Forequarter amputation | 5 |
| | Hemipelvectomy | 13 |
| | Hip disarticulation | 4 |
| | Stump revision | 2 |
| Endocrine: | Adrenalectomy | 3 |
| | Excision pheochromocytoma | 3 |
| | Mediastinal exploration | 6 |
| | Parathyroidectomy | 35 |
| | Parathyroid autograft implant/removal | .5 |
| | Thyroidectomy - complete/subtotal | 17 |
| Vascular: | Arterial/venous bypass/vascular reconstruction | 3 |
| | A-V shunt/shunt revision/shunt removal | 18 |
| | Broviac/Hickman catheter placement | 33 |
| | Endarterectomy/embolectomy | 2 |
| | Ligation IVS | 1 |
| | Subclavian CVP line placement | 3 |

TABLE I (continued)

| | | |
|---|---|---|
| Thoracic: | Unilateral thoracotomy | |
| | Biopsy nodules | 48 |
| | Chest wall resection | 1 |
| | Lobectomy | 10 |
| | Drainage, empyema | 2 |
| | Bilateral thoracotomy/median sternotomy | 33 |
| | Bronchoscopy | 15 |
| | Crycopharyngeal myotomy | 1 |
| | Mediastinoscopy | 10 |
| | Esophagoscopy/dilatation/± bronchoscopy | 20 |
| | Esophagogastrectomy | 9 |
| | Esophageal bypass/colon interposition | 4 |
| | Repair chest hernia | 1 |
| | Rib biopsy, open | 1 |
| | Thymectomy | 2 |
| Minor: | Biopsy: Node | 109 |
| | Excision toenail | 1 |
| | Incision and drainage of abscess | 22 |
| | Biopsy: Tissue mass, NOS | 76 |
| | Insertion feeding tube | 1 |
| | Insertion/removal abdominal catheter for chemotherapy | 28 |
| | Replacement valve in Laveen shunt | 1 |
| | Temporal artery biopsy | 2 |
| | Wound repacking/debridement | 13 |

TABLE II
Consultants - Surgery
APRIL 1, 1981 - MARCH 31, 1982

| | | |
|---|---|---|
| Plastic Surgery: | Major skin grafting | 3 |
| | Myocutaneous skin flap transfer | 3 |
| | Mammoplasty Augmentation/Revision after mastectomy | 6 |
| Vascular: | Creation A-V fistula | 4 |
| Gynecological: | D & C | 3 |
| | Laparoscopy | 4 |
| | Total abdominal hysterectomy | 1 |
| | Exploratory laparotomy | 1 |
| | Cystoscopy | 1 |
| Orthopedic: | Bone Biopsy | 13 |
| | Prosthetic limb replacement | 4 |
| | Arthroscopy | 6 |
| | Silastic arthroplasty | 2 |
| | Joint exploration | 2 |
| | Carpal tunnel release | 1 |
| | Osteotomy | 1 |
| ENT: | Antrostomy | 5 |
| | Laryngoscopy/pharyngoscopy/esophagoscopy bronchoscopy | 7 |
| | Myringotomy, bilateral | 4 |
| | Polypectomy | 2 |
| | Biopsy | 3 |
| | Septoplasty | 5 |
| | Debridement | 2 |
| | Tonsillectomy | 1 |
| | Parotidectomy | 2 |
| | Tracheostomy | 2 |
| | Tympanoplasty/Mastoidectomy/Sinusotomy | 3 |
| | Partial glossectomy | 1 |

## STATISTICAL REPORT

### SURGICAL SERVICES DEPARTMENT
### April 1, 1981 - March 31, 1982

| Patient | | NCI | NHLBI | NINCDS | | NIAMDD | NIAID | NIDR | NIMH | NICHHD | NEI | Consult-ants | Total # Pts Operated | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Neuro. | Muscle | | | | | | | | | |
| NCI | Major | 786.5 | 2 | 7 | - | - | - | 2 | - | 1 | - | 30.5 | 829 | 1110 |
| | Minor | 266 | - | 2 | - | - | - | - | - | - | - | 13 | 281 | |
| NHLBI | Major | 23 | 264 | 1 | - | - | - | 2 | - | - | - | 7 | 297 | 334 |
| | Minor | 8 | 29 | - | - | - | - | - | - | - | - | - | 37 | |
| NINCDS | Major | 10 | 3 | 62 | 1 | - | - | 2 | - | - | 1 | 6 | 85 | 133 |
| | Minor | 2 | - | 9 | 36 | - | - | - | - | - | - | 1 | 48 | |
| NIAMDD | Major | 86 | - | 1 | - | - | - | - | - | - | - | 10 | 97 | 124 |
| | Minor | 19 | - | - | - | - | - | - | - | - | - | 8 | 27 | |
| NIAID | Major | 54 | - | 3 | - | - | - | - | - | 1 | 1 | 5 | 64 | 96 |
| | Minor | 24 | - | - | 5 | - | - | - | - | - | - | 2 | 32 | |
| NIDR | Major | - | - | - | - | - | - | 1 | - | - | - | 2 | 3 | 5 |
| | Minor | 1 | - | 1 | - | - | - | - | - | - | - | - | 2 | |
| NIMH | Major | 5 | - | - | - | - | - | - | - | - | - | 2 | 7 | 11 |
| | Minor | 3 | - | - | - | - | - | - | - | - | - | 1 | 4 | |
| NICHHD | Major | 12 | - | 5 | - | - | - | - | - | - | 1 | 2 | 20 | 23 |
| | Minor | 1 | - | - | - | - | - | - | - | - | - | 2 | 3 | |
| NEI | Major | 3 | - | - | - | - | - | - | - | - | 17 | - | 20 | 20 |
| | Minor | - | - | - | - | - | - | - | - | - | - | - | - | |
| MAJOR TOTAL | | 979.5 | 269 | 79 | 1 | 0 | 0 | 8 | 0 | 2 | 20 | 64.5 | 1422 | |
| MINOR TOTAL | | 324 | 29 | 12 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 434 | |
| GRAND TOTAL | | 1303.5 | 298 | 91 | 42 | 0 | 0 | 8 | 0 | 2 | 20 | 91.50* | 1856** | |

*Includes 13 anesthesia procedures.

**Of these 1,856 surgical procedures, 157 patients were on OPD status and 29 procedures were performed on the nursing units. There were 168 emergency procedures and 120 non-emergency procedures added.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 03800-12 SURG |
|---|---|---|

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Surgical Consultants and Collaborative Research Involving Surgical Services at the National Institutes of Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | S. A. Rosenberg | Chief of Surgery, NCI | Surg | NCI |
| | and entire staff of | Surgery Branch, NCI | Surg | NCI |
| Other: | G. D. Aurbach | Chief, Metabolic Dis. Br. | MD | NIAMDD |
| | J. L. Doppman | Chief, Diag. Radiol. Dept. | DR | CC |
| | E. Glatstein | Chief, Rad. Oncol. Br. | RO | NCI |
| | J. Robbins | Chief, Clin. Endocrin. Br. | CE | NIAMDD |
| | J. Costa | Lab. of Pathology | LP | NCI |
| | R. C. Young | Chief, Medicine Branch | M | NCI |
| | P. Pizzo | Chief, Ped. Oncol. Br. | PO | NCI |
| | J. Gardner | Chief, Digestive Dis. Br. | DD | NIAMDD |

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | 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 (200 words or less - underline keywords)

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 I. Endocrine Surgery

The Surgery Branch has provided endocrine surgery and consultation for a variety of Clinical Center endocrinological problems. These problems include hyperparathyroidism, thyroid nodules and Grave's disease, adrenal cortical tumors, pheochromocytomas, and occasional tumors with paraneoplastic hormonal activity. This year two new programs have evolved to improve the clinical results in two areas of endocrine surgery. The first of these involves an attempt to improve the results of adrenal cortical autotransplantation for

selected patients requiring total adrenalectomy, and the second involves the evolution of a plan for the management of anaplastic thyroid cancer and advanced papillary/follicular thyroid cancer through cooperative efforts of the Clinical Endocrinology Branch, NIAMDD, the Medicine Branch, NCI, and the Radiation Oncology Branch, NCI.

Part II. Thoracic and Vascular Surgery

Consultative services for thoracic and vascular surgical problems are handled through the Surgery Branch. One hundred-two major and twenty-two minor thoracic surgical procedures have been performed along with 35 minor cases.

Part III. Nutritional Support

The Surgery Branch continues to mount a major effort in supporting patients nutritionally by intravenous feeding throughout the National Institutes of Health. The Surgery Branch has been responsible for 1623 days of total parenteral nutrition between the period 2/1/80 and 2/1/81, involving 64 courses in 50 patients.

Part IV. Ovarian Cancer

Studies of ovarian carcinoma are undertaken in Medicine Branch protocols with the cooperation of the Surgery Branch. Adjuvant systemic melphalan chemotherapy is being compared with intraperitoneal radioactive phosphorus for high-risk patients following complete surgical tumor resections. Combination chemotherapy is being utilized to treat advanced-stage patients. Intraperitoneal chemotherapy is evaluated both as an adjuvant in early-stage patients and as definitive therapy in certain patients with advanced disease. Patients with incomplete surgical tumor resections or with disseminated disease are treated with various combinations of systemic chemotherapy. The Surgery Branch collaborates with the Medicine Branch 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 1981, the Surgery Branch performed 48 operative procedures on patients in ovarian cancer protocols.

Part V. Tenckhoff Catheters

Tenckhoff catheters have been used for several years for peritoneal dialysis in patients with chronic renal failure. Using these catheters, direct administration of chemotherapeutic agents is possible into the peritoneal cavity. The conduct of these studies has been under the general direction of Dr. Charles Myers, Chief of the Clinical Pharmacology Branch. Phase I and Phase II trials of intraperitoneal 5-FU and adriamycin have been completed for ovarian cancer. An adjuvant 5-FU trial for poor risk patients who have had a resection for colon and rectal cancer is currently in progress. Phase I trials with intraperitoneal 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-three catheters were inserted 4/1/81-3/31/82.

Part VI. Vascular Access

The Surgery Branch accepts consults from the Clinical Center to provide means of vascular access. Renal dialysis, plasmapheresis, drug and blood product infusion and blood withdrawal are some of the indications for vascular access procedures in selected patients. Between April 1981 and March 1982, 15 Hickman catheters, 4 arterial venous fistulae, and 8 Scribner shunts have been constructed.

PUBLICATIONS

1. Javadpour, N., Woltering, E., and Brennan, M.: Adrenal neoplasm. Current problems in surgery. 17:1-52, 1980.

2. Jones, R.B., Collins, J.M., Myers, C.E., Brooks, A.E., Hubbard, S.M., Balow, J.E., Brennan, M.F., Dedrick, R.L., and DeVita, V.T.: High volume intraperitoneal chemotherapy with methotrexate in patients with cancer. Cancer Res. 41:55-59, 1981.

3. Brennan, M.F., and Copeland, E.M.: Panel report on nutritional support of patients with cancer. Am. J. Clin. Nutr. 34:1199-1205, 1981.

4. Krudy, A.G., Doppman, J.L., Brennan, M.F., Saxe, A.W., Marx, S.J., and Parthemore, J.G.: Arteriographic localization of parathyroid adenoma in the presence of lingual thyroid. AJR 136:1227-1230, 1981.

5. Brennan, M.F.: Total parenteral nutrition in the management of the cancer patient. Am. Rev. Med. 32:233-243, 1981.

6. Brennan, M.F.: Total parenteral nutrition in the cancer patient. New Engl. J. Med. 305:375-383, 1981.

7. Popp, M.B., Fisher, R.I., Wesley, R., Aamodt, R., and Brennan, M.F.: A prospective randomized study of adjuvant parenteral nutrition in the treatment of advanced diffuse lymphoma: influence of survival. Surgery 90:195-203, 1981.

8. Lowry, S.F., Smith, J., and Brennan, M.F.: Zinc and copper replacement during total parenteral nutrition. Am. J. Clin. Nutr. 34:1853-1860, 1981.

9. Spiegel, A.M., Marx, S.J., Brennan, M.F., Brown, E.M., Downs, R.W., Gardner, D.G., Attie, M.F., and Aurbach, G.D.: Parathyroid function after parathyroidectomy: Evaluation by measurement of urinary CAMP. Clin. Endocr. 15:65-73, 1981.

10. Brennan, M.F., Marx, S.J., Doppman, J.L., Costa, J., Saxe, A., Spiegel, A., Krudy, A., and Aurbach, G.D.: Results of reoperation for persistent and recurrent hyperparathyroidism. Ann. Surg. 194:671-676, 1981.

11. Arbeit, J.M., Lowry, S.F., Line, B.R., Jones, D.C., and Brennan, M.F.: Deep venous thrombosis in patients undergoing inguinal lymph node dissection. Ann. Surg. 194:648-655, 1981.

12. Krudy, A.G., Doppman, J.L., Brennan, M.F., Marx, S.J., Spiegel, A.M., and Aurbach, G.D.: The detection of mediastinal parathyroid glands by computed tomography and angiography. An analysis of 17 proven cases. Radiology 140:739-744, 1981.

13. Brennan, M.F.: Nutritional support of the cancer patient. In DeVita, V., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott, 1982, pp 1628-1639.

14. Burt, M., and Javadpour, N.: Germ cell tumors in patients with apparently normal testes. Cancer 47:1911, 1982.

15. Brennan, M.F., Doppman, J.L., Krudy, A.G., Marx, S.J., Spiegel, A.M., and Aurbach, G.D.: Assessment of techniques for preoperative localization of functionally abnormal parathyroid tissue in patients undergoing reoperation for hyperparathyroidism. Surgery 91:6-11, 1982.

16. Demetrakopoulos, G.Ev., and Brennan, M.F.: Tumoricidal potential of nutritional manipulations. Cancer Research 42:7565-7655, 1982.

17. Levine, A.S., Brennan, M.F., Ramu, A., Fisher, R.I., Pizzo, P., and Glaubiger, D.L.: Controlled clinical trials of nutritional intervention as an adjunct to chemotherapy with a comment on nutrition and drug resistance. Cancer Res. (Suppl) 42:774s-781s, 1982.

18. Burt, M.E., Gorschboth, C., and Brennan, M.F.: A controlled prospective randomized trial evaluating the metabolic effects of enteral and parenteral nutrition in the cancer patient. Cancer 49:1092-1105, 1982.

19. Wagman, L.W., Burt, M.E., and Brennan, M.F.: The impact of total parenteral nutrition on liver function tests in patients with cancer. Cancer 49:1249-1257, 1982.

20. Lippert, M., and Javadpour, N.: Detection of cell surface antigen in cancer of the ureter and renal pelvis. Urology, in press.

21. Pretorius, H.T., Katikineni, M., Kinsella, T.J., Barsky, S.H., Brennan, M.F., Chu, E.W., and Robbins, J.: Thyroid nodules following high dose radiotherapy diagnosis and management. Am. J. Med., in press.

22. Dunnick, N.R., Doppman, J.L., Gill, J.R., Strott, C.A., Keiser, H.R., and Brennan, M.F.: Localization of functional adrenal tumors by computed tomography and venous sampling. Radiology, in press.

23. Kirkemo, A.K., Burt, M.D., and Brennan, M.F.: Maintenance of serum vitamin levels in cancer patients on total parenteral nutrition. Amer. J. Clin. Nutr., in press.

24. Saxe, A.W., Spiegel, A.M., Marx, S.J., and Brennan, M.F.: Deferred parathyroid autografts with cryopreserved tissue following reoperative parathyroid surgery. Arch. Surg., in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 03801-12 SURG |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Studies in Cancer Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant disease. The major emphasis of these studies is in the treatment of malignant melanoma, soft tissue sarcomas, osteogenic sarcomas, and colorectal cancer. The major emphasis in Surgery Branch cancer therapy is in adjunctive therapy with emphasis on the use of multiple treatment modalities in addition to surgery.

Project Description:

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|---|---|
| Part I. | Malignant Melanoma |
| Part II. | Soft Tissue Sarcomas |
| Part III. | Osteogenic Sarcoma |
| Part IV. | Testicular Cancer |
| 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 I. Malignant Melanoma

The Surgery Branch is currently concluding follow-up on patients entered into treatment trial of patients for Stage II melanoma conducted in conjunction with the Immunology and Medical Oncology Branches. Patients were randomized to receive either lymphnode dissection alone or lymphnode dissection followed by treatment with either methyl CCNU, BCG, or BCG plus allogeneic and melanoma cells. 181 patients were randomized into this protocol. Follow-up is continuing. There does not appear to be a difference between any of the adjuvant treatment groups and treatment with surgery alone.

Part II. Soft Tissue Sarcomas

The Surgery Branch is conducting a variety of protocols evaluating the treatment of patients with soft tissue sarcomas. 55 patients have been included in a randomized protocol evaluating the role of adjuvant chemotherapy with adriamycin and cytotoxin in the treatment of patients with soft tissue sarcomas of the extremities. A highly statistically significant improvement has been seen in patients randomized to receive chemotherapy. Follow-up is continuing. 43 patients have been included in randomized protocols evaluating the role of amputated surgery compared to limb-sparing surgery plus radiation therapy in the treatment of patients with extremity sarcomas. Limb-sparing surgery appears to be an effective treatment for these patients. No differences have been seen between patients randomized receiving limb-sparing surgery or patients randomized receiving amputated surgery.

Part III. Osteogenic Sarcoma

Fifty-five patients have been entered into a study evaluating the use of high-dose methotrexate for the adjuvant treatment of patients with osteosarcomas. Continuous disease-free interval has improved from 20% in historical controls to 38% in adjuvant chemotherapy treated patients. The aggressive use of thoracotomy in these patients has led to a dramatic increase in disease-free survival. Currently 68% of patients are alive and NED following diagnosis of osteogenic sarcoma. This represents a substantial improvement over survival figures prior to the onset of this protocol.

A new protocol has been initiated comparing, in a prospective randomized fashion, adjuvant therapy with high-dose methotrexate compared to surgery alone in patients with osteogenic sarcoma. Twenty-three patients have been entered into this study.

Part IV. Testicular Cancer

Thirty-nine patients with stage III nonseminomatous testicular cancer were treated in a prospective randomized trial comparing cytoreductive surgery followed by combination chemotherapy versus chemotherapy alone. All patients had one or more of the following poor prognostic signs: bulky retroperitoneal disease, liver involvement, invasion or obstruction of the inferior vena cava, or lung metastases >2 cm in diameter. Cytoreductive surgery was technically feasible in this group of patients as assessed radiographically and by the decline in serum levels of alphafetoprotein and human chorionic gonadotropin following surgery. However, there was no statistically significant improvement in overall response rate (75% vs 74%), complete response rate (50% vs. 37%) or in overall survival between the two groups. As an extension of this study a randomized clinical trial in conjunction with the Medical Oncology Branch is now ongoing using intensive chemotherapy and administration of autologous bone marrow. The new protocol randomized this subset of patients to either a regimen containing platinum, vinblastine, bleomycin (PVB) and VP-16 followed by surgery or the conventional PVB alone. 15 patients have been randomized to this ongoing protocol.

Part V. Colorectal Cancer

At the present time two protocols for the study of patients with colorectal cancer are operative. The first protocol concerns the postoperative management of patients who have had lymph node positive colon or rectal cancer removed. These patients are carefully followed with monthly CEA assays, 3-monthly abdominal ultrasound and CAT scans, and monthly physical examinations. By so doing we wish to pick up early recurrences for "second look surgery" as well as determine optimal follow-up regimens. In the second protocol the same group of patients are randomized to receive intravenous or intraperitoneal 5-FU. Forty one patients have been entered into these studies. In the second study patients with local or regional colon or rectal recurrences undergo second look surgery randomized to receive or not receive radiation therapy.

Part VI. Breast Cancer

Under protocol 79-C-111 which began accrual of patients in July 1979, 78 patients have been entered through March 1982. Patients with clinical Stage I or II unilateral breast cancer are randomly assigned to receive total mastectomy with axillary dissection, or excisional biopsy with axillary dissection followed by radiotherapy to the affected breast. Of the 78 patients, 38 have been randomized to the mastectomy group, and 40 to the radiotherapy group. With at least one positive axillary node, 32 patients (42%) have entered into adjuvant chemotherapy with IV Adriamycin and oral Cytoxan. With a median follow-up of 15 months there have been three recurrences; two of these have died. Patients who receive mastectomy will be given an opportunity to have reconstruction after 6 months. Ten mastectomy patients have had reconstruction. An increased rate of accrual is hoped for during the next year.

Part VII. Endoscopy

The Surgery Branch continues to collect clinical data to help define the appropriate role for laparoscopy in cancer diagnosis and treatment. Laparoscopic tubal ligation, as a consultative service, is available for appropriate patients. During the past year, 30 patients have undergone laparoscopy on the NCI surgical service. Surgery Branch colon and rectal cancer protocols promise to increase our utilization of this endoscopic procedure in the coming year.

Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The utility of this procedure from both the diagnostic and therapeutic point of view of colon lesions is well established. Twenty-four colonoscopic procedures were performed in the past year. These included 18 diagnostic examinations and 6 snare polypectomies. Direct visual access to and ability to histologically sample tissues from the entire lower GI tract offer excellent opportunities for earlier diagnosis of colon malignancy. Premalignant lesions in patients with ulcerative colitis can be identified. Colonoscopy will play a role in the preoperative assessment of appropriate patients to undergo colon and rectal surgery.

Part VIII. Computer Applications

Data for Surgery Branch research continued to be collected, stored, and reported for 3 primary systems: (a) Cancer Patient Research Information System (CAPRI), (b) Serum Inventory, and (c) 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.

These systems are maintained on computers at the Division of Computer Resources and Technology. On the IBM-370 system, the text-editor WYLBUR is used for data entry, program maintenance, and remote job submission. The Time Sharing Option is used for graphic displays and interactive computing. On the DECsystem-10 computer, various software packages are used for entry of laboratory data, graphic displays, and interactive computing. Two high-speed Atlanthus T-1222 terminals are used for data entry and retrieval. A Tektronix 4012 terminal is

used for data retrieval and graphic display of information. 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 preoperative radiation therapy and postoperative 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 2000 rads preoperatively or surgical resection alone. Following resection patients are then randomized to receive cis-platinum, bleomycin, and vindesine or follow-up. Sixteen patients have been evaluated so far. Six have been entered into the study and 8 received palliative treatment.

Part X. Intraoperative Radiotherapy

The Surgery Branch has initiated investigations of the role of combined surgery and intraoperative radiation therapy for the local control of abdominal malignancies. A series of pilot patients have been treated where the patients underwent surgical resection of a variety of abdominal tumors, were transported under anesthesia from the operating room to the radiotherapy treatment facility, were treated with electron radiation directly to the tumor bed having vital abdominal structures shielded from the radiation beam, and were returned to the operating room for completion of surgery. No technical difficulties were encountered in the pilot series, and therefore investigative prospective randomized protocols were initiated during 1980 to evaluate combined surgical resection and intraoperative radiation in certain poor-prognosis malignancies. Protocols for the treatment of gastric and pancreatic cancers were established, comparing intraoperative radiotherapy with conventional external beam postoperative radiation in patients able to have all gross tumor surgically resected. Protocol for the treatment of retroperitoneal sarcomas was developed, comparing intraoperative and postoperative radiotherapy with conventional external beam postoperative radiation in patients able to have all gross tumor surgically resected. Protocol for the treatment of retroperitoneal sarcomas was developed, comparing intraoperative and postoperative low-dose external beam radiation with conventional high-dose postoperative low-dose external beam radiation with conventional high-dose postoperative radiotherapy in patients with surgically resectable tumors; in addition, the use of adjuvant chemotherapy is evaluated in patients treated for retroperitoneal sarcomas. Protocol for the treatment of bony sarcomas of the pelvis was initiated, treating patients with combined surgical resection and intraoperative radiation therapy to the tumor bed, comparing disease control in protocol patients with historical experience. Protocol for evaluating an escalating dose schedule for intraoperative irradiation was developed for patients with various malignant diseases that have no conventional surgical or radiotherapeutic treatment options. As of 4/1/82, accrual status of the various intraoperative

radiotherapy studies was: gastric carcinoma 16 evaluated, 11 randomized, 6 on study; pancreatic carcinoma 46 evaluated, 22 randomized, 9 on study; retroperitoneal sarcoma 35 evaluated, 17 randomized, 13 on study; pelvic bony sarcoma 5 evaluated, 3 on study; escalating dose study 6 evaluated, 4 on study.

Part XI. Resection of Pulmonary Metastases

The Surgery Branch continues its protocol for the aggressive removal of metastatic sarcoma to the lung. After a thorough work-up to rule out metastatic disease at other sites, patients with locally controlled osteogenic or soft tissue sarcomas are subjected to pulmonary resection. An attempt is made to remove all gross evidence of tumor while preserving as much pulmonary parenchyma as possible. Multiple procedures are often required. Within the past 12 months 54 operations have been performed for resection of pulmonary metastatic disease. Thirty-four of these were median sternotomies and 24 were lateral thoracotomies. Ten ancillary procedures on these patients were also performed.

PUBLICATIONS

1. Javadpour, N.: Testicular germ cell tumor. Urol. Digest 18:19, 1979.

2. Baker, A.R.: Local procedures in the management of rectal cancer. Sem. in Oncol. 7:385-391, 1980.

3. Javadpour, N., Woltering, and Soares, T.: Simultaneous measurement of tumor cytosol and peripheral serum levels of human chorionic gonadotropin and Alphafetoprotein in testicular cancer. Investigative Urology 18:11, 1980.

4. Javadpour, N.: Recent advances in detection of metastatic testicular cancer. Internal J. Andrology 4:222, 1981.

5. Javadpour, N., and Goldenberg, D., et al: Radioimmunodetection of metastatic testicular cancer. JAMA 246:45, 1981.

6. Lippert, M., and Javadpour, N.: Role of lactic dehydrogenase in testicular cancer. Urology 18:50, 1981.

7. Lippert, M., and Javadpour, N.: Lactic dehydrogenase in the monitoring and programs of testicular cancer. Cancer 48:2278, 1981.

8. Emmott, R., Droller, M., and Javadpour, N.: The ABO (H) cell surface antigens in carcinoma in situ and nonmalignant lesions of the bladder. J. Urol. 125:32, 1981.

9. Javadpour, N., and Roy, J.: Combined chromosomal and cell surface antigen studies in bladder cancer. Urol. 19:29, 1981.

10. Sindelar, W.F., Javadpour, N., and Bagley, D.H.: Histological and ultrastructural changes in rat kidney after cryosurgery. J. Surgical Oncology 18:363, 1981.

11. Javadpour, N.: Recent advances in surgery of urologic oncology. J. Nephrology, Urology and Andrology 1:205, 1981.

12. Javadpour, N., and Soares, T.: False positive and false negative alpha protein and human chorionic gonadotropin assays in testicular cancer - a double blind study. 48:2279, 1981.

13. Burt, M., and Javadpour, N.: Germ cell tumors in patients with apparently normal testes. Cancer 47:1911, 1981.

14. Javadpour, N., and Chen, H.C.: Improved HCG detection utilizing the beta subunit carboxyl-terminal radioimmunoassay of concentrated 24 hour urine in patients with testicular cancer. J. Urol. 126:170, 1981.

15. Lippert, M., and Javadpour, N.: Lactic dehydrogenase in the monitoring and prognosis of testicular cancer. Cancer 48:2278, 1981.

16. Lippert, M., and Javadpour, N.: Role of lactic dehydrogenase in testicular cancer. Urology 18:50, 1981.

17. Javadpour, N.: Recent advances in surgery of urologic oncology. J. Nephrology, Urology & Andrology 1:205, 1981.

18. Sugarbaker, P.H.: Optomizing peritoneoscopic visualization of the liver utilizing a double telescope technique. Surg. Gynec. Obst. 152:655-657, 1981.

19. Sugarbaker, P.H., Auda, S., Webber, B.L., and Trisch, T.: Early distant metastases from epitheloid sarcoma of the hand. Cancer 48:186-189, 1981.

20. Speyer, J.L., Sugarbaker, P.H., Collins, J.M., Dedrick, R.L., Klecker, R.W., and Meyers, C.E.: Portal levels and hepatic chance of 5-fluoronacil after intraperitoneal administration in humans. Cancer Res. 41:1916-1922, 1981.

21. Chretien, P.A., and Sugarbaker, P.H.: Surgical technique of hemipelvectomy in the lateral position. Surgery 90:900-909, 1981.

22. Sugarbaker, P.H., and Chretien, P.A.: A surgical technique for hip disarticulation. Surgery 90:546-553, 1981.

23. Sugarbaker, P.H.: Carcinoma of the colon - prognosis and operative choice. Curr. Probl. Surg. 18:755-802, 1981.

24. Shull, J.H., Javadpour, N., Soares, T., and deMoss, E.V.: ABO cell surface antigen in malignant and benign breast disease. J. Surg. Oncol. 18:193-196, 1981.

25. Tepper, J., and Sindelar, W.: Summary of the workshop on intraoperative radiation therapy. Cancer Treat Rep. 65:911-918, 1981.

26. Sloan, G.M., Maher, M.M., and Brennan, M.F.: Nutritional effects of surgery, radiation therapy, and adjuvant chemotherapy for soft tissue sarcomas. Amer. J. Clin. Nutr. 34:1094-1102, 1981.

27. Chang, A.E., Shiling, D.J., Stillman, R.C., Goldberg, N.H., Seipp, C.A., Barofsky, I., and Rosenberg, S.A.: A prospective evaluation of delta-9 tetrahydrocannabinol as an antiemetic in patients receiving adriamycin and cytoxan chemotherapy. Cancer 47:1746-1751, 1981.

28. Rosenberg, S.A.: The treatment of soft tissue and bony sarcomas: Review of NCI studies. Natl. Cancer Inst. Mongr. 56:241-244, 1981.

29. Reichert, C.M., Rosenberg, S.A., Webber, B.L., and Costa, J.: Malignant melanoma: A search for occult lymph node metastases. Human Path. 12:449-451, 1981.

30. Rosenberg, S.A., and Glatstein, E.J.: Perspectives on the role of surgery and radiation therapy in the treatment of soft tissue sarcomas of the extremities. Semin. Oncol. 8:190-200, 1981.

31. Gottdiener, J.S., Mathisen, D.J., Borer, J.S., Bonow, R.O., Myers, C.E., Barr, L.H., Schwartz, D.E., Bacharach, S.L., Green, M.V., and Rosenberg, S.A.: Doxorubicin cardiotoxicity: Assessment of late left ventricular dysfunction by radionuclide cineangiography. Ann. Int. Med. 94:430-435, 1981.

32. Shamberger, R.C., Sherins, R.J., and Rosenberg, S.A.: The effects of postoperative adjuvant chemotherapy and radiotherapy on testicular function in men undergoing treatment for soft tissue sarcoma. Cancer 47:2368-2374, 1981.

33. Shamberger, R.C., Sherins, R.J., Ziegler, J.L., Glatstein, E., and Rosenberg, S.A.: The effects of postoperative adjuvant chemotherapy and radiotherapy on ovarian function in women undergoing treatment for soft tissue sarcoma. J. Natl. Cancer Inst. 67:1213-1218, 1981.

34. Shamberger, R.C., Rosenberg, S.A., Seipp, C.A., and Sherins, R.J.: The effects of high-dose methotrexate and vincristine on ovarian and testicular function in patients undergoing postoperative adjuvant treatment for osteosarcoma. Cancer Treat. Rep. 65:739-746, 1981.

35. Fisher, R.I., Terry, W.D., Hodes, R.J., Rosenberg, S.A., Makuch, R., Gordon, H.G., and Fisher, S.G.: Adjuvant immunotherapy or chemotherapy for malignant melanoma: Preliminary report of the National Cancer Institute randomized clinical trial. Surg. Clinics N.A. 61:1267-1277, 1981.

36. Rosenberg, S.A., Tepper, J., Glatstein, E., Costa, J., Young, R., Seipp, C., and Wesley, R.: Adjuvant chemotherapy for patients with soft tissue sarcomas. Surg. Clinics N.A. 61:1415-1423, 1981.

37. Rosenberg, S.A., and Glatstein, E.: The Management of Local and Regional Soft Tissue Sarcomas. In Carter, S.K., Glatstein, E., and Livingston, R.B. (Eds.): Principles of Cancer Treatment. New York, McGraw-Hill, 1982, pp 697-706.

38. Rosenberg, S.A.: Principles of Surgical Oncology. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, Pa., J. B. Lippincott Co., 1982, pp. 93-102.

39. Rosenberg, S.A., Suit, H.D., Baker, L.H., and Rosen, G.: Sarcomas of the Soft Tissue and Bone. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, Pa., J. B. Lippincott Co., 1982, pp. 1037-1093.

40. Mastrangelo, M.J., Rosenberg, S.A., Baker, A.R., and Katz, H.R.: Cutaneous Melanoma. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, Pa., J. B. Lippincott Co., 1982, pp. 1124-1170.

41. Terry, W.D., Hodes, R.J., Rosenberg, S.A., Fisher, R.I., Makuch, R., Gordon, H.G., and Fisher, S.G.: Treatment of Stage I and II malignant melanoma with adjuvant immunotherapy or chemotherapy: Preliminary analysis of a prospective randomized trial. In Terry, W.D., and Rosenberg, (Eds.): Immunotherapy of Human Cancer, New York, Elsevier North-Holland, 1982, pp. 251-257.

42. Rosenberg, S.A.: Recent Advances in the Treatment of Sarcomas. Surgical Rounds, 5:36-58, 1982.

43. Rosenberg, S.A.: Soft Tissue Sarcomas: Natural History. In Silver, R.T. (Ed.): Clinical Topics in Cancer - Diagnosis and Treatment, New York, Le Jacq Publ. Co., 1982, pp. 433-438.

44. Sugarbaker, P.H., Barofsky, I., Rosenberg, S.A., and Gianola, F.J.: Quality of life assessment of patients in extremity sarcoma clinical trials. Surgery 91:17-23, 1982.

45. Sugarbaker, P.H., and Chretien, P.A.: A surgical technique for buttockectomy. Surgery 91:104-107, 1982.

46. Javadpour, N.: The technique of retroperitoneal lymphadenectomy in testicular cancer. In Book edited by D. E. Johnson, in press, 1982.

47. Dunnick, N.R., and Javadpour, N.: The value of CT and lymphography in detecting retroperitoneal metastases from nonseminomatous testicular cancer. AJR 136:1093, 1982.

48. Javadpour, N.: The management of urologic emergencies in cancer patients. In DeVita, Hellman, and Rosenberg (Eds.): Lippincott, 1982, page 1616.

49. Javadpour, N., Soares, and Princler, G.: In vitro synthesis of Alphafetoprotein and Human Chorionic Gonadotropin in Testicular Cancer. Cancer 49:303, 1982.

50. Sugarbaker, P.H., MacDonald, J., and Gunderson, L.: Colorectal cancer. In DeVita, V., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology, Philadelphia, Pa., J. B. Lippincott Co., 1982.

51. Sugarbaker, P.H., Gunderson, L., and MacDonald, J.: Carcinoma of the anal region. In DeVita, V., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology, Philadelphia, Pa., J. B. Lippincott Co., 1982.

52. Sugarbaker, P.H., Dunnick, N.R., and Sugarbaker, E.V.: Diagnosis and staging. In DeVita, V., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology, Philadelphia, Pa., J. B. Lippincott Co., 1982.

53. Smith, T.J., Kemeny, M.M., and Sugarbaker, P.H., et al.: A prospective study of hepatic imaging in the detection of metastatic disease. Ann. Surg. 195:486-491, 1982.

54. Kemeny, M.M., Sugarbaker, P.H., and Smith, T.J., et al.: A prospective analysis of laboratory tests and imaging studies to detect hepatic lesions. Ann. Surg. 195:163-167, 1982.

55. Sindelar, W.F.: Cancer of the small intestine. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, Pa., Lippincott, 1982, pp. 616-642.

56. Dunnick, N.R., Schwade, J.G., Martin, S.E., Johnston, M.R., and Glatstein, E.: Interstitial pulmonary infiltrate following combined therapy for esophageal carcinoma. Chest 81:453-456, 1982.

57. Kirkemo, A., and Johnston, M.R.: Percutaneous subclavian vein placement of the Hickman Catheter. Surgery 91:349-351, 1982.

58. Johnston, M.R., Pizzo, P.A., and Fauci, A.S.: Thoracic mass lesion in immuno-incompetent patients. Chest, in press.

59. Wagner, R.B., and Johnston, M.R.: The middle lobe syndrome; a collective review. Ann. Thorac. Surgery, in press.

60. Dresdale, A.R., Barr, L.H., Bonow, R.O., Mathisen, D.J., Myers, C.E., Schwartz, D.E., d'Angelo, T., and Rosenberg, S.A.: Prospective randomized study of the role of N-acetyl cysteine in reversing doxorubicin induced cardiomyopathy. Cancer Clin. Trials, in press.

61. Tepper, J., Glatstein, E., and Rosenberg, S.A.: Radiation therapy technique in soft tissue sarcomas of the extremity. Intl. J. Rad. Oncol., in press.

62. Rosenberg, S.A., Tepper, J., Glatstein, E., Costa, J., Young, R., Baker, A., and Wesley, R.: Prospective randomized evaluation of adjuvant chemotherapy in adults with soft tissue sarcomas of the extremities. Cancer, in press.

63. Vermess, M., Doppman, J.L., and Sugarbaker, P.H., et al.: Computed tomography of the liver and spleen with intravenous lipoid control material (EOE 13): A review of 60 examinations. Am. J. Roent, in press.

64. Sugarbaker, P.H., and Corlew, S.: Influence of surgical techniques on survival in patients with colorectal cancer. A review. Dis. Colon Rectum, in press.

65. Sugarbaker, P.H., and Chretien, P.A.: Surgical technique for anterior flap hemipelvectomy. Ann. Surg., in press.

66. Jenkins, J., Sugarbaker, P.H., Gianola, F.J., and Meyers, C.E.: Technical considerations in the use of intraperitoneal chemotherapy via a Tenckhoff catheter. Surg. Gynecol. Obstet., in press.

67. Kemeny, M.M., and Sugarbaker, P.H.: Host modifications of the skin allograft assay. J. Surg. Res., in press.

68. Sugarbaker, P.H.: Partial sarcretomy for en bloc resection of rectal cancer with posterior fixation. Dis. Colon Rect., in press.

69. Sugarbaker, P.H.: Quadriceps muscle group excision. Surgery, in press.

70. Mentzer, S.J., Sugarbaker, P.H., and Chretien, P.A.: Surgical technique for excision of the adductor muscle group. Surgery, in press.

71. Smith, T.J., Sloan, G.M., and Baker, A.R.: Epitrochlear node involvement in melanoma of the upper extremity. Cancer, in press.

72. McCauley, R., and Javadpour, N.: Supraclavicular biopsy in staging of testicular cancer. Cancer, in press.

73. Javadpour, N., and Ozols, R.F.: A randomized trial of cyto reductive surgery followed by chemotherapy versus chemotherapy alone in bulky stage III testicular cancer with poor prognostic features. Cancer, in press.

74. Sindelar, W.F., Tepper, J., Travis, E.L., and Terrill, R.: Tolerance of retroperitoneal structures to intraoperative radiation. Ann. Surg., in press.

75. Sindelar, W.F., Tepper, J., and Travis, E.L.: Tolerance of bile duct to intraoperative irradiation. Surgery, in press.

76. Rosenberg, S.A., Tepper, J., Glatstein, E., Costa, J., Baker, A., Brennan, M., deMoss, E., Seipp, C., Sindelar, W.F., Sugarbaker, P., and Wesley, R.: The treatment of ST sarcomas of the extremities: Prospective randomized evaluations of 1) limb-sparing surgery plus radiation therapy compared to amputation and 2) the role of adjuvant chemotherapy. Ann. Surgery, in press.

77. Tamarkin, L., Danforth, D., Lichter, A., deMoss, E.V., Chabner, B., and Lippman, M.: Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. Science, in press.

78. deMoss, E.V., Lichter, A.S., Lippman, M., Gerber, N.L., Reichert, C.M., Edwards, B.K., Schain, W.S., Gorrell, C.R., D'Angelo, T., and Rosenberg, S.A.: Complete axillary lymph node dissection prior to radiotherapy for primary breast cancer. Proceedings of the Symposium. Alternatives to Mastectomy. 1982, in press.

79. Lichter, A.S., Lippman, M.E., Gorrell, C.R., d'Angelo, T., Edwards, B., deMoss, E.V.: Adjuvant chemotherapy in patients treated primarily with irradiation for localized breast cancer. Proceedings of the Symposium. Alternatives to Mastectomy, 1982, in press.

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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Immunotherapy of Animal and Human Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| | S. Schwarz | Biologist | SURG NCI |
| | H. Wexler | Biologist | SURG NCI |
| | P. Spiess | Biologist | SURG NCI |
| | C. Hyatt | 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 MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 8.5 | 5.0 | 3.5 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed studies of tumor-host immune interactions in animals and humans with sarcomas are being performed in an attempt to develop new immunodiagnostic and immunotherapeutic techniques for diagnosis and treatment of these tumors. Immune response to murine sarcomas has been extensively evaluated and both tumor- specific and fetal antigens have been identified. Attempts are being made to develop adoptive immunotherapeutic techniques utilizing transfer of cells grown in long term culture in T cell growth factor. Techniques for the prolonged growth of cytotoxic T cells have been developed. These cells have been shown to mediate immunologic rejection of allografts and syngeneic tumors and attempts to use these cells for the adoptive immunotherapy of mouse and human tumors are in progress.

Publications:

1. Rosenberg, S.A., Schwarz, S., Spiess, P.J., and Brown, J.M.: In vitro growth of murine T cells. III. Method for separation of T cell growth factor (TCGF) from Concanavalin A and biological activity of the resulting TCGF. J. Immunol. Meth. 33:337-350, 1980.

2. Mathisen, D.J., and Rosenberg, S.A.: The in vivo distribution of adoptively transferred syngeneic, allogeneic, and xenogeneic lymphoid cells: Implication for the adoptive immunotherapy of tumors. J. Immunol. 124:2295-2300, 1980.

3. Mathisen, D.J., and Rosenberg, S.A.: Comparison of in vivo cell distribution following either intraperitoneal or intravenous injection of lymphoid cells. Transp. 29:347-349, 1980.

4. Lotze, M.T., Strausser, J.L., and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. II. Use of T cell growth factor (TCGF) to clone human T cells. J. Immunol. 124:2972-2978, 1980.

5. Yron, Ilana, Wood, Thomas A., Spiess, Paul, and Rosenberg, S.A.: In vitro growth of murine T cells: V. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. J. Immunol. 125:238-245, 1980.

6. Rosenberg, S.A., Spiess, P.J., and Schwarz, S.: In vitro growth of murine T cells. IV. Use of T cell growth factor (TCGF) to clone lymphoid cells. Cell. Immunol. 54:293-306, 1980.

7. Lotze, M.T., Line, B.R., Mathisen, D.J., and Rosenberg, S.A.: The in vivo distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF): Implications for the adoptive immunotherapy of tumors. J. Immunol. 125:1487-1493, 1980.

8. Spiess, P.J., and Rosenberg, S.A.: A simplified method for the production of murine T cell growth factor free of lectin. J. Immunol. Meth. 42:213-222, 1981.

9. Lotze, M.T., and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. III. The preparation of lectin free T cell growth factor (TCGF) and an analysis of its activity. J. Immunol. 126:2215-2220, 1981.

10. Sener, S.F., Brown, J.M., Hyatt, C.L., Terry, W.D., and Rosenberg, S.A.: Serologic analysis of human tumor antigens. III. Reactivity of patients with melanoma and osteogenic sarcoma to cultured tumor cells and fibroblasts using the immune adherence assay. Cancer Immunol. & Immunother. 11:243-250, 1981.

11. Lotze, M.T., Grimm, E.A., Mazumder, A., Strausser, J.L., and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. IV. Lysis of fresh and cultured autologous tumor by lymphocytes cultured in T cell growth factor (TCGF). Cancer Res. 41:4420-4425, 1981.

12. Rosenstein, M., Eberlein, T., Kemeny, M.M., Sugarbaker, P.H., and Rosenberg, S.A.: In vitro growth of murine T cells: VI. Accelerated skin graft rejection caused by adoptively transferred cells expanded in T cell growth factor. J. Immunol. 127:566-571, 1981.

13. Eberlein, T.J., Rosenstein, M.M., and Rosenberg, S.A.: Successful adoptive immunotherapy of a disseminated murine cancer using immunized cells expanded in T-cell growth factor. Surg. Forum 32:452-454, 1981.

14. Strausser, J.L., Mazumder, A., Grimm, E.A., Lotze, M.T., and Rosenberg, S.A.: Lysis of human solid tumors by autologous cells sensitized in vitro to alloantigens. J. Immunol. 127:266-271, 1981.

15. Rosenberg, S.A., Eberlein, T., Grimm, E., Mazumder, A., and Rosenstein, M.: Adoptive transfer of lymphoid cells expanded in T-cell growth factor: Murine and human studies. In Fefer, A., and Goldstein, A.L. (Eds.): The Potential Role of T Cell Subpopulations in Cancer Therapy New York, Raven Press, 1982.

16. Grimm, E.A., and Rosenberg, S.A.: Production and properties of human IL-2. In Fathman and Fitch (Eds.): Isolation, Characterization and Utilization of T Lymphocyte Clones, New York, Academic Press, 1982, in press.

17. Rosenberg, S.A., Grimm, E.A., Lotze, M.T., and Mazumder, A.: The growth of human lymphocytes in T-cell growth factor: Potential applications to tumor immunotherapy. In Mizel, S.B. (Ed.): Lymphokines, New York, Academic Press, Inc., 1982, in press.

18. Rosenberg, S.A.: Potential Use of Expanded T-Lymphoid Cells and T-Cell Clones for the Immunotherapy of Cancer. In Fath and Fitch (Eds.): Isolation, Characterization and Utilization of T Lymphocyte Clones, New York, Academic Press, Inc., 1982, in press.

19. Brown, J.M., Shoffner, P.C., Tondreau, S.P., Matthews, E.J., Terry, W.D., and Rosenberg, S.A.: Cytotoxic reactivity in the sera of melanoma patients to paired autologous and allogeneic cultured tumor and skin fibroblasts. Cancer Res., in press.

20. Mazumder, A., Grimm, E.A., Zhang, H.Z., and Rosenberg, S.A.: Lysis of fresh human solid tumors by autologous lymphocytes activated in vitro with lectins. J. Immunol., in press.

21. Eberlein, T.J., Rosenstein, M., Spiess, P.J., and Rosenberg, S.A.: The generation of long term T-lymphoid cell lines with specific cytotoxic reactivity for a syngeneic murine lymphoma. J. Natl. Cancer Inst., in press.

22. Grimm, E.A., Mazumder, A., and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. V. Generation of allospecific cytotoxic lymphocytes to nonimmunogenic antigen by supplementation of in vitro sensitization with partially purified T-cell growth factor (PP-TCGF). Cellular Immunol., in press.

23. Eberlein, T.J., Rosenstein, M., Spiess, P., Wesley, R., and Rosenberg, S.A.: Adoptive chemoimmunotherapy of a syngeneic murine lymphoma using long-term lymphoid cell lines expanded in T cell growth factor. Cancer Immunol. Immunoth., in press.

24. Rosenberg, S.A., Eberlein, T.J., Grimm, E.A., Lotze, M.T., Mazumder, A., and Rosenstein, M.: The development of long-term cell lines and lymphoid clones reactive against murine and human tumors: A new approach to the adoptive immunotherapy of cancer. Surgery, in press.

25. Rosenberg, S.A., Sharp, T.G., Sachs, D.H., Fauci, A.S., and Messerschmidt, G.L.: T-cell depletion of human bone marrow using monoclonal antibody and complement mediated lysis. Transp., in press.

26. Grimm, E.A., Mazumder, A., and Zhang, H.Z.: The lymphokine activated killer cell phenomenon: Lysis of NK resistant fresh solid tumor cells by IL-2 activated autologous human peripheral blood lymphocytes. J. Exper. Med., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03826-08 SURG

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biologic Tumor Markers in the Cells and Sera of Patients with Urologic Cancers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: N. Javadpour Senior Investigator SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | 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 (200 words or less - underline keywords)

Techniques have been developed to localize and quantitate biologic tumor markers in the cells and sera of patients with urologic cancers. These markers include human chorionic gonadotropin, alphafetoprotein, pregnancy specific glycoprotein ($SP_1$), placental alkaline phosphatases, placental protein #5, 10, 15 different isoenzymes of lactic dehydrogenase (LDH), ABO(H) antigens, and T-Antigen. These markers have been correlated with the histology, grade, stage, and management of patients with urologic cancers. Experimental animal models of cancer induction for the kidney, bladder, prostate, and testis have also been developed. These animal models are being utilized to study the role of cytoreductive surgery, chemotherapy, and immunotherapy of these urologic cancers. Athymic mice have been utilized for xenografts of testicular tumor that produce markers. By utilizing specific antibody labeled with $^{131}I$, the role of radioimmunodetection and radioimmunotherapy is being investigated in testicular and prostatic cancer.

PHS-6040
(Rev. 2-81)

PUBLICATIONS

1. Bergman, S., and Javadpour, N.: The cell surface antigen A, B, or O(H) as an indicator of malignant potential in Stage A bladder carcinoma. J. Urol. 119:49, 1978.

2. Javadpour, N., Hyatt, C.L., and Barone, J.J.: Common antigens found on fetal cells and viral transformed adult prostatic tissue. J. Surg. Oncol. 10:245, 1978.

3. Javadpour, N.: The value of biologic markers in diagnosis and treatment of testicular cancer. Sem. in Oncol. 6:37, 1979.

4. Javadpour, N., Bagley, D.H., and Zbar, B.: Failure of cryosurgical treatment of experimental intradermal tumors to eradicate microscopic lymph node metastases in guinea pig. J. Natl. Cancer Inst. 62:1479, 1979.

5. Woltering, E.A., Emmott, R.C., Javadpour, N., Marx, J., and Brennan, M.F.: ABO(H) cell surface antigens in parathyroid adenoma and hyperplasia. Surg. Forum 30:107-109, 1979.

6. Javadpour, N., Woltering, E.A., and Soares, T.: Simultaneous measurement of tumor cytosol and peripheral serum levels of HCG and AFP. Invest. Urol. 18:11, 1980.

7. Javadpour, N.: Immunocytochemical discordance in localization of pregnancy specific $_1$ glycoprotein ($SP_1$), alphafetoprotein (AFP) and human chorionic gonadotropin in certain testicular cancer. J. Urol. 124:615, 1980.

8. Javadpour, N.: Radioimmunoassay and immunoperoxidase of pregnancy specific $_1$ glycoprotein ($SP_1$) in certain testicular cancer. J. Urol., in press (April 1980).

9. Johnson, H., Flye, M.W., and Javadpour, N.: Serum $_2$ microglobulin levels in patients with testicular cancer. Urology 15:522, 1980.

10. Woltering, E.A., Knox, R.D., and Javadpour, N.: Detection of human chorionic gonadotropin in fresh and formalin-fixed testicular tumor tissue: a comparison of immunoperoxidase to radioimmunoassay. Urology 16:215, 1980.

11. Javadpour, N., Woltering, E.A., and Soares, T.: Simultaneous measurement of tumor cytosol and peripheral serum levels of human chorionic gonadotropin and alphafetoprotein in testicular cancer. J. Urol. 18: 11, 1980.

12. Javadpour, N.: Radioimmunoassay and immunoperoxidase of pregnancy specific $_1$ glycoprotein in sera and tumor cells of patients with certain testicular germ cell tumors. J. Urol. 123:514, 1980.

13. Mathisen, D.J., and Javadpour, N.: En bloc resection of inferior vena cava in cytoreductive surgery for bulky retroperitoneal metastatic testicular cancer. J. Urol. 16:51, 1980.

14. Javadpour, N.: Significance of elevated serum alphafetoprotein in seminoma cancer. September 1980.

15. Javadpour, N.: The role of biologic tumor markers in testicular cancer. Cancer 45:1755, 1980.

16. Javadpour, N., Utz, M., and Soares, T.: Immunocytochemical discordance in localization of pregnancy specific $_1$ glycoprotein ($SP_1$), AFP and HCG in certain testicular cancers. J. Urol. 124:615, 1980.

17. Woltering, E.A., Knox, R.D., and Javadpour, N.: Detection of HCG in fresh and formaldehyde fixed testicular tissue: The sensitivity of immunoperoxidase vs. RIA. Urology 18:11, 1980.

18. Emmott, R., Droller, M.J., and Javadpour, N.: The ABO(H) cell surface antigens in carcinoma in situ and nonmalignant lesions of the bladder. J. Urol. 125:32, 1981.

19. Burt, M., and Javadpour, N.: Germ cell tumors in patients with apparently normal testes. Cancer 47:191, 1981.

20. Javadpour, N., Goldenberg, D.M., and Kim, E.: Radioimmunodetection of metastatic testicular cancer with radioactive antibodies to human chorionic gonadotropin and alphafetoprotein. JAMA 246:45, 1981.

21. Javadpour, N., and Chen, H.: Improved human chorionic gonadotropin detection with carboxyl-terminal radioimmunoassay of the beta subunit on concentrated 24-hour in patients with testicular cancer. J. Urol. 26: 176, 1981.

22. Lippert, M., and Javadpour, N.: Lactic dehydrogenase in monitoring and prognosis of testicular cancer. Cancer 48:2278, 1981.

23. Shull, J.H., Javadpour, N., and deMoss, E.V.: The ABO(H) cell surface antigens in carcinoma and benign lesions of the breast. J. Surgical Oncol. 18:193, 1981.

24. Javadpour, N., and Lippert, M.: The cell surface antigen in cancer of the bladder. In Progress in Cancer Research and Therapy, 1981, 18:107.

25. Javadpour, N., and Soares, T.: False positive and false negative alphafetoprotein and human chorionic gonadotropin assays in testicular cancer - a double blind study. Cancer 48:2279, 1981.

26. Javadpour, N., Soares, T., and Princler, G.: In vitro synthesis of AFP and HCG in nonseminomeatous testicular cancer. Cancer 49:303, 1982.

27. Javadpour, N., and Roy, J.B.: Combined cell surface antigens and chromosomal studies in bladder cancer. Urology 19:29, 1982.

28. Lippert, M., and Javadpour, N.: Detection of cell surface antigen in cancer of renal pelvis and ureter. Urol., in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06652-06 SURG |
|---|---|---|

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Immune Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | P. H. Sugarbaker | Senior Investigator | SURG NCI |
| Other: | W. Matthews, Jr. | Chemist | SURG NCI |
| | Y. Roth | Investigator | SURG NCI |
| | M. Kemeny | Medical Staff Fellow | SURG NCI |
| | C. McCullough | Clinical Associate | SURG NCI |
| | F. Gianola | Physician's Assistant | SURG NCI |

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | 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 (200 words or less - underline keywords)

The work in this laboratory includes three major projects: (1) Studies involve an assessment of cell-mediated immune responses after the host has responded to a variety of alloantigenic stimulation to the same and other antigens. The significance of this work comes from attempts to understand regulatory mechanisms of cellular immune responses. This work has resulted in the development of a new mechanism of T cell control - the alloantigen elimination hypothesis. (2) Studies on the use of activated killer cells in the destruction of tumor cells have been undertaken. The receptor on tumor cells for killer lymphocytes is the subject of current investigation. An adjuvant immunotherapy attack on cancer cells remaining after surgery is planned using cells activated in vitro to lyse tumor cells. (3) A new mechanism of specific suppression to facilitate the transplantation of tissue and skin allografts is being attempted. The use of antigen specific suicide with 3H-Thymidine to produce clonally depleted cell populations is under investigation.

Publications:

1. Smith, T.J., and Sugarbaker, P.H.: Xenogenic cellular immune responses and the control of microscopic tumor. Oncology 39:93-100, 1982.

2. Kemeny, M.M., and Sugarbaker, P.H.: Adoptive transfer of cells sensitized in vitro into mice in a skin allograft assay. Cell. Immunol. 67:197-206, 1982.

3. Kemeny, M.M., and Sugarbaker, P.H.: Host modifications of the skin allograft assay. J. Surg. Res. 32:540-546, 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06654-05 SURG |
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies in Malignant Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | W. F. Sindelar | Senior Investigator | SURG NCI |
| Other: | C. Kurman | Microbiologist | SURG NCI |
| | C. Hyatt | Biologist | SURG NCI |
| | C. Weiss | Clinical Associate | SURG NCI |
| | Y. Skornick | Visiting Fellow | SURG NCI |
| | T. Kinsella | Senior Investigator | ROB NCI |
| | A. M. DeLuca | Biologist | ROB NCI |

COOPERATING UNITS (if any)

Radiation Oncology Branch

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Surgery Branch

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with sarcomas and gastrointestinal carcinomas are studied for evidence of reactivity against tumor-associated determinants expressed on both fresh and cultured syngeneic or allogeneic tumor cells using immunofluorescence and immunoperoxidase staining techniques. Various human malignant cell lines have been established in vitro and are being characterized morphologically and immunologically. An experimental model of pancreatic carcinoma has been developed in hamsters. Tumor-associated antigens have been isolated from both animal and human pancreatic cancers and are being investigated for possible applications to immunotherapy or methods of immunodiagnosis. Tissue-specific antigens have been isolated and are being investigated for possible use in immunotherapy of pancreatic carcinoma. Intraoperative radiotherapy is evaluated in dogs to determine responses of both normal and surgically-manipulated tissues to direct single-dose electron-beam irradiation in approaches to adapt operative radiotherapy to the treatment of human abdominal malignancies.

PUBLICATIONS

1. Tepper, J., Sindelar, W.F., and Glatstein, E.: Phase I study of intra-operative radiation therapy combined with radical surgery for intra-abdominal malignancies. Proc. Am. Soc. Clin. Oncol. 21:395, 1980.

2. Sindelar, W.F., Javadpour, N., and Bagley, D.H.: Histological and ultra-structural changes in rat kidney after cryosurgery. J. Surg. Oncol. 18:363-379, 1981.

3. Tepper, J., and Sindelar, W.F.: Summary of the workshop on intra-operative radiation therapy. Cancer Treat. Rep. 65:911-918, 1981.

4. Dresdale, A., Sindelar, W.F., and Kurman, C.: Preliminary identification of a pancreatic tumor associated antigen (TAA) in an experimental animal model. Proc. Am. Assoc. Cancer Res. 22:297, 1981.

5. Sindelar, W., Tepper, J., Travis, E., Schwade, J.G., Padikal, T., and Terrill, R.: Normal tissue tolerance to intraoperative irradiation in canines. Proc. Am. Soc. Clin. Oncol. 22:429, 1981.

6. Skornick, Y., Sindelar, W., and Shinitsky, M.: Effective tumor immunization in mice and positive skin test in cancer patients induced by tumor cells of increased membrane lipid microviscosity. Proc. Am. Assoc. Cancer Res. 22:289, 1981.

7. Sindelar, W.F., Kinsella, T., Tepper, J., Rosenberg, S.A., and Glatstein, E.: Intraoperative radiotherapy and radical surgical resection of various intra-abdominal malignancies: Report on first 20 patients treated at the National Cancer Institute. Proc. Am. Assoc. Cancer Res. 23:133, 1982.

8. Sindelar, W.F., Tepper, J., Travis, E.L., and Terrill, R.: Tolerance of retroperitoneal structures to intraoperative radiation. Ann. Surg., in press.

9. Sindelar, W.F., Tralka, T.S., and Gibbs, P.S.: Evidence for acute cellular changes in human hepatocytes during anesthesia with halogenated agents: An electron microscopic study. Surgery, in press.

10. Skornick, Y., Gorelik, E., and Sindelar, W.F.: Reduction of metastases in murine malignancies by immunotherapy with syngeneic tumor cells treated with cholesterol hemisuccinate. Surg. Forum, in press.

11. Sindelar, W.F.: Cancer of the Small Intestine. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, J. B. Lippincott, in press.

12. Sindelar, W.F.: Demonstration of specific serologic reactivity in human osteosarcoma. Cancer, in press.

13. Sindelar, W.F., Tepper, J., and Travis, E.L.: Tolerance of bile duct to intraoperative irradiation. Surgery, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06655-02 SURG |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Analyses of Factors Influencing Host Cellular and Humoral Immune Responses to Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | J. A. Roth | Senior Investigator | Surg NCI |
| Other: | D. Davidson | Clinical Associate | Surg NCI |
| | J. Putnam | Clinical Associate | Surg NCI |
| | S. Keller | Clinical Associate | Surg NCI |
| | P. Scuderi | Staff Fellow | Surg NCI |
| | E. Trahan | Chemist | Surg NCI |
| | R. Ames | 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 MANYEARS: | PROFESSIONAL: | OTHER: |
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| 1.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our laboratory has focused on factors that influence host responses to tumors and may adversely influence responses to immunotherapy. We have identified an immunoregulatory factor produced by a variety of human tumors that profoundly inhibits in vitro cell mediated immune responses. We are in the process of characterizing and purifying this factor and also determining its role in vivo. We are developing sensitive new techniques to measure humoral immune responses to human tumor-associated antigens including enzyme-linked immunabsorbant solid-phase assay using soluble human tumor-associated antigens. We have collected over 100 extracts from human primary sarcomas as well as metastases. Using autologous patient sera, we are analyzing the distribution of tumor associated antigens on primary tumors and their metastases. Concurrently, we have established a murine melanoma model with cloned primary and metastatic cell lines to test various therapeutic options. We are preparing hybridoma monoclonal antibodies to human and murine tumor-associated antigens in an attempt to develop specific serotherapy for metastatic tumors.

PUBLICATIONS

1. Roth, J.A., Golub, S.H., Cukingnan, R.A., Brazier, J., and Morton, D.L.: Cell-mediated immunity is depressed following cardiopulmonary bypass. Ann. Thor. Surg. 31:350-356, 1981.

2. Roth, J.A.: Humoral immune responses to human primary and metastatic sarcomas detected by an enzyme-linked immunoabsorbant solid-phase assay. Surg. Forum 32:454-456, 1981.

3. Roth, J.A., Grimm, E.A., Gupta, R.K., and Ames, R.S.: Immunoregulatory factors derived from human tumors. I. Immunological and biochemical characterization of factors that suppress lymphocyte proliferative and cytotoxic responses in vitro. J. Immunol. 128:1955-1962, 1982.

4. Roth, J.A., and Wesley, R.A.: Human tumor-associated antigens detected by serologic techniques: Analysis of autologous humoral immune responses to primary and metastatic human sarcomas by an enzyme-linked immunoabsorbant solid-phase assay (ELISA). Cancer Res., in press.

5. Roth, J.A., and Osborne, B.A.: Detection of an immunosuppressive immunoregulatory factor in the sera of sarcoma patients by enzyme-linked immunoassay (ELISA) and correlation with clinical course. Surg. Forum, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06656-01 SURG |
|---|---|---|

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Definition and Modification of Neoplastic Tissue Sterol Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | P. D. Schneider | Senior Investigator | Surg NCI |
| Other: | C. M. Gorschboth | Medical Technologist | Surg NCI |
| | S. B. Edge | Clinical Associate | Surg NCI |

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

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NCI, NIH, Bethesda, Maryland 20205

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 1.0 | | |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Techniques and methods have been installed to investigate tumor sterol metabolism including: radioisotope determination of hepatic cholesterol synthesis in human subjects as an indicator of malignant transformation of the liver; specimens from a spectrum of thyroid disease have been collected for examination of membrane isoprenoid content to delineate effects on sterol synthetic pathways of various thyroid diseases; and in vitro studies with a rat hepatoma line to demonstrate a differential inhibiting effect of oxidized sterols on hepatoma growth as opposed to normal liver growth. Techniques for liposome targeting of sterol suppressors to take advantage of this latter effect in vivo are being developed simultaneously with techniques for lipsome delivery of other agents in a rat model investigating hepatic dearterialization and protein degradation in liver with and without metastatic rat colon carcinoma.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE BIOLOGICAL RESPONSE MODIFIERS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1981 through September 30, 1982

## INTRODUCTION

The Biological Response Modifiers Program (BRMP) is a comprehensive program of the Division of Cancer Treatment, National Cancer Institute (NCI), involved in clinical and laboratory research with both extramural and intramural components to investigate, develop and bring to clinical trials potential therapeutic agents which may alter biological responses important in the biology of cancer growth and metastasis. This program was conceived as a focused approach in DCT to support further basic research in biological response modfiers and to rapidly apply potential leads from that research to the treatment of cancer in man. The classes of agents to be investigated in this program include immunoaugmenting, immunomodulating and immunorestorative agents, interferons and interferon inducers, lymphokines, cytokines, antigrowth factors, thymic factors, tumor antigens and modifiers of tumor antigen cell surface components, antitumor antibodies, antitumor cells, and maturation and differentiation factors. It is recognized that considerable research is underway in each of these areas but a focused, coordinated approach by the NCI may result in the rapid acquisition of knowledge and a more rapid application of information to the treatment of cancer.

## BACKGROUND

The National Cancer Institute has been evaluating the potential role of biological response modifying agents in the treatment of cancer over the past several years. In March, 1975, Dr. Frank Rauscher, then director of the NCI, established the interferon working group to "monitor developments in interferon." The following November, the National Cancer Advisory Board recommended that the NCI purchase interferon for basic clinical studies. In November, 1975, a report to the DCT Board of Scientific Counselors recommended that interferon be further investigated as an antitumor agent. The Board recognized the emerging importance of biological response modifying agents and directed the staff of DCT, NCI to carefully monitor this important new field.

In mid 1976, the NCI purchased human leukocyte, lymphoblastoid and fibroblast interferons through the Division of Cancer Biology and Diagnosis (DCBD). Six investigators were given interferons for clinical trials and 33 investigators received interferon for basic laboratory research. The DCT Board of Scientific Counselors was again presented with information on the clinical use of interferon as an anticancer agent in October, 1976, with a review of the Karolinska Institute trials.

The increase in availability of interferon and the recognition that it might be a useful antitumor agent prompted the NCI to ask the Board of Scientific Counselors of the Division of Cancer Treatment to review the data on interferon as well as other biologicals that might have some influence on tumor growth and metastasis. In October of 1978, the Board recommended that a more concerted biological response modifiers program be developed within the Division of Cancer Treatment (DCT). The BRMP began with the appointment of the Subcommittee on Biological Response Modifiers (BRM Subcommittee) by the Board of Scientific Counselors, DCT in October, 1978. This Subcommittee was established because of the recognition that BRM were destined to play an increasing role in the treatment and understanding of cancer. The initial charge of this Subcommittee was to review existing clinical and laboratory data and to review ongoing investigations to develop guidelines for a focused program within DCT which could subsequently encourage, support and direct the NCI effort in this area.

This Subcommittee completed the very difficult task of reviewing the BRM background information and made programmatic recommendations in their interim report of September, 1979.

Through a series of meetings among the Subcommittee members, workshops organized by the Subcommittee, informal discussions with scientists conducting research in the area, extensive travel to and attendance at scientific meetings on BRM and through extensive contacts with consultants with expertise in the laboratory and clinic relevant to these agents, the Subcommittee completed an interim report for the Board of Scientific Counselors. This interim report defined BRM as those agents or approaches which modify the relationship between tumor and host by modifying the host's biological response to tumor cells with resultant therapeutic effects. Included in this definition were several approaches:

- to increase the host's antitumor responses through augmentation and/or restoration of effector mechanisms or decrease that component of the host reaction which may be deliterious;
- to increase host defenses by the administration of natural or synthetic effectors or mediators;
- to augment host responses to modified tumor cells which might stimulate a greater host response or increase tumor cell sensitivity to an existing response;
- to decrease the transformation and/or increase a differentiation (maturation) of tumor cells;
- to increase the ability of the host to tolerate damage by cytotoxic modalities of cancer treatment.

The interim report of the Subcommittee consisted of two documents. The Appendix to the interim report reviewed the pertinent literature and provided scientific background on BRM. This selective review attempted to identify promising approaches from previous studies with these agents. The interim report described the Subcommittee's view of how the BRMP should be organized, initiated and administered.

As the Subcommittee deliberated, DCT began to identify resources to support laboratory research and clinical trials of BRM. An interim administrative program was established in the Office of the Director, DCT and a search for the Program Director was initiated. The Subcommittee was consulted frequently by DCT Program Staff for assistance in decision making relative to the initial organization of the BRMP and relative to the design and initial organization of BRM research contracts, grants and clinical trials. As a result of these deliberations, DCT and the NCI made available 13.5 million dollars in fiscal year FY 80 to establish the BRMP and to begin initial work under this Program. Initially, there was a strong emphasis on interferon and interferon related research. There were lesser, though substantial, resources applied to other biological response modifiers.

The Program Director for the BRMP began working at the NCI in October, 1980 and the semifinal report by the BRM Subcommittee was presented to the Board in the same month. In consultation with DCT and those program officials involved in the initial organization of the BRMP, the Program Director set up and organized the BRMP according to the guidelines established by the BRM Subcommittee. This report will describe the progress to date and will describe future initiatives currently under consideration at the NCI.

PROGRAM OBJECTIVES

- Establish a well focused program within DCT to promote laboratory and clinical research in biological response modifiers.

- To support both basic and clinical research in the extramural community through a balanced program of grants and contracts. Requests for Applications (RFA), Program Announcements (PA) and Requests for Proposals (RFP) will all be used to support these efforts.

- Establish the BRMP as a resource for both the extramural and intramural community to provide certain BRM for laboratory and clinical use in further investigations as to mechanism of action and therapeutic efficacy.

- Establish a screening program and a BRM development network similar to that now in effect for drug development in DCT. Both a broad based screening program to identify potentially active agents (common track) and assays of more specific BRM (specific tracks) will be developed to assist the BRMP Decision Network Committee in making judgments about which BRM should be pursued through further preclinical work and formulated for clinical trials.

- Establish an intramural laboratory and clinical program to pursue investigations on basic mechanisms of action of BRM in animal tumor models, in in vitro assays and in man. Produce BRM through the genetic engineering and fermentation resources of the BRMP for potential use in clinical trials in man.

## INITIAL PROGRAM DEVELOPMENT

The BRMP was initially set up in the Office of the Director, DCT, in 1980. The Associate Director for the BRMP was identified in 1980 and officially came on board October 20, 1980. Much of the initial ground work, personnel actions, equipment purchases and other arrangements to begin the program were under way at the time of the announcement by Health and Human Services Secretary, Richard Schweiker of the official establishment of the BRMP in April, 1981. During the initial year, the BRMP grew and over 40 individuals including more than 14 scientists were working on BRMP projects. The laboratories were located in approximately 6,000 square feet of interim space as the BRMP building was being renovated. The clinical program was established at the Frederick Memorial Hospital. Clinical trials began in April, 1981 and the renovated clinical facility was officially opened and dedicated in October, 1981. Most of the sections and a majority of the activities planned for the BRMP were operational by spring, 1981.

## SPECIFIC ACTIVITIES OF THE ASSOCIATE DIRECTOR, BRMP

A major focus in FY 81 had been the development of the BRMP as a DCT program. The majority of the objectives for program development including personnel acquisition, equipment purchases, space acquisition and program development have been accomplished. The BRM screening program has been established and currently represents an evolving system to evaluate experimental model systems the therapeutic efficacy of biological response modifiers. A total of six workshops were sponsored by the BRMP in FY 81. The topics covered were as follows:

- Augmenting Agents in Cancer Therapy
- Workshop on Growth and Maturation Factors
- Role of NK, ADCC and Macrophages in Tumor Rejection and as Indicators of BRM Activity
- BRMP Hybridoma Monoclonal Antibody Workshop
- Potential Utilization of Lymphokines in Cancer Therapy
- Potential Role of T-cell Subpopulation and Their Modulation in the Therapy of Cancer

In addition to initial program development and workshop coordination, the office of Associate Director has been heavily involved with establishing the clinical trials of the BRMP. Our clinical program has been functional since April, 1981 with an inpatient unit, an outpatient unit and the appropriate supporting services to conduct clinical experimental research with BRM at Frederick Memorial Hospital. This clincial unit represents the sole clinical outlet for the Frederick Cancer Research Facility (FCRF) and is the major thrust of the BRMP. Finally, the extramural program has been established and is fully functional. A balanced program of grants and contracts is in place and further initiatives are currently being developed to further expand the scope of work being supported by the BRMP.

In December, 1981 the decision was made to merge the intramural BRMP with the Laboratory of Immunodiagnosis which had been headed by Dr. Ronald Herberman. This decision was made concomitant with the move of Dr. Herberman's laboratory to the FCRF. These laboratory personnel were located in Building 560, occupying approximately 11,000 square feet of Wing Three. An active process of scientific review of all ongoing projects within the two components of the intramural BRMP was initiated by Dr. Herbérman in November and December of 1981 and upon completion in January, 1982, a proposed reorganization plan for the intramural BRMP was presented to Dr. Bruce Chabner, Acting Director, DCT. Under this organization plan, the intramural branch was renamed the Biological Research and Therapy Branch (BRTB) and several sections were reorganized or established under the new branch as is described later in this report. Many of the research projects were continued but consistent with the plans to develop a more focused and restricted intramural program, many projects were discontinued. The extramural program was presented to the Board of Scientific Counselors in February, 1982 and the intramural program was reviewed for the board in June, 1982. By June of 1982, the BRMP intramural and extramural programs were at full strength and further changes were anticipated with additional personnel coming on board only as replacements for existing personnel. All scientific projects as listed in this report are underway and the initial publications of the program have indicated that excellent progress is being made.

The renovated facility, Building 567, was completed in June, 1982 and the Biochemistry Section, Lymphokines/Cytokines Section (Proposed) and the Monoclonal Antibody/Hybridoma Section were moved from interim space into their new laboratory space in Building 567. These laboratories were fully occupied and functional in June, 1982. The animal facility of the BRMP is also located in Building 567. This facility opened in June, 1982 and now provides all the animals used by the program. This animal facility provides animals for research by intramural scientists and will provide a certified facility for the production of monoclonal antibody for clinical use by the program. The facility includes both conventional animal rooms and a barrier. At the time of its opening in June, 1982, it is anticipated that over the subsequent, several months all BRMP animal work would be moved from the interim animal space that had been occupied at the FCRF and that all animal projects would be reinitiated through this animal facility.

The Associate Director and the BRMP administrative staff moved into the administrative offices in Building 567 in March, 1982. These facilities provide conference room and office space for the administrative staff for the BRMP. The administrative staff for the Biological Resources Branch (BRB) was moved from the Landow Building in Bethesda to interim space at the FCRF in late 1981.

With the move of the BRB administrative staff into Building 567, the offices vacated in Building 426 were made available for the BRB staff and they have occupied that area since March, 1982. Therefore, as it is currently constituted, the BRMP intramural laboratories are located in Building 560, Wing Three and in Building 567. The administrative staff is in permanent BRMP space and there are no further moves anticipated for BRMP personnel. This report will describe, project by project, the research activities of the BRTB and the research areas supported by the BRB. As of June, 1982, the BRMP can be considered as a fully functional program within the Division of Cancer Treatment. Although many biological response modifiers are being investigated, a major thrust for FY 81 was the use of interferon in therapeutic trials. The major thrust for FY 82 has been the development of monoclonal antibody as an anticancer agent. The major focus in the initial year of the BRMP was to establish the program as a working unit. This has been accomplished and the BRMP should now be able to approach most of the priorities established by the Division of Cancer Treatment and the Board of Scientific Counselors of DCT.

SUMMARY REPORT

BIOLOGICAL RESOURCES BRANCH

October 1, 1981 Through September 30, 1982

## INTRODUCTION

The Biological Resources Branch (BRB) of the Biological Response Modifiers Program (BRMP) is composed of two sections:

1. Procurement, Formulation and Preclinical Trials Section (PFPTS).
2. Clinical Trials Section (CTS).

The BRB supports, through a balanced program of grants and contracts, preclinical and clinical biological response modifiers (BRM) research in the biomedical community. The branch monitors Phase I and early Phase II clinical studies which assess biological effects of BRM in patients and correlate changes in the biological modifications with antitumor activity. The BRB has established a preclinical screening program for the selection and preclinical assessment of efficacy of BRM. In addition, by providing a BRM distribution resource system for both the general public biomedical and the NIH in-house biomedical communities to supply biological response modifiers for preclinical and clinical research, the branch has significantly furthered investigations into the mechanisms of action and therapeutic efficacy of BRM. This resource distribution system encompasses both information acquisition and assessment, as well as agent acquisition, testing, and distribution.

The Branch moved from Bethesda to Frederick in December 1981. Except for the Head, PFPTS, all personnel have joined the staff during FY 1982.

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|---|---|
| Chief, BRB ---------- | Richard V. Smalley, M.D. |
| Head, PFPTS ---------- | Cedric W. Long, Ph.D. |
| Acting Head, CTS -------- | Meyer R. Heyman, M.D. |
| Program Analyst --------- | James C. Vennetti |
| Secretary ---------- | Paula F. Wolfe |
| Health Scientist Admin.-- | Andrew J. Vargosko, Ph.D. |

## BIOLOGICAL RESPONSE MODIFIERS PROGRAM, PROCUREMENT, FORMULATION AND PRECLINICAL TRIALS SECTION

Cedric W. Long, Ph.D. assumed the role of Section Chief for this section of the BRB in September, 1980. This section is responsible for the identification of BRM of interest to the BRMP through literature reviews in addition to coordinating information access in such a manner that relevant information on potential agents is directed toward appropriate program personnel and working groups. The PFPTS also assists in the development of BRM through animal toxicology and therapeutic trials and serves as a liaison for this activity in the Developmental Therapeutics Program. Other responsibilities include the development of appropriate experimental systems for detection and evaluation of potential BRM and

the coordination, planning and monitoring of detailed evaluations of biological response modifiers in relevant systems. The Section Head serves as Project Officer on all BRB contracts in the preclinical area and as Program Director on all BRB preclinical grants.

BIOLOGICAL RESPONSE MODIFIERS CLINICAL TRIALS SECTION

The BRM Clinical Trials Section monitors clinical trials involving the use of BRM and administers clinical grants. An important aspect of this function is the close liaison with CTEP in assessing correlations between changes in immunological reactivity and clinical efficacy and toxicity in these studies.

Dr. Meyer R. Heyman, a clinical oncologist who was hired as of July 1, 1982, heads this section. Monitoring of clinical trials with CTEP personnel is an ongoing arrangement in which the BRMP and CTEP work conjointly.

SUMMARY OF FY 82 ACTIVITIES

In the clinical area, Phase I studies of three agents initiated in September of 1980 under the Master Agreement have been completed. Trials of MVE-2 were completed in December 1981 and recommendations to Cancer Therapy Evaluation Program (CTEP) concerning Phase II trials have been made. Phase I interferon studies were completed as of April 1982 and ongoing Phase II studies using leukocyte interferon have been organized and coordinated by the BRB. Thymosin Phase I studies were completed in June and recommendations made to CTEP concerning Phase II studies. It is anticipated that a considerable body of knowledge will accumulate by the end of FY 1982 with respect to these three types of biological response modifiers.

During this year the BRB has also been involved in the following:

- Issued 4 Request for Awards (RFAs) and funded 10 grants as a result.
- Maintained 12 contracts that test Phase I clinical BRM.
- Initiated 4 Request for Proposals (RFPs) and awarded 4 new contracts in FY 1982.
- Initiated 1 Master Agreement and awarded one Task Order under it.
- Initiated and awarded 2 new clinical Task Orders under the Master Agreement held by the 27 institutions.
- Monitored 7 compounds in the BRM preclinical Screen.

GRANTS AND CONTRACT ADMINISTRATION

A major focus of the BRB has been the development of a system of grant and contract support and administration. The current and future initiatives of the extramural program should establish the BRMP as a major support mechanism for preclinical and clinical research into BRM. The branch staff maintains liaison with all pertinent peer review groups involved in grant and contract review. It also provides planning, direction, implementation, and evaluation of research supported by grants and contracts.

Liaisons have been established and maintained with other programs in the NCI, including the Immunology Program and the Tumor Biology Program in the Division of Cancer Biology and Diagnosis (DCBD), to minimize overlap in the grant and contract area. A regular working relationship is maintained with the Developmental Therapeutics Program in order that ongoing evaluations in the preclinical area can be jointly discussed as to efficacy and toxicity of BRM. A cooperative clinical evaluation system with the CTEP has been established to coordinate Phase I and Phase II BRM Clinical Trials.

GRANTS PROGRAM

In FY 1982 active grants numbered 78 which included those awarded and funded the previous fiscal year. Approximately $8.6 million was awarded in FY 1982 to 60 grants. These grants included 54 ROls, one R23, and five POls. An additional $2 million was awarded for approximately ten grants that were funded from the 72 applications received in response to four RFAs.

The grants can be categorized in specific areas as follows:

1. Interferons
2. Thymic Factors
3. Lymphokines
4. Adjuvants
5. Antibodies
6. Antigens
7. Lymphoid Cells
8. Growth and Maturation Factors
9. Miscellaneous Approaches to Biological Response Modification (Bone marrow transplantation, immunization by various altered cells, organ transplants, viral components and immune or necrosis factors)
10. Complications, Adverse Effects and Related phenomena Attending the Use and Evaluation of BRM in Cancer Therapy (Includes studies relevant to the safety of BRM when used therapeutically.)

Table I lists each grant title by referral area category.

CONTRACTS

The branch staff initiates contracts and provides programmatic direction, evaluation and monitoring for contracts supported by the BRB. In addition, BRB has assumed responsibility for seven contracts transferred from the Immunology Program, DCBD, and one contract transferred from the Developmental Therapeutics Program, Division of Cancer Treatment (DCT). Five of the DCBD contracts have expired and the remaining two will terminate January 1983.

In FY 1982, 37 contracts were active. The BRB awarded approximately $5 million to 22 extramural contracts including $1.7 million for eleven clinical task orders contracts and $3.4 million in eleven other non-clinical contract awards during FY 1982.

The contracts active and projected for funding in FY 1982 are shown in Table II. These contracts in general, provided (1) interferon for clinical and preclinical studies, (2) for the collection, storage, testing and quality assurance of BRM, and (3) for initial exploration into the monoclonal antibody and cytokine areas.

NEW INITIATIVES

The following RFAs were issued in September 1981:

1. Monoclonal Antibody in Animal Tumor Models
2. Monoclonal Antibody in Cancer Therapy
3. Therapeutic Use of Lymphokines in Cancer
4. Animal Tumor Models for Antipeptide Growth Factor And Maturation Factor Therapy

Four new grant program announcements have been prepared for issuance in FY 1982 and FY 1983 funding:

1. Development of cell lines producing lymphokines and cytokines with therapeutic effects as biological response modifiers.
2. Therapeutic effects of growth factors, maturation factors, and monoclonal antibody to growth factors on the growth and metastasis of cancer in animal tumor models.
3. Development of genetically engineered cell products for therapeutic application as biological response modifiers.
4. Development of methods of immunization that evoke effective in vivo anti-tumor immunity using purified tumor associated antigens as immunogens.

The following RFPs and Task Orders have been issued and are in various stages of review:

1. Chemical Coupling of Cytotoxic Agents Monoclonal Antibodies
2. Technical Support for Review and Evaluation of BRMs
3. Production of Hybridomas Secreting Monoclonal Antibody to Human Lymphokines
4. Production and Isolation of Human Macrophage Activating Factor
5. Production and Isolation of Type I/II Mouse Interferon (Master Agreement)
6. Phase I/II Clinical Evaluation of Anti-T-Cell Monoclonal Antibody and Ex Vivo Treatment of Plasma (Task Order under the clinical Master Agreement)
7. Phase I/II Clinical Evaluation of Anti-T-Suppressor Cell Antibody in Therapy (Task Order under the clinical Master Agreement)

The following RFPs are pending with contract awards anticipated in FY 1983 and are in different stages of genesis:

1. A Plan for the Acquisition and Distribution of BRM
2. The Procurement of Human Lymphoblastoid Interferon
3. The Use Of Lymphokines for Cancer Therapy
4. Phase I/II Clinical Evaluation of MDP Encapsulated in Liposomes

These new initiatives, in addition to those already in the BRMP, give the program the balanced approach recommended by the BRM subcommittee of the Board of Scientific Counselors.

CLINICAL PHASE I/II STUDIES UNDER A MASTER AGREEMENT

The BRMP established a Master Agreement mechanism in 1980 whereby 27 institutions were identified as capable of performing clinical studies using BRM (Table III). These studies are closely monitored by the BRB to assess significance of changes in immunological parameters observed in patients under treatment with BRM.

In previous fiscal years 14 task orders for testing interferons, thymosins and MVE-2 have been awarded under this Master Agreement (Table IV). Two task orders initially approved and funded to support the Phase I/II studies of fibroblast interferon have not been initiated because of unavailability of the product.

a). MVE-2

MVE-2, a potential macrophage stimulator and interferon inducer was tested in a Phase I clinical trial at Vanderbilt University and Ohio State University under contract with the Branch. A completed Phase I trial by investigators at Vanderbilt with 22 patients, in which MVE-2 was given intravenously up to a dose of 600 mg/$m^2$, noted significant proteinuria as a result of cumulative toxicity at around 2800 to 3000 mg total dose. At doses above 300 mg/$m^2$ there was some augmentation of NK reactivity and an occasional patient seemed to have an augmentation of interferon levels with MVE-2. However, these changes were not dramatic and inconsistencies were noted. The Ohio State results obtained in 15-20 patients were similar as to toxicity and inconsistent biological response modification although some monocyte mediated antibody dependent cytotoxicity augmentation was seen in some of the patients. Of the 35 or so patients treated in these two trials, only one or two minor responses were noted.

b). Interferon

Phase I trials with Burroughs-Wellcome lymphoblastoid interferon were conducted at UCLA and Duke University in a total of 49 patients. Doses of up to 50 million units I.M./$m^2$ on different schedules have been achieved. The data are too preliminary to analyze for biological response. Georgetown has entered approximately 25 patients on a Phase I single dose trial utilizing Meloy leukocyte interferon in doses up to 60 million units/$m^2$. Toxicity was not seen. Because of formulation problems, larger doses could not be given. The Northern California Oncology Group has treated 15 patients with Meloy leukocyte interferon in doses up to approximately 36 million units daily for 30 days.

Finally, investigators at Sidney Farber have studied the Warner-Lambert preparation of leukocyte interferon primarily in approximately 30 breast cancer patients. Two trials evaluating fibroblast interferon, to be performed at Sloan-Kettering and at the University of Wisconsin have been delayed pending availability of fibroblast interferon.

c). Thymosin

The thymosin studies are near completion. Phase I trials have been done by the Northern California Oncology Group and by Sloan-Kettering using Fraction 5 thymosin. Two different schedules have been implemented. Doses of 240 mg/$m^2$ daily X 15 have been reached by the Northern California groups and doses of 600 mg/$m^2$, three times per week by Sloan-Kettering. Modification of T-cell numbers and function has been nil. Of interest is the fact that the Northern California group has seen several responses with thymosin Fraction 5 which were reported upon at the ASCO meetings. A randomized PhaseI/Phase II study is being done at George Washington University utilizing the alpha-1 thymosin fraction at 900 micrograms/$m^2$ in comparison with a placebo. Alpha-1 serum levels are being measured and biological responses being carefully assessed in this study. Over 25 patients have been entered. Finally, Phase I trials utilizing both Fraction 5 and alpha-1 have been conducted at the University of California, San Diego and at the Fred Hutchinson Cancer Center in Seattle. The single doses utilized in California have produced little in the way of consistent biological responses and doses of Fraction 5 up to 960 mg/$m^2$ and of alpha-1 to 9.6 mg/$m^2$ have been used. In Seattle, the agents have been given daily for one week, Fraction 5 in doses to 960 mg/$m^2$ and alpha-1 to 1.2 mg/$m^2$. Although no responses have been seen in either of these two studies, allergic reactions with Fraction 5 (anaphylactic-like) in patients at higher dose levels have been seen in Seattle.

BRMP - DECISION NETWORK COMMITTEE (BRMPDNC)

The BRMPDNC has responsibility for guiding program staff in the overall development of the program; reviewing and selecting agents to be evaluated in the screen and to be studied clinically. The membership of this committee consists of representatives from DCT, Division of Cancer Biology and Diagnosis, Division of Cancer Cause and Prevention, the National Insitute of Allergy and Infectious Diseases, FCRF, the DCT Board of Scientific Counselors and its BRMP Subcommittee. Members have been included who have expertise in (1) toxicology (2) regulatory policies (3) basic research (4) pharmacology and (5) clinical medicine. Four of the ten rotating member slots will be reserved for extramural representation. The committee recently enlisted the prospective participation of a large number of consultants who have agreed to serve on a per request basis and will be available for consultation in their specific areas of expertise.

During this past year the BRMPDNC has sponsored a retreat on "Monoclonal Antibodies in Therapeutic Trials". During this one day retreat the state-of-the-science of monoclonal antibody research was reviewed and ideas formulated for future investigative approaches. The committee plans to hold annually 2-4 of these retreats on various topics.

## BRMP DECISION NETWORK COMMITTEE

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BRMP SCREEN

The BRB has developed a screening program which is designed to evaluate the activity of biologicals in the area of biological response. The screening program has a single "common track" and multiple "specific tracks". The "common track" screen consists of a sequential evaluation using both *in vitro* and *in vivo* systems and is designed to test the ability of the agents to augment T-cell, B-cell, NK-cells and macrophage functions in both *in vitro* and *in vivo* circumstances. Agents are also tested for their ability to prevent metastases in tumors and in primary hosts bearing autochthonous neoplasms. "Specific tracks" are designed to test the capabilities of specialized biologics such as monoclonal antibodies and cytokines. Since October 1981, the following agents have been entered in the BRM screen:

1. MVE-2
2. MDP
3. Azimexon
4. Thymosin alpha 1 & Fraction 5
5. Interferon
6. NED 137

In vitro studies for drugs numbered 1, 2 and 3 have been completed.

The broad based screening program identifies potentially active agents (common track) and assays of more specific BRM (specific tracks) will be developed to assist the BRMPDNC in making judgements concerning which BRM should be pursued through further preclinical work and formulated for clinical trials. Compounds reported to show BRM activity which are potential candidates for study in the screen are listed in Table V.

These agents are to be categorized and prioritized on the basis of review by the BRMP Operating Committee, a smaller committee which then passes on its recommendation to the BRMPDNC for subsequent consideration for entry into either the "common track" or a "specific track" of the screen. The BRMPDNC then monitors progress through the screen and decides upon recommendations for clinical studies with each BRM.

Publications

None.

Major Presentations

None.

TABLE I

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| INTERFERON | | |
| 16857-04 | Merigan, T. | Stanford University<br>"Effect of Interferon Therapy on Non-Hodgkin's Lymphoma" |
| 19061-06 | Pitha, P. | Johns Hopkins University<br>"Antitumor Effect of Interferon" |
| 24987-03 | Joklik, W. | Duke University<br>"Virus and Cells Inhibitory Activity of Interferon" |
| 26475 | Fleischmann, W.R. | University of Texas<br>"Modulation of Antitumor Effect of Interferon" |
| 26966-02 | Meyers, J.D. | Fred Hutchinson Cancer Research Ctr.<br>"Interferon for CMV Infection, Leukemia Relapse and GVHD" |
| 27590-02 | Johnson, H. | University of Texas<br>"Human Immune Interferon Preparation Antitumor Effect" |
| 29990-02 | O'Malley, J. | Roswell Park Memorial Institute<br>"Immune Interferon in Cellular,Viral and Immune Systems" |
| 29991-02 | Gupta, S. | Sloan-Kettering<br>"Interferon-Induced Proteins in Mouse and Human Cells" |
| 30517-01 | Havell, E.A. | Trudeau Institute<br>"Antitumor Actions of Interferons" |
| 31080-01 | Ozer, H. | Roswell Park Memorial Institute<br>"Clinical Phase II Trial of HFIF in Lymphoma and Myeloma" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| THYMIC FACTOR | | |
| 26023-03 | Patt, Y.Z. | University of Texas<br>"Evaluation of Thymic Hormones in Human Cancer" |
| R-23<br>26335-03 | Merluzzi, V.J. | Sloan-Kettering<br>"Immunopharmacological Studies of Cyclophosphamide" |
| 28657-02 | Krown, S.B. | Sloan-Kettering<br>"Phase I Trial of Thymopoietin Pentopeptide in Cancer" |
| 29943-02 | Goldstein, A.L. | George Washington University<br>"Role of Thymosin Peptides in T-Cell Differentiation" |
| LYMPHOKINES | | |
| 13599-11 | Klein, E. | Roswell Park Memorial Institute<br>"Relation of Immune Reactions to Cutaneous Neoplasia" |
| 25740-03 | Papermaster, B. | Cancer Research Center<br>"Lymphokine Mediated Tumor Specific Immunity" |
| 26023-03 | Bigazzi, P.E. | University of Connecticut<br>"Inflammatory Autoimmune Responses and Neoplasia" |
| 26224-03 | Papermaster, B. | Cancer Research Center<br>"Mouse Tumor Assays for Immunotherapy" |
| 29145-01 | Papermaster, B. | Cancer Research Center<br>"A Lymphokine Fraction for Testing in Cancer Patients" |
| 30172-02 | DiSabato, G. | Vanderbilt University<br>"Lymphocyte Factors in Normal and Leukemic Mice" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| ADJUVANTS | | |
| 15325-08 | Gray, G.R. | University of Minnesota<br>"Antitumor Active Components of BCG Cell Walls" |
| 16342-04 | Zwilling, B. | Ohio State University<br>"Immunoprophylaxis and Immunotherapy of Lung Cancer" |
| 23338-03 | Neifeld, J.B. | Virginia Commonwealth University<br>"Intratumoral Immunotherapy Prior to Surgery" |
| 23906-03 | Green, S. | Sloan-Kettering<br>"Tumor Necrosis Factor: Purification and Mode of Action" |
| 24326-03 | DiLuzio, N. | Tulane University<br>"Developmental Therapeutics: Glucan |
| 24729-08 | Cummins, C.S. | Virginia Polytechnic Institute<br>"Comparative Testing of Vaccines from C. parvum" |
| 26286-03 | Neidhart, J.A. | Ohio State University<br>"Polymerized Tumor Antigen-Adjuvant Immunotherapy" |
| 27922-02 | Lamm, D.L. | University of Texas<br>"BCG Immunotherapy of Superficial Bladder Cancer" |
| 29570-01 | Stein, J.A. | Bellinson Medical Center<br>"Intralesional BCG Immunotherapy in Malignant Melanoma" |
| 32155-01 | Reinisch, C. | Sidney Farber Cancer Institute<br>"Selective Manipulation of T-Cell Subset by Adjuvant" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| ADJUVANTS (CON'T) | | |
| P01<br>15665-07A1 | Medoff, G. | Washington Univ. School of Medicine<br>"Polyenes as Biologic Response Modifiers" |
| ANTIBODIES | | |
| 15952-07 | Reif, A. | Boston City Hospital<br>"Experimental Studies on Immunity to Cancer Cells" |
| 16699-03 | Hiramoto, R. | University of Alabama<br>"A Model for Multiple Modality Therapy of Osteosarcoma" |
| 19127-04 | Jansons, V.K. | New Jersey Medical School<br>"Liposomes as Carriers for Anti-tumor Agents" |
| 23865-03 | Mannick, J. | Peter Bent Brigham Hospital<br>"Transfer to Tumor Immunity with Immune-RNA" |
| 25854-02 | LoGerfo, P. | Columbia University<br>"Purified Homologous Antibody: Carrier for Isotopes" |
| 26386-02 | Bernstein, I.D. | Fred Hutchinson Cancer Research Ctr.<br>"Monoclonal Antibody Therapy of Cancer" |
| 28740-02 | Bast, R.C. | Sidney Farber Cancer Institute<br>"Specific Immunotherapy with Monoclonal Antibodies" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| ANTIBODIES (CON'T) | | |
| 29160-01 | Griffin, T. | University of Massachusetts Medical School<br>"Ricin A Chain/Anti-CEA Antibody Conjugates" |
| 29544-01A1 | Wang, M.C. | Roswell Park<br>"Immuno-Specific Chemotherapies of Prostate Cancer" |
| 29660-02 | Vaage, J. | Roswell Park<br>"Immunotherapy of Mammary Tumors" |
| 30520-01 | de Noronha, I.M. | Cornell University<br>"Serotherapy of Virus-Induced Feline Sarcoma or Leukemia" |
| 30573-01 | McCune, C.S. | University of Rochester<br>"Specific Immunotherapy of Renal Carcinoma" |
| 31288-01 | Primus, J.F. | University of Kentucky<br>"Tumor Localization and Therapy with Immunoliposomes" |
| R-23<br>33573-01 | Merritt, W.D. | George Washington University<br>"Anti-glycolipid Monoclonal Antibodies: Cytotoxin Target" |
| P01<br>25863-02 | Bolognesi, D. | Duke University<br>"Control of Neoplasia by Passive Serum Therapy" |
| ANTIGENS | | |
| 22513-03 | Steele, G. | Sidney Farber Cancer Institute<br>"Minimal Residual Disease After Rat Colon Carcinoma" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| ANTIGENS (CON'T) | | |
| 28498-02 | Stolfi, R.L. | Catholic Med. Ctr. Brooklyn Queens Nursing<br>"Tumor Immunotherapy with Pharmacological Agents" |
| 28696-02 | Oettgen, H. | Sloan-Kettering<br>"Experimental Therapy of Human Melanoma with Vaccines" |
| 28738-01A1 | Bortin, M. | Mt. Sinai Med. Ctr., Milwaukee<br>"Alloimmunization for Induction of Antitumor Immunity" |
| 29597-02 | Vosika, G. | University of North Dakota<br>"Bacterial Component Immunotherapy in Cancer" |
| 30276-01 | Niederkorn, J.Y. | University of Texas<br>"Immunological Modulation of Ocular Tumor Metastasis" |
| LYMPHOID CELLS | | |
| 10777-13 | Fefer, A. | University of Washington<br>"Adoptive Chemoimmunotherapy of Tumor in Mice" |
| 25184-02 | Klein, E. | Karolinski Institute<br>"Autoreactive Cells in Tumor Patient" |
| R-23<br>25486-03 | Maki, T. | New England Deaconess Hospital<br>"Generation of Effector Cells for Tumor Immunotherapy" |
| 25608-02 | Merluzzi, V.J. | Sloan-Kettering<br>"Specific Repair of Drug-Induced Immune Cellular Deficits" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| LYMPHOID CELLS (CON'T) | | |
| 25765-02 | Mitchell, J. | University of Southern California<br>"Effects of Therapy on Tumor Specific Immunity" |
| 29005-01 | Mannick, J. | Peter Bent Brigham Hospital<br>"Adoptive Immunotherapy in Renal Cell Carcinoma Patients" |
| 30558-01 | Cheever, M.A. | University of Washington<br>"Specific Immunotherapy of Murine Tumors" |
| GROWTH AND MATURATION FACTORS | | |
| 15581-07 | Dennert, G. | Salk Institute<br>"Immune Response to Cell Surface Antigens" |
| 21790-03 | Takayama, K.K. | University of Wisconsin<br>"Antitumor Activity of Glycolipid Endotox Mix" |
| 27765 | Hiramoto, R.N. | University of Alabama<br>"Exploitable Mechanisms in Combination Cancer Therapy" |
| MISC. APPROACHES TO BIOLOGICAL RESPONSE MODIFICATION | | |
| 17393-07 | Donahoe, P. | Massachusetts General Hospital<br>"Mullerian Inhibiting Substance" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY / GRANT NUMBER | PRINCIPAL INVESTIGATOR | INSTITUTION AND GRANT TITLE |
|---|---|---|
| MISC. APPROACHES TO BIOLOGICAL RESPONSE MODIFICATION (CON'T) | | |
| 17467-06 | Emeson, E.E. | Montefiore Hospital and Med. Ctr. "Adoptive Immunotherapy of Cancer by Allogeneic Marrow" |
| 18105-06 | Applebaum, F.R. | Fred Hutchinson Cancer Research Ctr. "Immunotherapy Studies of Spontaneous Malignancy" |
| 24553-03 | Huang, L. | University of Tennessee "Targeting of Liposomes to Tumor Cells" |
| 24936-03 | Proctor, J.W. | Allegheny-Singer Research Center "Bioassay for Antitumor Effects of R.E. Stimulants" |
| 27330-02 | Nishioka, K. | University of Texas "Antitumor Activity of Tuftsin" |
| 27579-02 | Cohen, E. | University of Illinois Med. Ctr. "Use of Syngeneic Hybrid Cells in Leukemia Therapy" |
| 27615-02 | Barth, R. | Ohio State University "Immunity to Tumor Localized Chemically-Modified Drugs" |
| 28630-02 | Hoffman, M. | Sloan-Kettering "Activation and Function of QAS + NK Cells" |
| 28835-02 | Cohen, S. | State Univ. of N.Y. at Buffalo "Cancer Chemotherapy and Murine Natural Killer Cells" |
| 29769-01 | Hersh, E. | University of Texas "Biological Response Modifier Therapy of Human Cancer" |

TABLE I (CONTINUED)

GRANTS CLASSIFIED BY REFERRAL AREA CATEGORY

| CATEGORY<br>GRANT NUMBER | PRINCIPAL<br>INVESTIGATOR | INSTITUTION AND<br>GRANT TITLE |
|---|---|---|
| MISC. APPROACHES TO BIOLOGICAL RESPONSE MODIFICATION (CON'T) | | |
| 33776-01 | Arai, K. | University of Pennsylvania<br>"Mechanisms of Tumor Regression by Hapton Treatment" |
| PO1<br>18221-06 | Storb, R.F. | Fred Hutchinson Cancer Research Ctr.<br>"Marrow Grafting in Treatment of Hematologic Malignancies" |
| PO1<br>23766-04 | O'Reilly, R.J. | Sloan-Kettering<br>"Marrow Transplantation in Aplastic Anemia and Leukemia" |
| COMPLICATIONS, ADVERSE EFFECTS AND RELATED PHENOMENA ATTENDING THE USE AND EVALUATION OF BRM IN CANCER THERAPY | | |
| 27598-02 | Harris, J. | Rush University<br>"Cancer Drug Effects on Patient Suppressor Cells" |

TABLE II

BIOLOGICAL RESPONSE MODIFIERS PROGRAM
BIOLOGICAL RESOURCES BRANCH

CONTRACTS PROJECTED FOR FUNDING IN FY 1982

| CONTRACTS | Estimates ($000) |
|---|---|
| Characterization and Analysis of Proteinaceous Materials | 108 |
| Phase I/II Clinical Trials of BRMs (Includes 11 Task Orders) | 1,682 |
| Purchase of Lymphoblast Interferon | 622 |
| Purchase of Type II Human Interferon | 270 |
| Collection, Storage and Quality Assurance of BRM | |
| Task A - Repository, Quality Assurance and Testing for IND | 108 |
| Task B - BRM Activity Testing | 189 |
| Technical Support for the Review and Evaluation of BRM | 430 |
| Production and Isolation of Type I/II Mouse Interferon | 500 |
| Chemical Coupling of Cytotoxic Agents to Tumor Reactive Monoclonal Antibody | 200 |
| Production and Isolation of Human MAF | 500 |
| Production of Monoclonal Antibody to Human Cytokines | 150 |
| Immunotherapy Contracts (DCBD Transfer of 2 Projects) | 320 |
| Extramural Contract Total | $5,079 |

TABLE III

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

INSTITUTIONS IN BRMP CLINICAL MASTER CONTRACT

University of California/Los Angeles
University of California/San Diego
University of Cincinnati
Dartmouth University
Duke University
Fox Chase Cancer Center
Fred Hutchinson Cancer Research Center
Georgetown University
George Washington University
Hahnemann Medical School
Illinois Cancer Council
Institute de Cancerlogie, France
Mayo Clinic
University of Minnesota
Northern California Cancer Program
Ohio State University
Ontario Cancer Institute, Canada
University of Pittsburgh
Roswell Park Memorial Institute
Sidney Farber Cancer Institute
Sloan-Kettering Institute
University of Southern California
Temple University (Southeastern Study Group)
University of Texas (M.D. Anderson)
Vanderbilt University
Wayne State University
Wisconsin University

TABLE IV

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

CLINICAL TASK ORDERS UNDER THE CLINICAL MASTER AGREEMENT - BRMP[1]

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 |
| University of California, San Diego | Thymosin |
| Fred Hutchinson Cancer Research Center | Thymosin |
| George Washington University | Thymosin |
| Sloan-Kettering Institute | Thymosin |
| Northern California Cancer Program | Thymosin |
| Vanderbilt University | MVE-2* |
| Ohio State University | MVE-2* |

PHASE II TASK ORDERS

| | |
|---|---|
| Georgetown University | Lymphoblastoid Interferon |
| Sidney Farber Institute | Lymphoblastoid Interferon |
| University of California, Los Angeles | Lymphoblastoid Interferon |
| Duke University | Lymphoblastoid Interferon |
| University of Wisconsin | Lymphoblastoid Interferon |
| Sloan-Kettering Institute | Lymphoblastoid Interferon |

[1]Initially Funded FY 1980
*Inactive after December 1981

TABLE V

BIOLOGICAL RESPONSE MODIFIERS PROGRAM DECISION NETWORK COMMITTEE

POTENTIAL BRM AGENTS AND APPROACHES

A. Immunomodulator Agents

*Azimexon
Cimetidine
Therafectin (SM1213)
DTC (Sodium diethyldithiocarbamate)
Bestatin
*NED-137
Krestin
Picibanil
Thiabenzimidazole
Alkyl lysophospholipids (ALP)
BAI 7433
CP 46665
Pretazetin
Thiazolidine-14-carboxylic acid (thioproline "Norgamem")
Isoprinosine
NPT 15392
Russian glucomannan
Amphotericin B

B. Immunostimulator Agents

*MDP
*Tuftsin
Lentinan
Glucan (soluable)
Mannozyme
Levan
Tilorones

C. Immunorestorative Agents

Levamisole
Thiabenzimidazole

D. Miscellaneous Chemicals

6-Ayrlpyrimidinoles
C353
Substituted pyrimidine from Upjohn Company
CL-246,738 (Cyanamid)
Prostaglandin Inhibitors (Aspirin, Indomethacin)

E. Miscellaneous Bacterial Extracts or Bacteria

BCG, (P3, CWS)
Nocardia rubra CWS
Staph. phage lysate
Pseudogen
Brucella abortus (Bru-pel)
C. Parvum
Aerobacteria polysaccharide
Corynebacterium equi.
Microbicyclic peptide (Pseudomona)
Mixed Bacterial Vaccines

F. Miscellaneous Biologicals

Tumor necrosis factor
Endotoxin
"Immune" RNAs

G. Interferons and Interferon Inducers

*Interferons
*MVE-2
Poly IC/LC
Bru Pel
Tilorones
Levan
Blue tongue virus
Mumps Virus

H. Thymic Factors

*Thymosins - Alpha 1
*Thymosins - Fraction 5
Other thymic factors

I. Lymphokines - Cytokines

Macrophage activation factor/MAF
Lymphocyte activation factor/LAF
Cytotoxic factor (lymphotoxin - Ll)
Transfer factor
Colony stimulating factor
T helper cell replacing factor (TRF)
Macrophage chemotactic factor
T-cell growth factor (TCGF)
Thymocyte mitogenic factor (TMF)
Macrophage inhibiting factor (MIF)

J. Antibody

*Monoclonal antibodies against:
Anti-T-cell
Anti-T-suppressor cell
Anti-tumor Ab
Antibody to lymphokines, cytokines, etc.
Cytophilic antibody

K. Antigens

Tumor Associated Antigens

L. Alloantigen Effector Cells

Effector cells - T-cell clones
T-helper cells

M. Growth/Maturation Factors

Chalones
Antigrowth Factors
Maturation Factors
Growth Factors

N. Miscellaneous Approaches

Bone Marrow Transplantation and Reconstitution
Plasmapheresis
Allogenic Immunization
Virus Infection of Cells
Vaccinia

NOTE: Agents are listed in order of priority under each category as assessed by the BRMPOC and BRMPDNC in 1981. Categories are not in any order of priority.

* Identified agents currently approved (per BRMPDNC) to enter in the BRM screen.

SUMMARY REPORT

BIOLOGICAL RESEARCH AND THERAPY BRANCH

October 1, 1981 Through September 30, 1982

## INTRODUCTION

The Biological Research and Therapy Branch (BRTB) of the Biological Response Modifiers Program (BRMP), was formed as a result of a merger in December 1981 of the Biological Development Branch of the BRMP with the Laboratory of Immunodiagnosis, DCBD, NCI. At that time the entire staff of the Laboratory of Immunodiagnosis moved to the NCI-Frederick Cancer Research Facility and began to occupy space in proximity with the Biological Development Branch. The staffs of both branches were merged and a reorganization plan was developed in which all of the personnel were assimilated. Dr. Ronald B. Herberman became chief of the new branch and the scientific personnel were divided into seven sections: Clinical Investigations Section (Stephen A. Sherwin, M.D., Acting Head), Monoclonal Antibodies/Hybridoma Section (Kenneth A. Foon, M.D., Proposed Acting Head), Biochemistry Section (James A. Braatz, Ph.D., Acting Section Head), proposed Lymphokines/Cytokines Section (Section Head to be appointed), Natural Immunity Section (Ronald B. Herberman, M.D., Acting Section Head), Immunopharmacology Section (Michael A. Chirigos, Ph.D., Section Head), and proposed Monocyte Macrophage Section (Section Head to be appointed). The following is a summary of the major research findings of the Branch during this fiscal year.

The Clinical Investigations Section of the Biological Research and Therapy Branch is responsible for the investigation of the therapeutic efficacy of BRMs and the analysis of biologic response modification and toxicity of BRMs. This section of the BRMP was established to facilitate the early clinical trials of biologic products with potential as anticancer agents. Agents being tested initially include interferons, lymphokines, immunomodulators, and monoclonal antibodies. The Clinical Investigations Section is particularly concerned with in-depth Phase I and II trials of biological response modifiers involving small numbers of patients. Optimal immunomodulatory doses as well as maximum tolerated doses of these new agents are being determined.

The Clinical Investigations Section of the BRMP began clinical trials in early May 1981. During the past year, the Clinical Investigations Section has continued to concentrate on Phase I trials of various types of interferons. A major part of the work of this section during the past year has involved early clinical trials of two representative alpha interferons: highly purified nonrecombinant lymphoblastoid interferon (provided to the National Cancer Institute by the Burroughs Wellcome Company) and highly purified recombinant leukocyte A interferon (provided to the National Cancer Institute by Hoffmann - La Roche, Inc.). These two interferon preparations are currently available in a large quantity and could conceivably be tested on a long-term basis in cancer therapy.

Phase I trials of highly purified recombinant and nonrecombinant alpha interferons were completed during this year. Recombinant leukocyte A interferon was tested in two multiple-dose Phase I trials, in which dosages were escalated

in groups of 5 or more patients. Patients were extensively monitored for toxicity, antitumor effect, immunologic function and serum interferon activity. Eighty-one patients were entered by the Clinical Investigations Section on a three times weekly multiple-dose Phase I trial of this agent. The BRMP also participated in a collaborative manner with the Baltimore Cancer Research Center in a similar multiple-dose trial of this agent on a twice daily schedule of administration. These trials showed that recombinant leukocyte A interferon could be administered safely in doses up to $54 \times 10^6$ units daily and $118 \times 10^6$ units three times weekly. The side effects seen with this agent included fever, chills, fatigue, anorexia, myalgia, nausea, vomiting, headache and dose-dependent reversible leukopenia and hepatotoxicity. These toxicities resembled those previously reported for nonrecombinant leukocyte interferons of considerably lower specific activity. This suggests that such toxicities are probably a direct result of the interferon molecule itself. Antitumor effect was seen in 9 out of 81 patients (partial remissions) with diagnoses of non-Hodgkin's and Hodgkin's lymphoma, chronic lymphocytic leukemia, breast cancer and melanoma. Serum interferon activity was found to be comparable to that previously reported for natural Cantell alpha interferon. Dose-dependent pharmacokinetics were demonstrated and evidence for steady-state accumulation on the three times weekly schedule of administration was found in doses greater than $50 \times 10^6$ units. Analysis of immunologic monitoring of patients revealed that most patients had no change or a decrease in their natural killer cell mediated cytotoxic activity. This decreased NK activity might be attributed, at least in part, to inhibitory factors present in the serum of these patients, a result which is being further explored. Approximately 80% of the patients demonstrated increased monocyte function as measured in a growth inhibition assay. Finally, lymphocyte blastogenesis was uniformly decreased in patients treated with this agent, as has been previously reported for other interferons.

Highly purified nonrecombinant lymphoblastoid interferon was tested in an escalating dose trial by both intravenous and intramuscular routes of administration. Twenty-nine patients were treated by the intravenous route and thus far 10 patients by the intramuscular route. These studies have indicated that the toxicities seen with lymphoblastoid interferon are similar to those described above for recombinant leukocyte A interferon. A maximum tolerated dose has been defined for the intravenously treated patients at $30 \times 10^6$ units daily x 5 days. Very high levels of serum interferon activity were seen with the intravenously treated patients. Antitumor effects (partial responses) were seen in 3 patients with non-Hodgkin's lymphoma, undifferentiated carcinoma and hypernephroma.

The primary aim of the Monoclonal Antibody/Hybridoma Section (MAHS) is to develop new hybridomas producing monoclonal antibodies directed toward tumor-associated antigens. This laboratory is attempting to raise both murine and human monoclonal antibodies. The major objective is to utilize such monoclonal antibodies for diagnosis and therapy of cancer. It is expected that these antibodies will be used diagnostically in vitro by studying patient's tumor cells with a panel of monoclonal antibody and in vivo by conjugating them to radionuclides such as $^{125}$-I and $^{111}$Indium for radionuclide imaging. In addition, some of these antibodies will be infused into appropriate cancer patients to study their potential beneficial effects on the patient's tumor. Antibodies will also be conjugated to drugs such as doxorubicin and methotrexate and to toxins such as diphtheria toxin and ricin, to investigate their anticancer

activity. Such therapies could be used to treat established tumors or perhaps may have their most important effect in the adjuvant setting. To better understand the role of monoclonal antibodies and immunoconjugates in cancer diagnosis and therapy, a number of guinea pig models for both solid tumors and leukemias have been established.

The MAHS has developed an IgG1 murine monoclonal antibody designated D3 that recognizes a 200,000 molecular weight antigen expressed on the surface membrane of the Line 10 hepatocellular carcinoma in strain 2 guinea pigs. This antibody is not found on the similarly derived Line 1 hepatocellular carcinoma and it is not found on any normal guinea pig tissues. Infusion of this monoclonal antibody into a guinea pig demonstrates specific binding by immunoperoxidase techniques to the Line 10 tumor but not to normal tissue. Radionuclide imaging using Iodine-125 and Indium-111 conjugated to the D3 monoclonal antibody demonstrates specific uptake in the Line 10 tumor and imaging of the tumor by radionuclide scanning. The D3 monoclonal antibody has been conjugated to doxorubicin and this conjugate has been demonstrated to bind to Line 10 cells and is toxic to 100% of these cells in vitro. D3 has also been conjugated to diphtheria toxin and in vitro studies also demonstrated specific binding and toxicity to Line 10 cells in vitro. In vivo studies using the D3 diphtheria toxin conjugates have demonstrated dramatic inhibition of tumor growth.

The MAHS has raised a number of murine monoclonal antibodies to human bronchogenic carcinoma cell lines. These monoclonal antibodies are all IgG1 and are recognizing different antigens on the surface of bronchogenic carcinoma cells. Some of the antibodies are more restricted to one type of bronchogenic carcinoma while others react with numerous subtypes of bronchogenic carcinoma (e.g. small cell, squamous cell) and carcinomas derived from other organs.

Various methods of screening these antibodies against tumor cell lines and tissues are being used. Most of the screening is done by a radioimmunoassay using either live cells, glutaraldehyde fixed cells, soluble antigens or membrane preparations. Enzyme-linked immunosorbent assays (ELISA) are also being used to study these various cell preparations. Tissue sections taken from tumors and normal tissues are also being studied with these monoclonal antibodies using immunoperoxidase techniques. In addition, immunofluorescence using flow cytometry (Becton-Dickinson FACS 4 and Ortho Cytofluorograph) is also utilized.

A different approach to raising monoclonal antibodies is being investigated for colon carcinoma. Tumors are either prepared in single-cell suspension for immunization and testing or they are grown in nude mice and the established tumor is then prepared into cell membranes or soluble antigens for immunization and testing. B cell lines are also established from patients by Epstein-Barr transformation. The objective is to raise monoclonal antibodies that not only have broad reactivity against colon carcinoma cells but some that may react uniquely with an individual patient's colon carcinoma. These antibodies could be used at a later time to treat the patient whose tumor was used for immunization.

Because of the potential adverse reactions to murine monoclonal antibodies and the possibility of developing antibodies with more specific activity, we have

been attempting to raise human monoclonal antibodies. Recent studies in collaboration with the Lymphokine Section have demonstrated successful in vitro sensitization of human lymphocytes with tetanus toxoid. These sensitized B lymphocytes can subsequently be fused to human myelomas and produce specific monoclonal IgG antibody that binds to tetanus toxoid. Currently, attempts are being made to adapt this to human tumor systems.

Monoclonal antibodies are being produced against human granulocytes, monocytes, eosinophils, and platelets. While human monoclonal antibodies to human B lymphocytes and T lymphocytes have been extensively described, antibodies to monocytes, granulocytes, eosinophils and platelets have been less completely studied. Under active study are three IgG1 monoclonal antibodies to human monocytes, five IgG1 monoclonal antibodies to human granulocytes, and eight monoclonal antibodies to human eosinophils that have various isotypes including IgG1, IgG2A, IgG2B, and IgM. A complete characterization of these antibodies against panels of normal and malignant cells, bone marrow cells, stem cells, and molecular characterization of the antigens is currently being completed. They will also be studied for their effect on various immune functions including phagocytosis, chemotaxis, natural killer activity, and superoxide production. The platelet antibody has been demonstrated to abrogate such platelet functions as aggregation, adhesion and serotonin release.

The Biochemistry Section conducts research on the isolation, purification, biochemical and biological characterization of human tumor-associated antigens, soluble mediators of the immune response and cellular growth factors; 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 immuno-diagnostic assays; and uses purified proteins to assay for amino acid sequence as a first step to determine nucleotide sequence and obtain the cDNA coding for the protein.

A human lung tumor antigen (LTA) has been purified to homogeneity and an improved version of a radioimmunoassay (RIA) for its quantitation has been developed. This RIA was used to measure LTA levels in a panel of 215 sera from patients with lung and other cancers, benign lung disease as well as normal controls. The results obtained were encouraging and suggest the possible usefulness of this technique for the diagnosis or monitoring of lung cancer. More specifically, LTA elevations above an established cutoff value (1.7 g/ml) were found in 2% of the normals, 13% of patients with non-lung malignancies and none of the patients with benign lung disease. Lung cancer patients showed more frequent elevations which was dependent on the histologic type of tumor. Thus, patients with squamous cell and adenocarcinoma tumors were more frequently elevated (42% and 60%, respectively) than those with large cell or small cell tumors (17% and 19%, respectively). To continue these studies, a constant and reproducible source of LTA was identified. Thus the tissue culture lung tumor cell line ChaGo has been maintained for this purpose. A relatively simple purification procedure has been developed which we plan to soon apply to a large batch of cells. This cell line is also being used as a model system for studies of the subcellular localization of LTA and the mechanisms involved in its biosynthesis. Cell-free protein synthesis directed by isolated mRNA from ChaGo is being carried out as an attempt to determine the origin of the multiple size and charge forms of LTA detected in crude tumor and cultured cell extracts.

In an alternate approach to the study of protein or other antigens associated with human lung tumors, a previously described monoclonal antibody (MoAb) 503-D8, with specificity for squamous, adeno, and large cell (but not small cell) lung carcinomas has been extensively used. The antigen recognized by this MoAb is a protein which is associated with the cell membrane, although a component can also be identified in the spent medium of cultured lung tumor cells. Isolated protein subunits of 15,000, 18,000, 70,000 and 150,000 daltons have been identifed using the HUT-125 lung tumor cell line which are immunoprecipitated by 503-D8. The largest form appears only in the supernatant and preliminary evidence suggests it is the only form which is glycosylated.

In a related area, antigens associated with human melanoma cells are being studied. With the aid of a monoclonal antibody, a 250K dalton glycoprotein has been identified which is found primarily associated with the plasma membranes of melanoma cells. Until recently this glycoprotein was thought to be melanoma specific. However, recent evidence indicates that this antigen is shared by both cultured and biopsied tumors of glial cell origin. The expression of this glycoprotein appears to be associated with malignant transformation but there may be heterogeneity in expression and possible molecular forms.

A second melanoma-associated antigen is being studied, again with the aid of a monoclonal antibody. This antigen is a 94K dalton glycoprotein which is found primarily in spent culture medium of both melanoma, carcinoma and fetal uveal melanocytes but not of a variety of normal adult tissues and cells. Recently, the 94K oncofetal antigen has been observed in normal human serum. Detectable expression of the antigen has been confined to *in vivo* derived and cultured tumor cells and fetal cells. Detailed analysis of the antigen in human serum indicates that at least some of the antigen circulates complexed with IgG antibody. In addition, free antibody to the 94K antigen can be detected and corresponds in molecular weight to an $F(ab^1)_2$ fragment.

The proposed Lymphokines/Cytokines Section has made significant progress in the development of the capability of production and/or isolation of lymphokines and cytokines, ranging from macrophage activating factor (MAF) to tumor-associated transforming growth factor. Assays for these and other factors are either under development or are fully developed and in use. Methods of large-scale production of factors such as MAF and macrophage migration inhibitory factor (MIF) are being developed to provide substantial quantities of these lymphokines for experimental purposes. Partial purification of tumor-associated transforming growth factor (TGF) has been accomplished and an animal model that can mimic the observations seen in cancer patients has been developed. A correlation between TGF activity in the urine and tumor burden has been found in this guinea pig mammary carcinoma model. This observation, coupled with the demonstration of TGF activity in the urine of a high percentage of patients with disseminated cancers, suggests that further evaluation of TGF activity in urine of cancer patients may provide information helpful in following a patient's course and response to treatment. Work on a fully purified and biochemically characterized thymic hormone (thymosin $_1$) has led to designation of a 6 amino acid region that evidently contains the active region of the molecule when it is included with the rest of the C-terminal portion of the polypeptide. This particular sequence seems to be unique to thymosin $_1$ and may be a crucial sequence for the development and maturation of lymphocytes.

Work on the effects of lymphokines on macromolecular synthesis by macrophages has shown that activation for cytotoxicity is associated with an inhibition of macrophage RNA synthesis, and mainly with decreased levels of 28S ribosomal RNA. Compounds that are specific inhibitors of RNA induce cytotoxicity in macrophages, suggesting that a decreased rate of RNA synthesis and the acquisition of cytotoxic activity may be casually related.

A mouse monoclonal antibody (MoAb), produced by injections of human cultured T cells (CTC), was shown to totally abrogate the proliferative responses of CTC to IL-2 and appears to react against the cell-surface receptor for IL-2. Several MoAb against cell surface structures of T lymphocytes or subsets of T cells were screened for their ability to stimulate proliferation of fresh T cells or CTC, or to inhibit the response of $10^5$ CTC to optimal dose of IL-2 in a 3-day microwell $^3$H-thymidine assay (Bonnard et al., in "Immunology 80"). One MoAb reacted with very fresh T cells and with only a fraction (15-80%) of CTC by FACS analysis. However, it completely blocked CTC response to IL-2 in a quantitative manner, between the $10^{-4}$ and $10^{-6}$ dilutions (using ascites fluid). Like the antibody itself, the inhibitory activity could be readily absorbed out by CTC abd PHA-blasts ("activated T cells"), but not by fresh T cells or by T lymphoblastoid cell lines, and could not be attributed to contamination by mycoplasma or viruses. Overnight incubation of CTC with MoAb at 37°C capped a receptor of CTC for IL-2, as assessed by the subsequent inability of these CTC to absorb IL-2 or to proliferate with IL-2. CTC similarly treated with OKT4, OKT8 or anti-HLA MoAb were not affected. We conclude that this MoAb may detect the cell-surface receptor for IL-2. The known distribution of the MoAb-defined antigen on "activated" T cells and its preliminary biochemical characteristics are consistent with this antigen being the cell-surface receptor for IL-2.

Very promising results were obtained when human lymphokines were utilized to modulate the sensitization of human peripheral blood lymphocytes to a soluble antigen, tetanus toxoid in vitro. The fusion of these cells to human or murine myeloma cell lines led to the development of hybridomas that make specific antibody against tetanus toxoid. The numbers of antibody-producing cells produced during the sensitization could be markedly increased with lymphokine preparations in both human and murine systems. In addition, and of particular practical importance, these procedures have led to the production of human hybridomas making high levels of specific antibodies, in contrast to previous results in other laboratories, in which only low levels of antibody secretion were detected. The role of macrophages was shown to be very important in the in vitro immunization procedure and the effects of lymphokines on macrophages during these reactions to antigens appear to be crucial for augmentation or suppression of the antibody response.

Evaluation of various cell lines for lymphokine production has led to the adaptation of a human lymphoblastoid cell line RPMI-1788 as the cell line to be used for the initial isolation of these factors. Our results indicate that MIF, MAF and chemotactic factors are constitutively produced by this cell line, greatly simplifying the production methods.

The research activities of the National Immunity Section are primarily focused on the role of natural effector cells in resistance against cancer. There have been extensive studies on natural killer (NK) cells, with emphasis on the characteristics of these cells, the factors regulating their activity, and their in vivo role in resistance against tumor growth.

A major advance in the characterization of human NK cells has come from the finding of their close association with LGL, that possess a distinct morphology with Giemsa staining, containing azurophilic granules, with pale, blue-staining cytoplasm. More than half of these LGL form lytic conjugates with NK-susceptible targets. These cells have been isolated from peripheral blood lymphocytes using discontinuous Percoll density gradients and subsequent further purification by rosette formation with sheep erythrocytes at 29°C, to minimize contamination with small typical T lymphocytes. Using these procedures, we have been able to reproducibly obtain fractions containing >90% LGL by morphological analysis and containing <1% classical T cells as determined by morphology and monoclonal antibody analysis. Virtually all of the LGL have Fc receptors for IgG and they account for most, if not all, of the NK cell activity against NK-susceptible targets. We have extended our previous results by examining in detail a large variety of NK-susceptible targets, including both lymphoid and anchoragedependent lines. High density fractions of cells, which were enriched for classical T cells but devoid of LGL, could not be induced to become cytotoxic by the addition of activating agents such as IFN or IFN-inducers. Coupling our purification procedure with a single cell cytotoxicity assay, we have estimated the >70% of the LGL are functionally active NK cells.

Determination of the possible association of mouse NK cells with LGL has been considerably more difficult. However, during the past year, by modification of the conditions used for Percoll density gradient centrifugation, it has been possible to enrich fairly well for LGL in low density fractions and to obtain high density fractions of lymphocytes that are virtually devoid of LGL. The LGL-enriched subpopulations have been shown to be enriched for NK activity and the LGL-depleted subpopulations have had no NK activity.

Highly purified populations of LGL, which were essentially devoid of mature typical T lymphocytes, as well as classical T cells, maintained rapid growth when cultured in the presence of IL-2.

The cultured LGL demonstrated a pattern of cytotoxicity that was similar to that of fresh LGL, reacting mainly against NK-susceptible targets, and both fresh and cultured NK cells exhibited antibody-dependent and lectin-induced cytotoxicity.

It has been possible to also maintain the proliferation of mouse cytotoxic cultured lymphoid cells (CLC) for 1-12 months in the presence of IL-2. Clones from these cultured lymphoid cells were established by either limiting dilution or soft agar techniques. Most of the clones had strong cytotoxic activity against a variety of syngeneic and allogeneic tumor target cells. The clonal populations generally exhibited a more restricted pattern of cytotoxic activity than the parental CLC and the pattern varied among the clones.

Another approach to isolate a large number of highly enriched LGL would be to find naturally occurring LGL tumors which maintain their morphological and functional characteristics. These cells would be available in essentially unlimited quantities and could be used for those studies requiring a large number of cells for detailed analysis. Recently, we have demonstrated that there are LGL tumors in a high percentage of aged (greater than 24 months old) F344 rats. These tumor cell lines are obtained from animals with spontaneous mononuclear cell leukemia. A significant percentage (30-40%) of these cell lines demonstrate very high cytolytic potential.

IFN has been shown to have a variety of effects on immune reactivity, including the ability to rapidly augment cell-mediated cytotoxic responses such as the reactivity of NK cells, and macrophages, or monocytes.

To obtain better insight into the nature of the diversity of such effects by IFN, more than 12 preparations of human leukocyte IFN (homogenous or partially purified species of natural recombinant and hybridized recombinant) as well as highly purified beta (fibroblast) and immune (gamma) IFN were tested for their ability to augment the reactivity of NK cells and monocytes. All of the IFN species tested were shown to be able to significantly augment the cytotoxic reactivity of both NK cells and monocytes. However, when low levels of IFN were imployed, appreciable quantitative differences among the various species were seen.

Studies have been performed to determine the mechanism of IFN effects on NK cells. The IFN-induced increase in human NK activity was shown to be paralleled by an increase in (2'-5')oligo A synthetase, a major pathway involved in the antiviral action of IFN. By introducing the product of the enzyme, (2'-5') pppApApA, into human NK cells by the calcium phosphate precipitation method, the effect of IFN was mimicked.

We have found that partially purified preparations of IL-2, when present in the cytotoxicity assay, or after pretreatment of the effector cells, could substantially augment NK activity, to levels at least as high as that induced by IFN. The possibility was then considered that IL-2 might be an important factor for the spontaneous reactivity of human NK cells. To explore this possibility, we preincubated human LGL for various periods of time with monoclonal antibodies against human IL-2. Preincubation for 1-4 hours caused a significant decrease in spontaneous NK activity, and after 20 hours of preincubation with these antibodies, virtual elimination of reactivity was observed.

We have begun a detailed biochemical analysis of initial binding steps of purified LGL to NK-susceptible targets. We have found that solubilized material from the membranes of K562 target cells, inserted into lipid vesicles, can efficiently inhibit effector-target cell interactions, but does not inhibit the subsequent cytolytic reaction. Target cell structures, isolated from K562 (a myeloid leukemia cell line) has been demonstrated to be a glycoprotein (sensitive to 65°C, sensitive to trypsin, adherent to Con-A columns) of 100-150,000 MW. The specific activity of the material (based on 50% inhibition of binding) can be increased 25 to 100 fold by lectin column purification and elution with alpha-methylmannoside.

The cytotoxic activity of NK cells against primary tumors remains an important issue. Because of the ability to isolate highly purified populations of LGL and T cells, we have begun to examine NK activity of patients against autologous and allogeneic primary human tumors.

LGL had significant reactivity against most primary tumors. In contrast, no cytotoxicity was mediated by T cell or adherent cell fractions against either primary tumors or tumor cell lines.

With the ability to depress NK activity in vivo and specifically reconstitute animals with highly enriched populations of LGL, the rat provides a very useful model system for investigating the role of NK cells in the natural resistance

to tumors. We have recently shown that it is possible to specifically reconstitute the cytolytic function of animals treated by agents which depress NK activity (X-irradiation or anti-asialo GM1) by the transfer of 3-6 x $10^6$ LGL but not T cells or monocytes.

Results using the MADB106 mammary adenocarcinoma line have shown that IV injected tumor cells will colonize the lungs and grow at an increased rate in rats with depressed NK activity (anti-asialo GM1 treated). Preliminary results with LGL transfer indicates that LGL can significantly reduce the number of lung colonies and the rate of tumor growth in reconstituted animals. These results, although preliminary, are the first direct evidence for a role of LGL (NK cells) in control of metastasis.

The findings that in vivo administration of anti-asialo GM1 could selectively inhibit the cytotoxic activity of mouse NK cells without detectable inhibition of T cell-mediated immunity, also provided a good approach to further study the role of NK cells in the control of metastatic spread and growth of tumors. Treatment of mice with such antiserum made them considerably more susceptible to the development of lung metastases from intravenously inoculated B16 melanoma cells.

Studies have also been continued to explore the possible importance of NK cells in immune surveillance. As one model of primary carcinogenesis in mice, we have injected urethane into young mice. Strains of mice susceptible to development of pulmonary tumors after urethane showed an early, profound depression in NK activity after receiving urethane. In contrast, urethane treatment of strains of mice that are resistant to pulmonary carcinogenesis did not show depression of NK activity. The number of detectable tumor nodules in the lungs of mice transplanted with normal syngeneic bone marrow or spleen cells was significantly less than in control urethane-treated mice. Also, young mice of susceptible strains have been found to be more sensitive to the carcinogenic effects of urethane than older mice, and this was paralleled by greater sensitivity of the young mice to the NK-suppressive action of urethane.

As a second model of primary carcinogenesis, C57BL/6 mice were treated with a schedule of multiple, low doses of x-irradiation. Such treatment was found to result in a substantial deficit in NK activity and this depressed NK activity could be restored by transfer of normal bone marrow cells, a procedure which has been shown to interfere with radiation-induced carcinogenesis. As a further indication for the importance of NK depression in carcinogenesis in this model, mice of different ages were irradiated. It was previously demonstrated that susceptibility to carcinogenesis decreases rapidly after one month of age. In parallel, we have found that older mice do not have as long-lasting or profound a depression in NK activity after the treatment. Thus, in both of the primary carcinogenesis models studied to date, it appears that NK cells play an important role in resistance against development of tumors.

The major area of research for the proposed Monocyte/Macrophage Section is the study of monocytes and their tissue counterparts, macrophages, and the role of these cells in the defense against cancer. The investigators in the proposed Monocyte/Macrophage Section performs a wide variety of research investigations that fall into six general categories: investigations on the phenotypic, biochemical and functional characteristics of monocytes and macrophages; in-depth in vitro investigations into the ability of monocytes and macrophages to destroy tumor targets; analysis of the factors regulating the development and

activation of macrophages and monocytes; analysis of the role of monocytes and macrophages in the immune response and the interaction of these cells with other components of the immune response; investigations on the production of biologic response modifiers (BRMs) by monocytes and macrophages and the effects of BRMs on the functional activities of these and other component cells of the immune response; and development of in vivo models to test the relevance of of monocytes and macrophages to host defense against malignancy.

Animal models have been developed which allow us to compare the contribution of macrophage cytotoxicity against tumor cells vis-a-vis the cytotoxicity exerted by natural killer cells. Animals that were treated in a fashion that reduced their natural killer cell function (anti-asialo GM1 treatment) showed a much greater degree of metastatic disease following i.v. inoculation with B16 tumor cells. When these animals were treated with adoptive transfer of activated tumoricidal macrophages, the numbers of metastases in these partially NK-cell deficient animals was reduced. In contrast, the adoptive transfer of unactivated macrophages into animals resulted in a greater number of metastases found in these animals. Additional in vivo animal experimentation indicates that activated macrophages when given in adoptive transfer systems have different trafficking patterns from unactivated macrophages.

Two monoclonal antibodies directed against murine macrophage cell surface antigens have been recently produced, 36 and 52. These monoclonal antibodies react strongly with bone marrow derived macrophages and thioglycollate-elicited peritoneal macrophages, but react weakly with resident peritoneal cells. These monoclonal antibodies are being characterized in an attempt to provide reagents that can identify subpopulations of murine macrophages based on their membrane antigenic characteristics. An assay for plasminogen activator activity has been developed and is being used as an indicator for murine macrophage activation when these cells are treated with macrophage activating factor (MAF). Although there is a correlation between the cytotoxic function of macrophages treated with MAF and their plasminogen activator production, there is no positive correlation between macrophages activated by endotoxin and their plasminogen activator activity. Two murine T cell hybrids have been produced that produce MAF.

The ability of human monocytes to destroy tumor targets is an area of continued interest. Human monocytes have been shown to be capable of killing a wide range of human and mouse tumor targets. It has been documented that monocytes have a baseline spontaneous cytotoxicity against many types of tumor cells, and that this baseline spontaneous level of monocyte cytotoxicity can be augmented by a variety of agents including alpha interferon and MAF-containing human lymphokine preparations. Studies into the lymphocyte-monocyte interactions have shown that there are helper lymphocytes that are capable of secreting soluble factors that augment monocyte cytotoxicity and similarly there are suppressor lymphocytes and macrophages which under the appropriate circumstances can down-regulate monocyte cytotoxic function. Studies have been performed that document the role of liposomes in human monocyte activation. It has been shown that various activating factors, including MAF-containing lymphokines, can be packaged into multilamellar liposomes and that these packaged activating substances will be preferentially phagocytized by human monocytes. Following ingestion of MAF-containing liposomes (but not empty liposomes), macrophages have enhanced tumoricidal properties.

Detailed analysis of membrane events that are observed on monocytes and macrophages following activation signals have been performed. These studies show that changes occur at the cell membrane level which alter the ability of the cell to undergo transmethylation reactions of phospholipid pathways. These changes in transmethylation activity are correlated with the activation status of the monocyte. It has been demonstrated that some highly purified interferon preparations are capable of inhibiting transmethylation reactions at the level of the monocyte membrane. Similarly, tumor promotors such as phorbol myristate acetate were shown to decrease phospholipid methylation in a variety of peripheral blood mononuclear cells but this effect was most prominent in monocytes.

The Immunopharmacology Section 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.

Poly ICLC was examined in-depth for its immunoregulatory capacity. This agent was found to induce a high titer of interferon which correlated with enhanced macrophage and natural killer cell tumor lytic activity. A combination of Poly ICLC with cytoreductive treatment with Cytoxan resulted in a synergistic antitumor effect. Azimexon was found capable of reconstituting the number of bone marrow cells accompanied by a similar increase in granulocyte-monocyte colony-forming units in mice which were exposed to various doses of irradiation (100 to 400 rads). The results indicate a direct stimulatory effect of azimexon on the proliferation of nucleated bone marrow cells which is due to stimulation of hemopoietic precursor cells, mediated by a colony stimulating factor(s) for granulocytes and macrophages. Six BRMs were examined for their capacity to stimulate the delayed type hypersensitivity response (DTHR), which is a T cell driven response. Levan, Lentinan, Mannozym, $MVE_2$, Poly ICLC and IFN enhanced the DTHR and were found to correlate with their capacity to regulate macrophage and NK cell tumor lytic activity. Significant therapeutic response was achieved against an alveolar lung carcinoma when $MVE_2$ was combined with cytoreductive chemotherapy. The synergistic antitumor effect was considered to be due to the capacity of $MVE_2$ to enhance alveolar macrophage tumor lytic activity. Results of pharmacokinetic studies of $MVE_2$ indicate that the scheduling of treatment is critical in regulating effector cell responses. Single or multiple treatment with $MVE_2$ increased macrophage tumoricidal activity, equally; however NK cell augmentation was best following single dose treatment. $MVE_2$ and Poly ICLC were found to be effective adjuvants in combination with drug chemotherapy (Cytoxan, Adriamycin, 5-fluorouracil) in treating a mammary adenocarcinoma.

## CLINICAL INVESTIGATIONS SECTION

The Clinical Investigations Section of the Biological Research and Therapy Branch is responsible for the investigation of the therapeutic efficacy of BRMs and the analysis of biologic response modification and toxicity of BRMs. This Section of the BRMP was established to facilitate the early clinical trials of biologic products with potential as anticancer agents. Agents being tested initially include interferons, lymphokines, immunomodulators, and monoclonal antibodies. The Clinical Investigations Section is particularly concerned with in-depth Phase I and II trials of biological response modifiers involving small numbers of patients. Optimal immunomodulatory doses as well as maximum tolerated doses of these new agents are being determined.

The facilities of the Clinical Investigations Section include a four-bed inpatient unit at Frederick Memorial Hospital and a ten-bed outpatient unit located in the Medical Pavilion adjacent to the hospital. The well-equipped outpatient unit has greatly expanded the patient treatment capacity of the Clinical Section. In addition, a leukapheresis unit has been established in the outpatient unit to permit development of this treatment modality in cancer therapy.

The personnel of the Clinical Investigations Section include the following physicians:

Stephen A Sherwin, M.D., Acting Head
James A. Knost, M.D., Expert
Paul G. Abrams, M.D., Expert
Mehmet Fer, M.D., Visiting Scientist
Jeffrey Ochs, M.D., Medical Staff Fellow
Gino Bottino, M.D., Medical Staff Fellow

Patients entered into trials of the Clinical Investigations Section have been largely referred by their primary physician (generally a medical oncologist) with the understanding that they are to be referred back to this physician upon completion of their treatment. No attempt is made to provide multimodality, ongoing cancer care to these patients beyond the experimental treatment period. The referred patients have disseminated malignancies for which no effective curative therapy exists and yet are relatively early in their disease course with good performance status. A considerable effort in terms of correspondence and speaking engagements has been devoted to generating referrals from local and regional oncologists with considerable success. In addition, a Special Ambulatory Care Program (SACP) was also established to permit referral of patients from greater distances when otherwise appropriate.

The Clinical Investigations Section of the BRMP began clinical trials in early May, 1981. During the past year, the Clinical Investigations Section has continued to concentrate on Phase I trials of various types of interferon and has begun to examine monoclonal antibodies in Phase I trials as well. This represents an attempt to focus on two biological response modifiers with clear potential as anticancer agents. Within the area of interferon testing, the research program has limited its attention to an examination of representatives

of each of the three classes of interferons, namely alpha, beta and gamma. A major part of the work of this Section during the past year has involved early clinical trials of two representative alpha interferons: highly purified nonrecombinant lymphoblastoid interferon (provided to the National Cancer Institute by the Burroughs Wellcome Company) and highly purified recombinant leukocyte A interferon (provided to the National Cancer Institute by Hoffmann-La Roche, Inc.). These two interferon preparations are currently available in a large quantity and could conceivably be tested on a long-term basis in cancer therapy. Other clinical trials of representative interferons initiated during this year include a Phase I trial of immune interferon in cancer patients and a Phase I trial of genetically engineered fibroblast interferon. In this manner, the Clinical Investigations Section will have had an opportunity to study, in at least Phase I testing, an interferon of each of the three major types.

Phase I trials of highly purified recombinant and nonrecombinant alpha interferons were completed during this year. Recombinant leukocyte A interferon was tested in two multiple-dose Phase I trials, in which dosages were escalated in groups of 5 or more patients. Patients were extensively monitored for toxicity, anti-tumor effect, immunologic function and serum interferon activity. Eighty-one patients were entered by the Clinical Investigations Section on a three times weekly multiple-dose Phase I trial of this agent. The BRMP also participated in a collaborative manner with the Baltimore Cancer Research Center in a similar multiple-dose trial of this agent on a twice daily schedule of administration. These trials showed that recombinant leukocyte A interferon could be administered safely in doses up to $54 \times 10^6$ units daily and $118 \times 10^6$ units three times weekly. The side effects seen with this agent, including fever, chills, fatigue, anorexia, myalgia, nausea, vomiting, headache and dose-dependent reversible leukopenia and hepatotoxicity. These toxicities resembled those previously reported for non-recombinant leukocyte interferons of considerably lower specific activity. This suggests that such toxicities are probably a direct result of the interferon molecule itself. Antitumor effect was seen in 9 out of 81 patients (partial remissions) with diagnoses of non-Hodgkin's and Hodgkin's lymphoma, chronic lymphocytic leukemia, breast cancer and melanoma. Serum interferon activity was found to be comparable to that previously reported for natural Cantell alpha interferon. Dose-dependent pharmacokinetics were demonstrated and evidence for steady-state accumulation on the three times weekly schedule of administration was found in doses greater than $50 \times 10^6$ units. Analysis of immunologic monitoring of patients revealed that most patients had no change or a decrease in their natural killer cell mediated cytotoxic activity. This decreased NK activity might be attributed, at least in part, to inhibitory factors present in the serum of these patients, a result which is being further explored. Approximately 80% of the patients demonstrated increased monocyte function as measured in a growth inhibition assay. Finally, lymphocyte blastogenesis was uniformly decreased in patients treated with this agent, as has been previously reported for other interferons.

Highly purified nonrecombinant lymphoblastoid interferon was tested in an escalating dose trial by both intravenous and intramuscular routes of administration. Twenty-nine patients were treated by the intravenous route and thus far 10 patients by the intramuscular route. These studies have indicated that the toxicities seen with lymphoblastoid interferon are similar to those described above for recombinant leukocyte A interferon. A maximum tolerated dose has been

defined for the intravenously treated patients at 30 x $10^6$ units daily x 5 days. Very high levels of serum interferon activity were seen with the intravenously treated patients. The results of similar assays in the intramuscularly treated patients are currently pending. Antitumor effect (partial responses) were seen in 3 patients with non-Hodgkin's lymphoma, undifferentiated carcinoma and hypernephroma. Immunologic monitoring data from these trials are currently being analyzed.

Other interferon trials expected to begin during this year include Phase I trials of nonrecombinant immune (gamma) interferon, recombinant fibroblast (beta) interferon, and recombinant leukocyte A interferon administered intralesionally. In addition, Phase II trials were initiated of recombinant leukocyte A interferon in breast cancer, lymphoma and chronic lymphocytic leukemia. These latter trials were undertaken collaboratively with the Clinical Oncology Program, DCT, NCI. These trials will include further immunologic monitoring of the patients being treated in order to identify potential in vitro correlates of tumor response, and to determine the mechanisms responsible for the alterations in function. Finally, a small Phase II efficacy trial of lymphoblastoid interferon in patients with hypernephroma is scheduled to begin this year as well.

Early Phase I trials of antitumor monoclonal antibodies remain a high priority of the Clinical Investigations Section. Three trials of this type are scheduled to begin during this year, including Phase I trials of an anti-T cell monoclonal antibody in patients with chronic lymphocytic leukemia and cutaneous T cell lymphoma, an anti-melanoma monoclonal antibody being developed in the Intramural Laboratory Program of the BRMP, and anti-idiotype monoclonal antibodies in patients with refractory malignant lymphoma. Other antitumor monoclonal antibody trials are currently in the planning stages.

Although the Clinical Investigations Section will remain focused on the early clinical testing of interferons and antitumor monoclonal antibodies for the foreseeable future, on occasion other biological response modifiers with potential as anticancer agents will be tested. Along these lines, a small Phase I trial of 13-cis-retinoic acid as an immune modifier in cancer patients was initiated during this year. In this trial patients will receive doses of 13-cis-retinoic acid below that which has been previously shown to be the maximum tolerated dose. These low doses will perhaps have more favorable effects on the immune system and patients will be monitored accordingly. This approach to the Phase I testing of a biological response modifier should allow the determination of optimal biological doses which could, in fact, have more pronounced antitumor effects. This approach to the Phase I testing of potential biological response modifiers will remain a strong commitment of the Clinical Investigations Section in the future.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09200-02 BRTB |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Phase I Trials of Recombinant and Nonrecombinant Interferons in Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
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COOPERATING UNITS (if any)

Hoffmann-La Roche, Inc., Nutley, NJ: Burroughs-Wellcome Co., Research Triangle Park, NC; NCI-FCRF

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
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| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 10.0 | 6.0 | 4.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various recombinant and nonrecombinant interferons have been tested in Phase I trials in cancer patients in order to study the toxicity, antitumor effect, immunomodulatory effect and pharmacokinetics of these preparations. The initial Phase I trials employed highly purified recombinant leukocyte A interferon and human Namalva cell lymphoblastoid interferon. These trials, which are now complete, demonstrated that both interferons could be administered safely to patients with disseminated cancer and that the toxicities encountered resembled those previously reported for less purified Cantell alpha interferon. Limited antitumor effect was seen in both trials. Pharmacokinetic analysis" reveals dose-dependent levels of serum interferon activity. Immunologic monitoring data indicate unchanged or decreased natural killer cell mediated cytotoxicity, increased monocyte function in a growth inhibition assay and decreased lymphocyte blastogenesis. Other Phase I interferon trials recently initiated include trials of recombinant leukocyte A interferon as an intralesional agent and escalating dose trials of nonrecombinant gamma (immune) interferon and beta (fibroblast) interferon.

PROJECT DESCRIPTION

OBJECTIVES

Phase I trials of various recombinant and nonrecombinant interferons were conducted in order to determine a maximum tolerated dose and optimal biological response modifying dose which could be employed in future Phase II efficacy trials of these agents. Antitumor effect was noted, thereby giving some indication in which specific malignancies these agents might be active. These trials were also designed to study the in vivo immunologic effects of the various interferons being administered and also allowed for extensive pharmacokinetic monitoring of the patients being treated.

METHODS EMPLOYED

In these Phase I trials of recombinant and nonrecombinant interferons, patients with a variety of histologically proven malignancies refractory to standard curative therapy were treated with escalating dosages of the agents being tested. Patients were extensively monitored for clinical and laboratory signs of toxicity in order to determine and objectively define maximum tolerated dose for the interferon preparation. Antitumor effect was determined by serial measurements of indicator lesions on physical exam or on appropriate radiologic studies or serial measurements of tumor markers. Patients were also monitored extensively for immunologic effect in natural killer cell mediated cytotoxicity assays, monocyte growth inhibition assays, lymphocyte blastogenesis assays and enumeration of T-cell subpopulations by FACS analysis. Serum interferon activity was determined in full 24-hour profiles and periodically for steady-state determination in all patients treated with the agents and was measured by a bioassay consisting of inhibition of plaque formation by vesicular stomatitis virus. Two Phase I trials of two different highly purified alpha interferons were conducted. A multiple-dose trial of recombinant leukocyte A interferon, supplied by Hoffmann-La Roche, Inc. (specific activity $2 \times 10^8$ U/mg protein), was conducted in which patients received a fixed multiple dose on a 3 times weekly or twice daily schedule for a period of 28 days. Dosages were escalated after groups of 5 or more patients were treated at a given dose. Dosages employed ranged from $1\text{-}136 \times 10^6$ units intramuscularly 3 times weekly. In the other Phase I trial of an alpha interferon preparation, individual patients received escalating doses of highly purified nonrecombinant Namalva cell interferon (supplied by Burroughs-Wellcome Co.) by intravenous infusion 5 days weekly over a 5-week period of time. The dosages employed ranged from 0.1 to $50 \times 10^6$ units i.v. daily times 5 days. Other Phase I trials initiated during this year include an escalating single-dose trial of nonrecombinant immune interferon (Meloy Laboratories, Inc.), a multiple-dose trial of genetically engineered fibroblast interferon (Hoffmann-La Roche, Inc.), and an escalating-dose trial of recombinant leukocyte interferon as an intralesional agent.

MAJOR FINDINGS

I. Clinical Results

The fixed multiple-dose Phase I trial of recombinant leukocyte A interferon in patients with disseminated cancer involved a total of 81 patients. Of these 81 patients, 54 completed the trial and 12 patients were removed from study for reasons of drug toxicity, 8 for reasons of tumor progression, and 7 for other unrelated medical problems. The toxicities observed with this preparation included fever, chills, fatigue, anorexia, myalgia, headache, nausea and vomiting, and dose-dependent reversible leukopenia and hepatic transaminase elevations. A maximum tolerated dose for this agent on a 3 times weekly was defined as 118 x $10^6$ units based on the occurrence of unacceptable hepatic transaminase elevations at higher doses. Objective evidence of antitumor effect qualifying as a standardly defined partial remission was seen in 9 of 76 evaluable patients. These 9 patients included 5 patients with non-Hodgkin's lymphoma and 1 patient each with chronic lymphocytic leukemia, Hodgkin's disease, breast cancer and malignant melanoma. Most of these patients had been heavily pretreated with chemotherapy and/or radiation therapy. Responding patients were continued on a maintenance therapy regimen of recombinant leukocyte A interferon. Maintained partial remission lasted up to 7 months. The patients on this trial received full 24-hour pharmacokinetic profiles on days 1, 15 and 27 as well as frequent monitoring of serum interferon activity at other times in order to determine if steady-state accumulation occurred. Dose-dependent pharmacokinetics were observed on this trial, with increased peak levels of serum interferon activity at increasing doses. The half-life of serum interferon was determined to be between 6 and 9 hours. Evidence for steady-state accumulation of interferon was apparent at doses in excess of 50 x $10^6$ units i.m. 3 times weekly.

In the single escalating dose trial of human Namalva cell lymphoblastoid interferon, patients were treated by either intravenous infusion or intramuscular injection over a 5-week period of time during which the dose increased on a weekly basis from 0.1 to 1.0, 10.0, 30.0 and 50.0 x $10^6$ units i.v. daily times 5 days. This interferon is a highly purified nonrecombinant alpha interferon which is a mixture of eight or more molecular species of alpha interferon including that of the recombinant leukocyte A interferon discussed above. The specific activity of this preparation is 1-2 x $10^8$ units/mg protein. Twenty-nine patients were entered on the intravenous portion of this trial and 10 patients thus far on the intramuscular portion. The toxicities encountered with this interferon preparation are similar to those mentioned above for recombinant leukocyte interferon and previously reported for other less purified interferon preparations. The maximum tolerated dose appeared to be 30 x $10^6$ units i.v. daily x 5, based on unacceptable leukopenia and hepatic transaminase elevation. Objective evidence of antitumor effect qualifying as a partial remission was observed with this type of interferon as well. Three patients had partial remissions lasting 2-4 months including a patient with undifferentiated carcinoma of the pelvis, nodular poorly differentiated lymphocytic lymphoma, and hypernephroma. Patients were extensively monitored for serum interferon activity on this trial with full 24-hour pharmacokinetics being determined on day 1 of each treatment cycle for each patient and subsequent monitoring for steady-state activity. High levels of serum interferon activity

were seen with this particular preparation and evidence of steady-state accumulation was noted in doses in excess of 10 x $10^6$ units i.v. daily for at least 24-36 hours.

II. Immunological Monitoring

All patients entered onto the Phase I trials of recombinant leukocyte A interferon and highly purified nonrecombinant lymphoblastoid interferon were monitored extensively for immune function both prior to starting therapy and at several time points following the initiation of therapy. The studies were designed to determine if possible not only a maximum tolerated dose but also an optimum biological response modifying dose. A number of immunological assays were performed including assays for natural killer cell activity, monocyte function as measured in a monocyte growth inhibition assay, and T lymphocyte function as measured by the proliferative reponse of lymphocytes to mitogens and mixed leukocyte culture. In addition to these functional assays, determinations of lymphoid subpopulations were made using monoclonal antibodies and flow microfluorometry. To be certain that any changes observed after interferon administration were due to the interferon and not to other non-treatment related fluctuations in function, all assays were standardized and day-to-day variability in activity was assessed for each individual by repeated testing over several days prior to the start of therapy to determine baseline values. This allowed analysis in terms of significant alteration from the pretreatment range for each function being measured. An unexpected finding of this study was that in vivo administration of both types of alpha interferon did not lead to an augmented natural killer cell response but instead to no significant change in the majority and to a diminished response in about 30% of the patients. The number of patients who showed this diminished NK response was greater on the twice daily protocol than on the three times weekly protocol. This was not due solely to the administration of more interferon, as when the data were recalculated using a total weekly dose of interferon the depression that was seen was still greater on the twice daily protocol. The depression also was greater at the higher doses of interferon; with patients receiving greater than 80 million units per injection, almost all of the patients showed a diminished response. At the lower doses, although there was no augmentation of NK activity, the depression was not as great as at the higher doses. In contrast to the results with the NK cytotoxicity assay, monocyte function, as measured in a cytostatic assay, was seen to increase significantly in over 80% of the patients. Patients showed an increase when treated on any of the three protocols, and this increase was seen at all dose levels from 1 million units to 136 million units. The lymphocyte proliferation responses to mitogens and in mixed lymphocyte culture were seen to decrease in over 70% of the patients. This decrease was consistent at all dose levels with all schedules of administration. The analysis of the leukocyte subpopulations using monoclonal antibodies is not yet complete, but an initial evaluation of the data suggests an increase in the lymphocytes reacting with the OKT10 monoclonal antibody. There was no significant shift in the ratio of OKT4t (containing helper) to OKT8t (containing suppressor) lymphocytes following treatment with interferon. The reasons for the unexpected finding of decreased NK activity in patients receiving interferon in vivo are not yet clear. We have some preliminary evidence that the administration of interferon by the intramuscular route may induce circulating inhibitory factors which may interfere with the NK boosting effects of interferon. We have not

yet examined the serum of the patients treated by intravenous infusion of lymphoblastoid interferon to determine whether the decrease in NK activity seen in those patients has a corresponding inhibitory factor in the serum. In other studies where an augmentation of NK activity has been observed, the interferon was given as a single injection and not repeatedly as in these studies. Further studies are being undertaken to determine whether the lack of augmentation observed here was due to the type of interferon or to the route or timing of interferon administration.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH

These Phase I trials of various recombinant and nonrecombinant interferons in cancer patients will allow the determination of maximum tolerated doses that can be used in Phase II efficacy trials in specific types of cancer. These Phase II efficacy trials will in turn help to define the ultimate therapeutic role for these interferon preparations in a given malignancy. Moreover, the Phase I trials should in and of themselves serve as a model for the future early clinical trials of a variety of other biological response modifiers, genetically engineered and otherwise. Although these preliminary Phase I trials have not yet defined precise optimal biologic response modifying doses, the specific immunologic alterations seen in these patients indicate that it may be possible to design Phase II efficacy trials in which the dose and schedule employed reflect the immunomodulatory effects of the interferon being administered. Such trials may well indicate clinical activity for interferon that could otherwise not be appreciated.

## PROPOSED COURSE

Phase I trials of these various recombinant and nonrecombinant interferons will be completed. The information generated in these trials, including the definition of maximum tolerated dose, will be used to direct Phase II trials performed in larger clinical research facilities or in the cooperative group setting. These Phase I trials will have included examination of highly purified recombinant and nonrecombinant alpha (leukocyte or lymphoblastoid), beta (fibroblast) and gamma (immune) interferons in patients with different types of cancer.

## PUBLICATIONS

Sherwin, S., Fein, S., Whisnant, J., and Oldham, R.: Phase I trials of recombinant and nonrecombinant alpha interferons in cancer patients. In UCLA Symposium on Molecular and Cellular Biology, Vol. 25, in press.

Knost, J., Sherwin, S., Abrams, P., and Oldham, R.: Increased steroid dependence during therapy with recombinant leukocyte interferon. Lancet 2:1287-1288, 1981.

Maluish, A.E., Conlon, J., Ortaldo, J.R., Sherwin, S.A., Leavitt, R., Fein, S., Weirnik, P., Oldham, R.K., and Herberman, R.B.: Modulation of NK and monocyte activity in advanced cancer patients receiving interferon. In UCLA Symposium on Molecular and Cellular Biology, Vol. 25, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09233-01 BRTB |
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PERIOD COVERED: October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Phase II Trials of Recombinant Leukocyte Interferon in Patients with Breast Cancer and Lymphoproliferative Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | | |
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| Others: | Robert K. Oldham | Associate Director | | BRMP | NCI |
| | Dan Longo | Senior Investigator | | MB | NCI |
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| | Ian MaGrath | Senior Investigator | | POB | NCI |
| | Ronald Herberman | Chief | | BRTB | NCI |

COOPERATING UNITS (if any)

Hoffmann-La Roche, Inc., Nutley, NJ; NCI-FCRF

LAB/BRANCH: Biological Research and Therapy Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 2.0 | OTHER: 1.0 |
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year a Phase I trial of recombinant leukocyte interferon in patients with a variety of disseminated cancers revealed that this agent could be administered up to doses of 50 x $10^6$ units/$m^2$ i.m. 3 times weekly without unacceptable myelotoxicity or hepatic toxicity. This trial also showed objective evidence of antitumor response (partial remissions) in some patients with non-Hodgkin's lymphoma, breast cancer, chronic lymphocytic leukemia and Hodgkin's disease. Immunologic monitoring of patients receiving this agent failed to reveal any dose-dependent immunologic effect which correlated with tumor response. It was therefore decided to initiate Phase II efficacy trials of recombinant leukocyte interferon at a maximum tolerated dose with dose reductions as necessary for unacceptable toxicity. Phase II efficacy trials were initiated in patients with various lymphoproliferative disorders including non-Hodgkin's lymphoma, chronic lymphocytic leukemia and mycosis fungoides, as well as patients with refractory metastatic breast cancer. These trials are currently ongoing and results with regard to antitumor response and toxicity will be analyzed.

PROJECT DESCRIPTION

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 are encountered. No evidence is available at this time with regard to lower doses that might have an optimal biological response modifying effect. In the same Phase I trials, objective evidence of antitumor effect (partial remissions) was seen in several patients with non-Hodgkin's lymphoma as well as occasional patients with breast cancer, melanoma, chronic lymphocytic leukemia and Hodgkin's disease. It was therefore decided to initiate Phase II efficacy trials in lymphoproliferative disorders and refractory metastatic breast cancer using the maximum tolerated dose of 50 x $10^6$ units/$m^2$ i.m. 3 times weekly. These trials were intended to provide more precise information as to the antitumor efficacy of recombinant leukocyte interferon in these specific malignancies as well as to provide further information and insights into the immunologic effects of this agent and its longer term toxicities.

METHODS EMPLOYED

Two trials have been initiated. One involves patients with various lymphoproliferative disorders including previously treated patients with non-Hodgkin's lymphoma (both favorable and unfavorable histologies), chronic lymphocytic leukemia, American Burkitt's lymphoma, and mycosis fungoides. The other trial concerns patients with refractory metastatic breast cancer who have failed standard therapy with hormonal therapy (if ER positive) and combination chemotherapy. In both of these trials, patients receive 50 x $10^6$ units/$m^2$ i.m. 3 times weekly for a period of 3 months. Patients are monitored carefully for hematologic and hepatic toxicity as well as systemic toxicities known to be associated with interferon. These systemic toxicities include primarily fatigue and anorexia. Patients can have dose reductions to 50% and then 10% of their intial dose if unacceptable toxicity in any of these categories occur. Patients are monitored carefully for antitumor effect by periodic physical examinations and appropriate radiologic studies. Immunologic monitoring is being performed on these patients with periodic assays for natural killer cell mediated cytotoxicity, monocyte function as measured in a growth inhibition assay, lymphocyte blastogenesis assays, and enumeration of T cell subpopulations using FACS analysis. Patients are also being monitored for serum interferon activity following injections on a monthly basis in order to determine whether a drop in peak serum interferon activity might be predictive of interferon antibody formation.

MAJOR FINDINGS

These trials are currently ongoing and therefore no definite statements can be made at the present time with regard to antitumor efficacy, long-term

toxicity or immunologic effect of recombinant leukocyte interferon in these two malignancies.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH

These two trials will help to more precisely define the antitumor efficacy of recombinant leukocyte interferon in patients with refractory lymphoma and breast cancer. The dose chosen for these trials is the maximum tolerated dose as previously determined in a Phase I trial with appropriate dose reduction for unacceptable toxicity. Future Phase II efficacy trials or Phase III trials might employ doses below the maximum tolerated dose when a sufficient data base exists for determining which lower dose might be optimal for a biological response modifying effect. In addition to further defining the antitumor efficacy of this type of alpha interferon, these trials will further define the toxicities and immunologic effects of this interferon as well as its propensity to induce antibody formation in the recipients. The two trials described here are among the first Phase II efficacy trials of a genetically engineered biological response modifier in patients with cancer.

## PROPOSED COURSE

At the conclusion of these trials, the antitumor efficacy of recombinant leukocyte interferon in these specific malignancies will be analyzed. If signficant antitumor activity has been observed, then further trials in a Phase III setting are warranted. Such trials could compare the relative efficacy of interferon alone or in combination with other agents to standard therapy for the diseases in question. In addition, it might be worthwhile to examine the role of interferon in the adjuvant setting. At this point in the natural history of a malignancy, interferon as well as other biological response modifiers may well prove to be most effective since tumor burden is minimal and immunologic competence may be a relatively high level.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09234-01 BRTB |
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PERIOD COVERED October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Phase I Trial of 13 Cis-Retinoic Acid as an Immune Modifier in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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| Others: | Robert K. Oldham | Associate Director | BRMP | NCI |
| | James A. Knost | Expert | BRTB | NCI |
| | Paul G. Abrams | Expert | BRTB | NCI |
| | Jeffrey S. Ochs | Medical Staff Fellow | BRTB | NCI |
| | Ronald Herberman | Chief | BRTB | NCI |

COOPERATING UNITS (if any)

Hoffmann-La Roche, Inc., Nutley, NJ; NCI-FCRF

LAB/BRANCH Biological Research and Therapy Branch

SECTION Clinical Investigations Section

INSTITUTE AND LOCATION NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 2.0 | OTHER: 1.0 |
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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Retinoids, including 13-cis-retinoic acid, have been shown to have antitumor activity in man. The mechanism has been postulated to be a differentiating effect on the malignant cell. However, recently a variety of in vitro immunologic studies have shown that retinoids can increase cell-mediated cytotoxicity, natural killer cell activity, and complement-dependent cell lysis. In addition, retinoids can have various effects on monocyte function. It was therefore decided to initiate a Phase I trial of 13-cis-retinoic acid as an immune modifier in cancer patients. Patients will be treated with varying doses below the maximum tolerated dose, to determine if an optimal biologic response modifying dose exists for this compound.

PROJECT DESCRIPTION

OBJECTIVES

Although retinoids such as 13-cis-retinoic acid have been postulated to exert their antitumor effect by way of a differentiating effect on tumor cells, recent studies have shown that these compounds can also influence various components of the cellular immune system. It is therefore possible that at least some of the antitumor effect of retinoids may relate to their actions as immune modifiers. The Clinical Investigations Section therefore initiated a Phase I trial of 13-cis-retinoic acid as an immune modifier in patients with cancer. In this trial patients receive escalating doses of 13-cis-retinoic acid below the maximum tolerated dose and are monitored carefully for immunologic effect to determine whether this correlates with tumor response. The trial is intended to define an optimal biologic response modifying dose for this compound.

METHOD EMPLOYED

A total of 15 patients will be treated on this trial. Five patients each will receive doses of 5, 50 and 100 $mg/m^2$ p.o. daily x 6 weeks. Although these doses are less than or equal to the maximum tolerated dose for this compound, patients will be carefully monitored for the known toxicities of retinoids as well as antitumor effect. In addition patients will be monitored carefully for immunologic effect in a variety of assays including natural killer cell mediate cytotoxicity, monocyte function as measured in a growth inhibition assay, lymphocyte blastogenesis and T cell subset enumeration by FACS analysis. Patients will also be tested for the presence of retinoid metabolites to see whether the level of the compound in the serum correlates with the observed immunologic effects.

MAJOR FINDINGS

This trial was recently initiated and no definitive statements can be made at this time with regard to the antitumor or biologic response modifying effects of 13-cis-retinoic acid.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

13-cis-retinoic acid has been shown to have antitumor activity in patients with various epidermoid malignancies of the skin and head and neck as well as occasional patients with melanoma and colorectal carcinoma. In addition, patients with various premalignant lesions such as leukoplakia may benefit from the administration of this compound. Previous Phase I trials of 13-cis-retinoic acid have determined a maximum tolerated dose for this agent and this is the dose currently being employed in efficacy trials in patients with advanced cancer as well as in adjuvant therapy trials. The trial described here will provide information as to whether doses less than the maximum tolerated dose

having optimal effects on immune function might better be employed in studies of this type.

PROPOSED COURSE

The information generated in a Phase I trial of 13-cis-retinoic acid as an immune modifier in cancer patients will be used to direct further research in the anticancer effects of this class of agents. The Clinical Investigations Section of the BRMP began this trial in early May. An optimal biological response modifying dose for 13-cis-retinoic acid based on immunologic monitoring of patients in this trial could be used in future efficacy trials of this agent including trials which evaluate the use of 13-cis-retinoic acid in the adjuvant setting.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09235-01 BRTB |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Phase I Trials of Antitumor Monoclonal Antibodies in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| Others: | Kenneth Foon | Acting Head (Proposed) Monoclonal Antibody/Hybridoma Sect. | BRTB | NCI |
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| | James A. Knost | Expert | BRTB | NCI |
| | Paul G. Abrams | Expert | BRTB | NCI |
| | Jeffrey S. Ochs | Medical Staff Fellow | BRTB | NCI |
| | Gino Bottino | Medical Staff Fellow | BRTB | NCI |
| | Charles Morgan | Expert | BRTB | NCI |
| | Ronald Herberman | Chief | BRTB | NCI |

COOPERATING UNITS (if any)
NCI-FCRF

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 10.0 | 6.0 | 4.0 |

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoclonal antibodies reactive with various human tumor cells have been prepared from murine hybridomas according to standard techniques. Such antibodies have previously been administered to man by other investigators in limited Phase I trials and have been shown to be without serious toxicities and have occasional objective evidence of antitumor effect. Phase I trials of antitumor monoclonal antibodies initiated by the Clinical Investigations Section include studies of anti-T cell monoclonal antibodies in patients with chronic lymphocytic leukemia and cutaneous T cell lymphoma, anti-melanoma monoclonal antibody in patients with disseminated melanoma, and anti-idiotype monoclonal antibody in patients with refractory malignant lymphoma.

PROJECT DESCRIPTION

OBJECTIVES

Antitumor monoclonal antibodies with reactivity against human tumor cells may have significant potential as anticancer agents in man. These antibodies may be administered in their nascent form or could conceivably be vehicles for cellular toxins, chemotherapy agents, and alpha-emitter radioisotopes. The Clinical Investigations Section is strongly committed to the early clinical testing of antitumor monoclonal antibodies. Phase I trials to be initiated during this year include studies of an anti-T cell monoclonal antibody in patients with chronic lymphocytic leukemia and cutaneous T cell lymphoma, an anti-melanoma antibody in patients with disseminated malginant melanoma, and anti-idiotype monoclonal antibodies in patients with refractory malignant lymphoma. In these trials patients will be carefully monitored for antitumor effect and toxicity and in addition will be monitored for specific and non-specific effects on the immune system.

METHODS EMPLOYED

In the Phase I trial of an anti-T cell monoclonal antibody in patients with chronic lymphocytic leukemia and cutaneous T cell lymphoma, groups of three or more patients will be treated at escalating doses on a twice weekly schedule over a period of 28 days. Patients will be carefully monitored for antitumor effect, toxicity and immunomodulatory effect in a variety of assays. Patients will be monitored for the presence of free antigen and antibody and for the formation of anti-murine immunoglobulin. Antigenic modulation will be followed by FACS analysis of anti-T cell monoclonal antibody positive cells. Patients will be tested for reactivity with the antibody prior to its administration as well as during therapy. Immunologic monitoring of various other immunologic parameters will be performed as well, including assays for natural killer cell activity, monocyte function as measured in a growth inhibition assay, and lymphocyte blastogenesis. In the Phase I trial of an anti-melanoma monoclonal antibody, individual patients will receive escalating doses of this antibody and will again be 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 will be subjected to biopsy before therapy to demonstrate reactivity of their tumor cells with the monoclonal antibody as judged by immunofluorescence and immunoperoxidase techniques. The anti-idiotype monoclonal antibody trial for patients with malignant lymphoma will involve the administration of anti-idiotype antibodies prepared against immunoglobulins specifically being secreted by the patient's malignant cells. This will therefore involve the preparation of specific hybridomas against the tumor idiotype of each patient treated.

MAJOR FINDINGS

No findings can be reported at the present time in any of these Phase I monoclonal antibody trials.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The early clinical testing of monoclonal antibodies with antitumor reactivity is of key importance in terms of understanding the potential toxicity, immunomodulatory effect and antitumor activity of these agents. Monoclonal antibodies of this type may well be a major new therapeutic modality for patients with disseminated malignancy. In addition these antibodies may have importance in diagnostic tests for patients with disseminated cancer by means of radionuclide imaging.

PROPOSED COURSE

The early Phase I trials of monoclonal antibodies described above will be followed by further tests of these same antibodies coupled to various cellular toxins, chemotherapy agents or radioisotopes. In this fashion monoclonal antibodies may ultimately be developed as vehicles for the delivery of toxic agents directly into the tumor cell.

The primary aim of the Monoclonal Antibody/Hybridoma Section (MAHS) is to develop new hybridomas producing monoclonal antibodies directed toward tumor-associated antigens. This laboratory is attempting to raise both murine and human monoclonal antibodies. The major objective is to utilize such monoclonal antibodies for diagnosis and therapy of cancer. It is expected that these antibodies will be used diagnostically in vitro by studying patient's tumor cells with a panel of monoclonal antibody and in vivo by conjugating them to radionuclides such as $^{125}$-I and $^{111}$Indium for radionuclide imaging. In addition we would expect to infuse these antibodies into appropriate cancer patients to study the potential beneficial effect these antibodies might have on the patient's tumor. We will also conjugate these antibodies to drugs such as doxorubicin and methotrexate and to toxins such as diphtheria toxin and ricin and investigate their anticancer activity. Such therapies could be used to treat established tumors or perhaps may have their most important effect in the adjuvant setting. To better understand the role of monoclonal antibodies and immunoconjugates in cancer diagnosis and therapy, we have established a number of guinea pig models for both solid tumors and leukemias.

We have also used the hybridoma technology to pursue a number of other areas of investigation. First of all, we are developing a series of monoclonal antibodies to human leukocytes. Such antibodies are a powerful tool to dissect the human immune system, to better understand the mechanisms of cell-cell interaction. In addition, differentiation antigens are generally expressed on leukemia and lymphoma cells and can be used diagnostically and therapeutically in the treatment of leukemia and lymphoma. We are also interested in establishing human hybridomas by fusing human monocytes to the macrophage cell line U937 that has been treated with 8-azaguanine. Similarly we are attempting to make T-T hybridomas by fusing human T lymphocytes to an 8-azaguanine treated T cell line (HSB2) and are attempting to make natural killer hybridomas by fusing purified large granular lymphocytes (LGL), which represent the natural killer population, to either the U937 or HSB2 lines. The purpose of such fusions is to establish cell lines that have the biologic capabilities of the normal cell counterparts and that have been immortalized by the permanent cell line. Such hybridomas may produce cytokines and may function as effector cells in various cellular assays.

The members of the MAHS include Dr. Michael Bernhard, who arrived in December, 1980. Dr. Kenneth Foon arrived in July, 1981 as a Litton Bionetics research scientist, and is in the process of being converted to an NIH position, at which time he will become the Acting Section Head. Dr. Jack Pearson joined the Section in January, 1982.

## HIGHLIGHTS OF ACHIEVEMENTS IN THE LAST YEAR

### I. Guinea Pig Tumor Models

Dr. Michael Bernhard has developed an IgG1 murine monoclonal antibody designated D3 that recognizes a 200,000 molecular weight antigen expressed on the surface membrane of the Line 10 hepatocellular carcinoma in strain 2 guinea pigs. Dr. Bernhard has demonstrated that this antibody is not found on the similarly derived Line 1 hepatocellular carcinoma and it is not found on any normal guinea pig tissues. Infusion of this monoclonal antibody into a guinea pig demonstrates specific binding by immunoperoxidase techniques to the Line 10 tumor but not to normal tissue. Radionuclide imaging using Iodine-125 and Indium-111 conjugated to the D3 monoclonal antibody demonstrates specific uptake in the Line 10 tumor and imaging of the tumor by radionuclide scanning. The D3 monoclonal antibody has been conjugated to doxorubicin by Dr. Kou Hwang, and this conjugate has been demonstrated to bind to Line 10 cells and is toxic to 100% of these cells in vitro. D3 has also been conjugated to diphtheria toxin and in vitro studies also demonstrated specific binding and toxicity to Line 10 cells in vitro. In vivo studies using the D3 diphtheria toxin conjugates have demonstrated dramatic inhibition of tumor growth. In vivo studies with the doxorubicin conjugate are currently underway.

Dr. Jack Pearson has developed a mammary carcinoma in strain 2 guinea pigs that arises in mammary tissue and metastasizes to the lungs. The primary tumor can be removed prior to the identification of pulmonary metastasis with the subsequent development of pulmonary disease. This, therefore, represents an excellent model for adjuvant therapy and we are currently raising monoclonal antibodies to both the primary mammary carcinoma and the metastatic mammary carcinoma. We are also raising a monoclonal antibody to a chronic myelogenous leukemia in strain 13 guinea pigs, which was identified by Dr. Warren Evans. Such an antibody could be useful in serotherapy trials and also in in vitro treatment of bone marrow prior to bone marrow transplantation.

### II. Murine Monoclonal Antibodies Raised to Human Carcinomas

Drs. Paul Abrams, James Knost, and Jeffrey Ochs have raised a number of murine monoclonal antibodies to human bronchogenic carcinoma cell lines and to colon carcinoma cells. Monoclonal antibodies have been raised to the adenocarcinoma cell line HUT125, the large cell carcinoma cell line HUT157, and to the squamous cell carcinoma cell line SKMES. These monoclonal antibodies are all IgG1 and are recognizing different antigens on the surface of bronchogenic carcinoma cells. Some of the antibodies are more restricted to one type of bronchogenic carcinoma while others react with numerous subtypes of bronchogenic carcinoma (e.g. small cell, squamous cell) and carcinomas derived from other organs.

Various methods of screening these antibodies against tumor cell lines and tissues are being used. Most of the screening is done by a radioimmunoassay using either live cells, glutaraldehyde fixed cells, soluble antigens or membrane preparations. Enzyme-linked immunosorbent assays (ELISA) are also being used to study these various cell preparations. Tissue sections taken from tumors and normal tissues are also being studied with these monoclonal

antibodies using immunoperoxidase techniques. In addition, immunofluorescence using flow cytometry (Becton-Dickinson FACS 4 and Ortho Cytofluorograph) is also utilized.

A different approach to raising monoclonal antibodies is being investigated for colon carcinoma. A collaborative study with Dr. Michael Hanna of the Frederick Cancer Research Facility and Dr. Charles Hoover of Johns Hopkins University has been established to produce monoclonal antibodies to individual patient's tumor cells. Tumors are either prepared in single-cell suspension for immunization and testing or they are grown in nude mice and the established tumor is then prepared into cell membranes or soluble antigens for immunization and testing. B cell lines are also established from patients by Epstein-Barr transformation. We would hope that we could raise monoclonal antibodies that not only have broad reactivity against colon carcinoma cells but some that may react uniquely with an individual patient's colon carcinoma. These antibodies could be used at a later time to treat the patient whose tumor was used for immunization.

## III. Human Monoclonal Antibodies to Human Tumors

Because of the potential adverse reactions to murine monoclonal antibodies and the possibility of developing antibodies with more specific activity, we have been attempting to raise human monoclonal antibodies. There are a number of obstacles to raising such monoclonal antibodies, including 1) an inadequate source of immunized B cells for fusing and 2) a lack of suitable human myeloma lines. The sources for human B lymphocytes that we have used have been from draining lymph nodes removed at surgery, mainly those donated to us by Dr. Charles Hoover of Johns Hopkins University, and peripheral blood from patients on Dr. Hoover's and Hanna's colon carcinoma protocol who are being immunized with autologous tumor cells. Another source for immunized human B lymphocytes is from in vitro cultures. Recent studies in collaboration with Dr. Jeffrey Rossio of the Lymphokine Section of the Biological Response Modifiers Program have demonstrated successful in vitro sensitization of human lymphocytes with tetanus toxoid. These sensitized B lymphocytes can subsequently be fused to human myelomas and produce specific monoclonal IgG antibody that binds to tetanus toxoid. We are currently attempting to adapt this to human tumor systems.

Another problem with raising human monoclonal antibodies has been an adequate myeloma line. While there are excellent well established murine myeloma lines for developing hybridomas, this is not true for human myelomas. Currently we are studying four lines including the HMy2 myeloma line from Cambridge, England and the 4672 myeloma established by Carlo Croce from the Wistar Institute. In addition, Paul Abrams from our our laboratory has treated the U266 myeloma line and has made it HAT-sensitive and in addition has developed another myeloma cell line which he refers to as AMM2. The SK-007 line first described by Kaplan has not been used in our lab as it has been contaminated with mycoplasma. We are currently testing all of these cell lines in parallel experiments to see which will be the best fusing partner.

To date, approximately 75 human myeloma fusions have been performed in our laboratory. Approximately 50% of these fusions produced growing hybridomas

and approximately half of the growing hybridomas produced human immunoglobulins. Further testing and screening demonstrated that most of these immunoglobulins were either nonspecific in their reactivity or the hybridomas did not maintain their ability to secrete immunoglobulins. We are currently left with approximately 5% of the total fusions performed that have been cloned a number of times and are producing immunoglobulin with some specificity toward human tumors. We are hopeful that further screening of human myeloma lines to determine which is the best fusing partner and in addition developing better ways of immunizing human cells will lead us to a greater yield of human hybridomas.

## IV. Establishing Human T-T, Monocyte-Macrophage, Large Granular Lymphocyte-T, Large Granular Lymphocyte-Macrophage Hybridomas

We have treated the human macrophage cell line U937 with 8-azaguanine and have rendered it HAT-sensitive. We have attempted a number of fusions of this HAT-sensitive macrophage line to human monocytes. While we found no growth in the first five fusions performed, the sixth fusion is currently demonstrating a number of hybridoma clones. If these continue to grow they will be further cloned, tested for the production of interleukin-1 (lymphocyte activating factor) and interferon, and for their ability to function in various macrophage functional assays such as chemotaxis, migration inhibition, and tumor cytolysis.

We are attempting to develop human T-T hybridomas by fusing concanavalin-A stimulated peripheral blood T lymphocytes with a HAT-sensitive T lymphoblastoid cell line (HSB2). Hybridomas will be screened for the production of various lymphokines including macrophage activating factor, migration inhibition factor, chemotactic factor and interferon. We are also attempting to fuse purified large granular lymphocytes, which represent the natural killer population in human peripheral blood, to both the HAT-sensitive U937 and HSB2 cell lines. We would hope that these cell lines would function as natural killer cells and allow us to better understand the morphologic, antigenic, and functional characteristics of natural killer cells. In addition it is possible we could use these cell lines to isolate the receptor on target cells and biochemically characterize it.

## V. Anti-Idiotype Antibodies to Human B Cell Tumors

Investigators from Stanford University developed a murine monoclonal antibody to the idiotypic determinants on a B cell lymphoma. This antibody was eventually used to treat this particular patient at a time when the patient developed a progressive course of his disease which was unresponsive to standard therapies. Monoclonal antibody was infused on eight occasions over four weeks and the patient entered a complete remission that was durable for at least ten months. We are raising anti-idiotype monoclonal antibodies to patients with B cell tumors including chronic lymphocytic leukemia, lymphoma, and multiple myeloma. Following the development of these monoclonal anti-idiotype antibodies, at an appropriate time in the course of the patient's disease, we would hope to treat these patients.

VI. Leukocyte Antigens

We are currently raising monoclonal antibodies to human granulocytes, monocytes, eosinophils, and platelets. While human monoclonal antibodies to human B lymphocytes and T lymphocytes have been extensively described, antibodies to monocytes, granulocytes, eosinophils and platelets have been less completely studied. Furthermore, the effects of these antibodies on cell function and physiology have not been extensively studied. We are currently characterizing three IgG1 monoclonal antibodies to human monocytes, five IgG1 monoclonal antibodies to human granulocytes, and eight monoclonal antibodies to human eosinophils that have various isotypes including IgG1, IgG2A, IgG2B, and IgM. A complete characterization of these antibodies against panels of normal and malignant cells, bone marrow cells, stem cells, and molecular characterization of the antigens is currently being completed. They will also be studied for their effect on various immune functions including phagocytosis, chemotaxis, natural killer activity, and superoxide production. The platelet antibody has been demonstrated to abrogate such platelet functions as aggregation, adhesion and serotonin release.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER ZO1 CM 09204-02 BRTB |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Therapy of Guinea Pig Hepatocellular Carcinoma with Monoclonal Antibodies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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| PI: | Michael Bernhard | Expert | BRTB | NCI |
| Others: | Kenneth Foon | Acting Section Head (proposed) | BRTB | NCI |
| | Robert Oldham | Associate Director | BRMP | NCI |

COOPERATING UNITS (if any)
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SECTION
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have selected the D3 MoAb/Line 10 tumor system as a model for drug/toxin-MoAb solid tumor therapy. The similarity in the immune responses of guinea pigs and man, especially with regard to anaphylactic reactions, makes the guinea pig Line 10 tumor an especially appropriate model. We have produced and characterized the D3 MoAb and it appears to display adequate levels of "tumor specificity," both in vitro and in vivo. In addition, D3 is only slightly cytotoxic, allowing good assessment of the antitumor cytotoxicity of drug/toxin conjugates. D3 is currently in bulk production and has been sent to collaborators for drug/toxin conjugation and has been radiolabelled. In vitro assessment of the amount of drug/toxin per antibody molecule and in vitro cytotoxicity and specificity studies are underway. Both diphtheria toxin A chain and doxorubicin-conjugated D3 are specifically cytotoxic for L10 cells in vitro. Diphtheria A chain-conjugated D3 has shown striking effectiveness against established L10 tumors in vivo, with no observed side effects to date. In vivo radioimaging studies are in progress, with specific tumor imaging already demonstrated. By testing various coupling procedures and drugs/toxins, we hope to identify the procedures most likely to prove successful in other in vivo tumor systems, including in man.

PROJECT DESCRIPTION

OBJECTIVES

1. To develop an appropriate animal model to assess the therapeutic effectiveness of monoclonal antibody (MoAb)-drug or toxin conjugates in vivo.

2. To determine the most effective drug or toxin and coupling technique in this model.

3. To prepare affinity-purified antigen for therapy trials.

4. To develop monospecific conventional antiserum for similar studies if tumor heterogeneity presents problems.

METHODS EMPLOYED

1. Prepare appropriately "tumor-specific" MoAb, characterize and define the antibody and antigen.

2. Prepare this MoAb in bulk for radioisotope-labelling and drug/toxin coupling.

A. Ascites Production

BALB/c mice (6 week old females) were treated with Pristane (2,6,10,14-Tetramethylpentadecane, Aldrich Chemical Co., Milwaukee, WI.) 5-7 days prior to use. Twenty million tissue culture grown hybridoma cells were injected intraperitoneally, and ascites fluid was harvested several times beginning 7-14 days after inoculation. Twenty to 100 mice were treated at a time, and each lot of ascites fluid consisted of pooled fluids from a group inoculated simultaneously. Each lot was handled separately.

B. Purification of Ascites Fluid

Fluids were clarified by centrifugation at 10,000 X g/60 mins, filtered (0.2 m), diluted 1:2 in endotoxin-free NaCl and precipitated with 20.4% (w/v) sodium sulfate. Precipitated material was pelleted at 12,500 X g/30 mins, resuspended in 0.1 M Tris, pH 7.3, reprecipitated in 14% (w/v) sodium sulfate, and pelleted as above. After resuspension in Tris and exhaustive dialysis against PBS, the 'purified' monoclonal antibody was clarified by centrifugation at 100,000 X g/1 hour, passed throuh 0.2 m Millipore filter for sterility, aliquoted and stored at -85°. The Bio-Rad protein assay was used to determine total protein before and after purification, $IgG_1$ levels were quantitated by radial immunodiffusion, and endotoxin levels were determined by limulus lysate assay.

C. Isoelectric Focusing

D3 hybridoma cells were grown in serum-free medium for 72 hours. After clarification of the supernatant (10,000 X g/30 mins), samples at various

dilutions (from undiluted to 1/100) were run on LKB PAGplate (LKB, Rockville, MD.) pH 3.5-9.5 on an LKB Multiphore. Bands were stained with Coomassie Blue R250.

D. Two Dimensional Gel Electrophoresis

Strips containing the stained band from isoelectric focusing (covering pH range from 3.5 to 9.5) were cut out and equilibrated overnight in SDS-PAGE buffer containing 10% 2-mercaptoethanol. A single strip was placed in an appropriately cut area of the stacking gel of a 10% SDS-PAGE slab gel and run as described by Laemmli.

E. Radioimmunoprecipitation

Fresh L1 and L10 ascites cells were washed 3 times in PBS and iodinated by the lactoperoxidase technique or metabolically labeled with $^{3}H$ leucine (1 mCi/2x$10^{7}$ cells, s.a. 110 Ci/mmol). Cells were washed 4 times in 50 ml PBS and lysed in PBS containing 0.5% NP-40 and 1 mM phenylmethyl sulfonylfluoride (PMSF). After vigorous vortexing, cells were held on ice for 30 minutes and rotated an additional 30 minutes at 4 degrees. Supernatants were clarified by centrifugation at 100,000 X g/1 hour, and held at 4°.

Protein-A-Sepharose (PAS, Pharmacia) was coupled to monoclonal antibody for immunoprecipitation. Four hundred microliters of PAS as a 10% suspension in TTB (0.1M Tris, pH 8.5, 0.5% Tween 20 and 0.1% BSA) was mixed with 20 µl D3 purified ascites or P3 control ascites fluid (BRL, Rockville, MD.) The volume was brought to 1.0 ml with TTB and tubes were rotated at 4° for 2 hours. After washing 3 times in TTB, D3 and P3 direct-coupled pellets were held dry at 4°.

Directly coupled (PAS-D3 and PAS-P3) PAS-monoclonal antibody were then rotated overnight at 4° with L10 and L1 labeled detergent extracts. Pellets were washed 3 times with TTB and 3 times with TT (TTB without BSA), boiled in SDS-PAGE buffer with and without 2-mercaptoethanol and and run on 5% or 7.5% SDS polyacrylamide slab gels.

F. In Vivo Localization of D3 Monoclonal Antibody

Dermal L10 tumors were initiated in guinea pigs by the intradermal injection of 1 X $10^{6}$ fresh ascites tumor cells. Four weeks later, tumor-bearing guinea pigs received no further treatment or were injected i.v. (using the dorsal penile vein) with 1 ml PBS containing 10 mg of D3 antibody or 10 mg of control P3 immunoglobulin. Ten minutes to 24 hours later tumors were surgically removed, fixed in 10% formalin, and embedded in paraffin. Analysis of the *in vivo* localization of D3 was performed on deparaffinized tissue sections using an immunoperoxidase technique for the detection of mouse immunoglobulin.

A similar analysis was performed on untreated animals, reacting tumor and normal tissues with D3 MoAb *in vitro* using unfixed, cryostat sections and a modification of this procedure. Unfixed cryostat sections were maintained at -85°C until used. Slides were rehydrated for 5 minutes in PBS and pretreated with PBS containing 10% normal horse serum and 0.1% BSA (10 minutes). Sections were

reacted with the monoclonal antibody (diluted in PBS containing 0.1% BSA) for 30 minutes, washed several times in PBS and then incubated in methanol containing 0.3% $H_2O_2$ to block endogenous peroxidase activity. The remainder of the assay follows the procedure outlined in the Vector Labs Vectastain ABC kit (Burlingame, CA.).

Live ascites L1 and L10 cells were treated in a similar fashion. Thoroughly washed cells were reacted with the D3 monoclonal antibody, fixed in Saccamono's solution, cytospin preparations made, and then processed in the same manner as the cryostat sections. In all of the peroxidase procedures (fixed, cryostat and live cell) P3 ascites fluid controls (at the same concentrations as the D3 monoclonal) and PBS controls were included as negative controls. All assays were performed at 1/1000, 1/5000 and/or 1/10,000 dilutions.

G. In Vivo Radioimaging Studies

Line 1 and Line 10 tumors were established in dermal sites in 500 gram guinea pigs. Tumor dose was adjusted so that after 7 days' tumor growth Line 10 tumors were 2 cm in diameter and Line 1 tumors, which are regressors, were at their maximal size. Radiolabeled D3 antibody ($^{111}$In and $^{125}$I) conjugated D3 antibody will be administered i.v. to these tumor-bearing animals. Initially 3 animals were used for each isotopically conjugated antibody: 2 animals bearing Line 10 tumors and 1 animal bearing a Line 1 tumor. Initially these animals were treated with 100, 150 and 250 microcuries of $^{111}$Indium-labelled antibody or $^{125}$Iodine-labelled antibody. Animals were imaged at the NIH Clinical Center at 24-hour intervals after infusion of antibody. This initial study helped determine appropriate amounts of label for accurate imaging and to decide what problems we will have with concentration of isotope in Line 1 animals (negative control). Animals bearing both Line 10 and L1 tumors (on contralateral hips) were also imaged successfully. Counts per gram tissue were determined at several time periods.

H. In Vitro Determination of Conjugate Effectiveness and Definition of Most Effective Parameters

Drug or toxin-conjugated MoAb will be assessed for in vitro cytotoxicity by measuring the incorporation of $C^{14}$-labelled leucine, trypan blue dye exclusion, and RNA polymerase inhibitory activity. Propidium iodide incorporation has also been used. Negative controls include P3 ascites fluid and unconjugated D3. If in vitro cytotoxicity is not demonstrated, binding studies using our usual live cell RIA will be conducted. Conjugates demonstrating $\geq$ 50% in vitro cytotoxicity will be considered for testing in vivo. Diphtheria toxin A chain-D3 and doxorubicin-D3 have shown good in vivo binding and cytotoxicity. Diphtheria toxin A chain-D3 conjugate has shown promising results in treating established tumors in vivo. $\alpha$-amanitin-D3 conjugate has shown reduced binding and limited cytotoxicity in vitro.

I. Comparative In Vivo Studies Using Drug or Toxin-MoAb Conjugates

Drug- or toxin-coupled D3, which has proven efficacious in in vitro studies will be administered (i.v.) to tumor-bearing guinea pigs. Studies will be

designed to assess both toxicity and specific tumor kill. Initial in vivo studies using unconjugated D3 have already been completed and have shown significant but undramatic reduction of tumor size with several therapeutic protocols. Similar protocols will be followed using the drug- or toxin-conjugated D3, expanding the dosage range to be studied from 0.5 mg to a maximum of 10 mg D3. Initial studies will probably utilize only a single dose of conjugated antibody and tumors will subsequently be measured twice weekly for several weeks. Results of this preliminary experiment will be analyzed before further experiments are undertaken.

J. Preparation of Adriamycin (Doxorubicin)-MoAb (D3) Conjugates

Adriamycin (doxorubicin) is first acylated by N-succinimidyl-3(2-pyridyl-dithio-propionate) forming doxorubicin-2-pyridyl-dithiopropionamide; this product is initially identified by thin-layer chromatography as having a $R_f$ = 0.90 (doxorubicin, $R_f$ = 0.09 in $CHCl_3$; MeOH - $H_2O$ = 0.09). The acylated Doxorubin is then reduced to form doxorubicin-mercapto-propianamide which is characterized as having $R_f$ = 0.75. Both of these intermediates will be further characterized by elementary analysis, NMR, IR and UV spectroscopies. The doxorubicin mercapto-propionamide is then coupled to monoclonal antibody (D3) which is also previously acylated according to the same method employed for doxorubicin. The separation of conjugates from free doxorubicin and free D3 is carried out by Sephadex GT-25 chromatography. The characterization of these three main products is by spectrophotometric analysis between 600Å -180Å. Each product has its own unique absorption spectrum. In addition, the characterization of conjugated antibody will also be performed by SDS-gel electrophoresis.

3. Preparation of affinity-purified antigen for therapy

NP40 extracts of Line 10 tissue culture cells will be passed over our D3 monoclonal antibody affinity column, and specific antigen eluted with sodium acetate, pH 2.5. Eluted fractions will be neutralized with 1 M Tris buffer, pH 8.5, immediately after elution from the column. Antigen will be frozen immediately following elution from the affinity column and when a sufficient amount of antigen has been collected, frozen materials will be pooled, dialyzed, lyophilized, and analyzed by SDS-PAGE and inhibition of the live cell RIA. This antigen will then be used for antigen therapy protocols (which have not yet been developed).

4. Use of affinity-purified L10 antigen for preparation of monospecific conventional antiserum for in vitro and in vivo cytotoxicity studies with antibody (C' and ADCC) and conjugates if antigenic heterogeneity of tumor poses problems for MoAb therapy.

Rabbits will initially be immunized with 2 x $10^6$ Line 10 cells emulsified in complete Freund's adjuvant and injected subcutaneously in a minimum of 4 sites. Subsequent boosts will be performed with purified Line 10 antigen (as described in 3. above). Booster immunizations will occur at 2-3 week intervals and immunized animals will be bled 1 week after each boost and antiserum assessed by live cell RIA to determine specificity and titer of the antiserum produced. Adequate antiserum should show a minimum titer of 1:10,000 in our live cell

RIA. When this titer has been reached, animals will be bled, serum will be prepared, precipitated once with 50% saturated ammonium sulfate, dialyzed against PBS, and passed through an affinity column prepared with cyanagen bromide-activated Sepharose 4B and the Line 10 antigen purified as in Method 3. above. Antibody eluted from this column will be assayed by live cell RIA for specificity and titer. If functionally monospecific antiserum can be produced in this manner, this serum will be stored at -80° until such time as the results of the in vivo monoclonal antibody trials are completed. If difficulties in in vivo treatment, possibly arising from antigenic heterogeneity, are encountered, similar trials may be repeated using the functionally monospecific rabbit antiserum.

MAJOR FINDINGS

A. Guinea pig Line 10 hepatocellular carcinoma (L10) was chosen as a model solid tumor. A large body of information is available concerning this tumor system, and a similarly derived yet antigenically distinct tumor (Line 1, L1) is available as a negative control.

B. A murine IgG1 monoclonal antibody (D3) was developed to this tumor. D3 is specific for L10, as shown by live cell radioimmunoassay (RIA) and immunofluorescence. Immunoperoxidase on cryostat sections of normal guinea pig tissues and L1 and L10 tumors has also demonstrated the L10 specificity of D3 and revealed cross reactions only with a cytoplasmic antigen in muscle tissue. The tumor-associated cell surface antigen recognized by D3 is a dimer, $M_r$ 290,000 (monomer 148,000).

C. In vivo immunoperoxidase studies, in which tumor-bearing guinea pigs were injected i.v. with 10 mg D3 demonstrated specific localization of D3 on L10 tumor cells (dermal tumor and lymph node metastases) within 10 minutes, with maximal tumor localization at 24 hours post-administration. However, D3 was also associated with the vasculature in all normal tissues for the first 18-24 hours, but with no staining associated with the cellular components of any normal tissues, including muscle. By 8 hours some proximal convoluted tubules of the kidney showed slight reactivity, with all proximal tubules lightly stained by 48 hours. Identical staining of normal tissues was obtained with control murine ascites of no known specificity, showing that these normal tissue reactions were not antigen specific and were related to clearance of this heterologous antibody. Staining patterns revealed no evidence of antigenic heterogeneity in this tumor and indicated the morphological characteristics of the tumor determined the intensity of staining which began staining at 10 mins and was heavily stained by 8 hrs. Tumor morphology and vascularity seemed to influence tumor cell staining: dermal tumors (loosely associated cells with lots of intracellular space) were 100% positive whereas lymph node metastases (tightly packed cells) were stained most heavily nearest blood supplies. Also of significance was the internalization of antibody in tumor cells by 48 hours (of importance in antibody-conjugate studies).

D. Radioimaging, using $^{111}In$ and $^{125}I$-labelled D3 showed 420-fold more D3 in L10 than L1 tumors with $^{111}In$ and 39 times more $^{125}I$-labelled antibody in L10 than L1 tumors (at 179 and 197 hours, respectively). Although 8.8% of the administered $^{111}In$ localized in L10 tumors, 1% was found in liver, kidney and spleen; similar observations were made with $^{125}I$-D3. However, in both instances L10 tumors were clearly imaged by external scanning.

E. Diphtheria toxin A chain and doxorubicin conjugated to D3 have shown specific in vitro cytotoxicity with L10 cells. DNA synthesis was inhibited by 24 hours and 100% of the tumor cells were killed by 7 days. in vivo experiments are underway. Preliminary experiments indicate that the diphtheria toxin A chain conjugated D3 has a highly significant effect on inhibiting tumor growth. Seven-day (established) dermal tumors were treated with 1 mg antibody-diphtheria toxin A chain conjugate. Measurable tumors were present in only 15% of controls at 14 days after tumor cell injection, slowly increasing with time as more treated animals developed tumors (70% of animals by 24 days). Mean tumor size in treated animals was 66 mm compared to 145 mm in controls at 24 days.

F. in vitro evaluation of doxorubicin-D3 conjugate. Two types of conjugates were obtained. One conjugate has coupled approximately 30 moles of doxorubicin; another has about 50 moles of doxorubicin. The stabilities of these conjugates have not yet been established. The high-substituted conjugates (1 mole of Ab/50 moles of doxorubicin) have been used to study in vitro activity.

## SIGNIFICANCE TO THE BRMP

The development of this model fulfills two of the major aims of the BRMP: 1) developing significant animal models for monoclonal antibody therapy with application to man; and 2) making the model available to collaborators throughout the country (Vic Raso, Sidney Farber Institute, Boston, Mass.; Tom Oeltmann, Vanderbilt University, Memphis, Tenn.: Jim Preston, University of Florida, Gainesville, Fla.; Gerald Vosika, University of South Dakota Medical School, Fargo, South Dakota).

The model was chosen to develop means of diagnosing and treating human tumors with monoclonal antibodies. We have been very successful in this first year. Preliminary imaging studies indicate this technique may be useful diagnostically; preliminary in vivo studies with drug and toxin-conjugated D3 indicate this may be an effective treatment modality for solid tumors. These studies will provide the basis for similar approaches in man.

## PROPOSED COURSE

A. in vitro and in vivo testing of ricin A chain and $\alpha$-amanitin conjugated D3.

B. Radioimaging with $^{111}$In-labelled D3, using various techniques to label the D3 to attempt to minimize nonspecific uptake in liver, kidney and spleen. Similar studies with $^{123}$I-labelled D3. The major aim is to determine the minimum size tumor that can be imaged.

C. Use of $^{123}$I-labelled D3 to treat tumors in vivo.

ABSTRACTS

Bernhard, M.I., Foon, K.A., Clarke, G.C., Christensen, W.L., Hayer, L., Key, M.E., Hanna, M.G., Jr., and Oldham, R.K. 1982. Monoclonal Antibody Serotherapy of Solid Tumors: A Guinea Pig Model. I. Characterization of Monoclonal Antibody. Hybridoma 1:204.

Bernhard, M.I., Foon, K.A., Clarke, G.C., Christensen, W.L., Hayer, L., Key, M.E., Hanna, M.G., Jr., and Oldham, R.K. 1982. Monoclonal Antibody Serotherapy of Solid Tumors. A Guinea Pig Model. Proc. Amer. Assoc. Cancer Res., p. 256.

Key, M.E., Peters, L.C., Bernhard, M.I., and Hanna, M.G., Jr. 1982. Restrictions imposed by vascular barriers on the intratumoral localization of monoclonal antibodies. Fed. Proc. 41:552.

Oldham, R.K., Bernhard, M.I., Key, M., Peters, L.C., Brandhorst, J., Hanna, M.G., Jr., and Foon, K.A. 1982. A Guinea Pig Model for Monoclonal Antibody Therapy of Solid Tumors. Proc. 13th Int. Cancer Congress.

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PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Efficacy of Monoclonal Antibody Therapy Involving Two Guinea Pig Model Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| Others: | Kenneth A. Foon | Acting Section Head (proposed) | BRTB | NCI |
| | Michael I. Bernhard | Expert | BRTB | NCI |
| | Richard Smalley | Chief | BRB | NCI |
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COOPERATING UNITS (if any)

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LAB/BRANCH

Biological Research and Therapy Branch

SECTION

Monoclonal Antibody/Hybridoma Section

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The administration of $5 \times 10^6$ mammary carcinoma tumor cells to strain 2 guinea pigs resulted in 100% tumor incidence regardless of the route of administration. Spontaneous metastases to the lungs were observed. The primary and pulmonary tumors were characterized as undifferentiated carcinomas. This tumor is weakly immunogenic following a challenge with $5 \times 10^5$ viable tumor cells to animals previously exposed to irradiated cells alone or admixed with BCG. Development of monoclonal antibodies against the primary and pulmonary metastatic tumors is currently underway. Drug studies against a chronic myelogenous leukemia (CML) in strain 13 guinea pigs are ongoing. This leukemia follows a reproducible (although accelerated) course as observed in man, i.e., chronic phase followed by blast crisis, and death. Three clinically active drugs, busulfan, hydroxyurea or Cytoxan are currently being tested for their ability to extend the chronic phase and/or prevent the development of blast crisis. Development of monoclonal antibodies against the chronic and blast tumor cells is in progress. Monoclonal antibodies against each tumor will be examined alone, i.e., passive immunotherapy, or as carriers of drugs, toxins or isotopes for antitumor therapy.

PROJECT DESCRIPTION

OBJECTIVES

The aim of this project is to develop effective therapeutic protocols for the control of a transplantable spontaneous mammary carcinoma in strain 2 guinea pigs and a transplantable chronic myelogenous leukemia in strain 13 guinea pigs. The therapeutic measures encompass the use of surgery or cytoreductive (drug) therapy followed by the application of specifically derived monoclonal antibodies used alone or in combination with drugs, toxins or isotopes for the elimination of pulmonary disseminated metastases (mammary carcinoma) or toward the elimination of leukemia (CML) in strain 13 guinea pigs.

RATIONALE

Since the established forms of cancer treatment for solid tumors, namely surgery, chemotherapy, and radiotherapy are generally applicable for the removal or destruction of the primary tumor mass, the challenge in man is the eradication of disseminated metastases. Recently, emphasis has been placed on the use of tumors that metastasize spontaneously and demonstrate low immunogenicity as models for human cancer. The transplantable guinea pig spontaneous mammary carcinoma appears to fulfill those criteria necessary for its acceptance as a good model for human cancer, i.e., syngeneic with its host, weakly antigenic and ability to spontaneously metastasize to the lungs in tumor-bearing animals following surgical excision of the primary breast tumor. In addition, there is a significant time interval between the time of tumor cell inoculation and the occurrence of spontaneous pulmonary metastases.

The chronic myelogenous leukemia in strain 13 guinea pigs is the only known animal model that closely mimics human chronic myelogenous leukemia (CML) both clinically and morphologically. This disease also has a chromosomal abnormality similar to the Philadelphia chromosome. This model has been standardized so that the disease follows a reproducible (although accelerated) course observed in man, i.e., the chronic phase followed by the development of blast crisis and eventual death.

METHODS EMPLOYED

I. Transplantable Spontaneous Mammary Carcinoma in Strain 2 Guinea Pigs.

A. Earlier studies have revealed that following the intravenous inoculation of $5 \times 10^6$ tumor cells, all animals died approximately 60 days later and at necropsy revealed massive tumor infiltration throughout the lungs. In support of subsequent drug studies, it will be important to test the effect of drugs against the metastatic tumor when the tumor load is minimal in the lungs. Therefore, drug therapy will be initiated on either day 1 or 10 post-tumor cell inoculation. The drugs of choice in this study will be adriamycin, 5-fluorouracil, methotrexate and cyclophosphamide. These drugs have been the choice of therapy against breast cancer in humans.

B. A series of drug toxicity studies will be initiated to determine the optimal dose that will be tolerated in normal strain 2 guinea pigs. The parameters to be followed, to determine the toxic effects of each drug, will be body weight loss, WBC and polynuclear neutrophil counts, and mortality. The drugs will be administered for two doses approximately 7-10 days apart.

C. Once the optimal dose of each drug has been determined, therapy studies will be initiated either one or ten days following the intravenous inoculation of $5 \times 10^6$ metastatic tumor cells. All drug-treated animals will be held for survival. Long-term survivors will be sacrificed at a time when there is at least a 100% increase in median survival time when compared to the survival time of the untreated control group. Another approach to test the effects of these drugs will be to utilize animals bearing established subcutaneous tumors following the inoculation of $5 \times 10^6$ metastatic tumor cells. In this case, when the tumor growth is approximately 10 mm in size, drug therapy is to be initiated to study the effects of chemotherapy on an established tumor.

## II. Transplantable Chronic Myelogenous Leukemia in Strain 13 Guinea Pigs

A. Initial studies will involve the application of three clinically active drugs for the sole purpose of extending and/or maintaining the chronic phase of this leukemia. The chronic phase occurs within 20-23 days after tumor cell inoculation which lasts for approximately 7-10 days followed by the subsequent development of blast crisis (30-32 days) and death of all animals within approximately 35 days.

B. A series of drug toxicity studies will be initiated in normal strain 13 guinea pigs. The drugs to be tested will be hydroxyurea, busulfan and Cytoxan. The drug regimen will vary depending on each drug.

C. Once optimal doses of these drugs have been determined, studies will be initiated in an attempt to increase and/or maintain the chronic phase of this leukemia.

## III. Monoclonal Antibody Therapy

Monoclonal antibodies will be raised and immunologically characterized by standard techniques to the primary and metastatic carcinoma tumor and to the chronic and blast phses of the CML (see reports by Foon and Bernhard). Conjugation of the candidate monoclonal antibodies to radionuclides, drugs and toxins will be done by Dr. Hwang. In vivo therapy experiments analyzing the efficacy of monoclonal antibodies developed for both model systems will then be examined alone, i.e., passive immunotherapy, or serve as carrier molecules in combination with drugs, toxins or isotopes for elimination of pulmonary metastases and for treatment of CML.

MAJOR FINDINGS

1. Transplantable Spontaneous Mammary Carcinoma

Over the last year, investigations have been underway to study the biologic and morphologic characteristics of a transplantable mammary carcinoma that arose spontaneously in a 2.5-year-old female strain 2 guinea pig (manuscript in preparation). The administration of $5 \times 10^6$ tumor cells resulted in 100% tumor incidence regardless of the route of administration. Spontaneous metastases to the lungs, but not to other visceral organs or lymph nodes, were consistently observed in most tumor-bearing animals. Furthermore, investigations revealed that following excision of the primary breast tumor, the capacity and/or incidence of lung metastases was related to the size of the primary tumor growth at the time of surgery. In this case, guinea pigs were injected into the breast area with $5 \times 10^6$ tumor cells and observed for tumor growth. Animals undergoing surgery for tumors either at 15-20 or 25-30 mm revealed a 40% incidence of macrosocpic lung metastases 3 months post-surgery. However, the incidence of pulmonary metastasis increased to 60% in animals undergoing surgery for tumors at the size of 30-35 mm and to 100% for animals with 35-40 mm tumors. The number of lung foci for all groups ranged between 1 to 5 with the nodules measuring approximately 0.1 to 0.4 mm in diameter. No gross metastatic involvement was observed on other organs. Microscopically, the primary and metastatic pulmonary tumors were characterized as undifferentiated carcinomas. This morphologic classification was supported by electron microscopy.

Preliminary studies have shown that this mammary tumor is weakly immunogenic. A significant difference ($P<.01$) in tumor growth was observed among animals treated with irradiated cells alone or admixed with BCG following a viable cell challenge of $5 \times 10^5$ tumor cells when compared to the untreated control group. However, after the initial occurrence of the tumor, the growth rates were progressive and similar to that observed in the untreated control group. Subsequent studies have shown that when graded doses of tumor cells ($10^4$ to $10^6$, respectively) were injected via the breast area all animals exhibited a 100% tumor incidence. No tumors developed in animals that received $1 \times 10^3$ tumor cells when the study was terminated at day 95.

Of interest was the confinement of metastases to the lungs in tumor-bearing animals or in animals following excision of the primary breast tumor. In these studies, the route of metastases appeared to be hematogeneous. The tumor emboli in the lungs were generally confined to the large pulmonary arteries. Investigations are ongoing to develop monoclonal antibodies against both the primary and metastatic tumors for subsequent use in therapy trials. Likewise, chemotherapy studies against both primary and pulmonary metastatic tumors are currently underway.

2. Transplantable Chronic Myelogenous Leukemia

Studies have shown that following the subcutaneous inoclulation of $3.0 \times 10^6$ leukemic blood leukocytes into the scapular area of strain 13 guinea pigs the chronic phase of this leukemia occurs within 20-23 days after tumor cell inoculation. This phase lasts approximately 7 to 10 days, followed by the

subsequent development of blast crisis (30-32 days) and death of all animals within approximately 35 days. The chronic phase is characterized by the development of leukemia (WBC > 30,000/$mm^3$) and the elevated count is due mainly to more mature granulocytes (myelocytes, bands and segmented neutrophils). Thereafter, the leukocyte count rises sharply with predominantly blast cells similar to CML blast crisis in man.

Recently, chemotherapeutic studies were initiated to study the effects of three clinically active drugs, when administered at a time before onset of the chronic phase of this leukemia. The goal here is to find an active drug, as well as an optimal dose, that will delay the occurrence of the chronic phase. Once this basic drug parameter has been established, the most active drug as well as optimal dose will be administered at a time when the animals are in the chronic phase of this leukemia for the sole purpose of extending the chronic phase and/or preventing the development of blast crisis.

The parameters being utilized to follow the activity of a specific drug are: 1) blood leukocyte counts; 2) time to development of the chronic phase and to eventual blast crisis; and 3) survival time of the treated animals when compared to the untreated control group. Thirteen days post-tumor cell inoculation (3.0 x $10^6$), groups of animals were treated intraperitoneally with either 0.25 or 0.50 mg/kg of busulfan or 630, 540 or 450 mg/kg of hydroxyurea. In this case, the drugs are being administered two times a week over a four-week period. In addition, another group of guinea pigs was treated with 150 mg/kg of cyclophosphamide. The drug in this case is given every 10 days over a four-week period. At the present time, only the untreated group is beginning to show signs of developing leukemia based on elevated leukocyte counts. The treated groups, regardless of drug or dose, still exhibit no signs in the development of leukemia. Studies are currently underway to develop MOAb's against both the chronic and blast cell populations involved in this disease.

PROPOSED COURSE

A. Transplantable Spontaneous Guinea Pig Mammary Carcinoma

1. Drug toxicity and the efficacy of antitumor therapy against the metastatic tumor will continue as previously described.

2. Studies will continue in the development and immunological characterization of MoAb's derived from both the primary and pulmonary metastatic tumors. Metastatic tumors may differ from the primary tumor with respect to antigenicity and other biologic properties; therefore, MoAb's produced against primary and secondary tumros may identify different antigenic determinants.

3. Once the two MoAb's have been fully characterized, serotherapy trials will be initiated for both the primary and metastatic tumors. Following inoculation of tumor cells (5 x $10^6$), MoAb therapy will be administered five days later and thereafter two times weekly, over a four-week period. During the course of therapy, sera will be obtained every other week from treated animals and checked for free antigens, cicculating murine antibody and guinea pig anti-

murine antibody. Periodically, animals will be sacrificed and all visceral organs, lymph nodes and tumors will be examined for localization of MoAb by immunoperoxidase staining (PAPS).

4. The eventual goal of this project will be to utilize the MoAb as a carrier molecule for an active drug, toxin or isotope directed toward the metastatic pulmonary foci following surgical removal of the primary breast tumor.

B. Transplantable Chronic Myelogenous Leukemia

1. Drug studies will continue to find an active drug that is capable of maintaining the chronic phase of this disease.

2. Once MoAb's have been fully characterized, serotherapy trials will be initiated against this disease. Animals are to be inoculated with $3.6 \times 10^6$ leukemic blood leukocytes (s.c.) followed two days later with the initiation of MoAb therapy (i.v.). The regimen of therapy will be twice weekly over a four-week period. Every other week the treated animals will be monitored for CBC, bone marrow morphology and sera obtained, for the presence of free antigen, circulating murine antibody and guinea pig anti-murine antibody. All treated animals are to be sacrificed two weeks after the last treatment and bone marrow and visceral organs examined histologically.

3. Once we have established the optimal dosage and schedule for MoAb serotherapy (and/or MoAb toxin and drug conjugates), we will attempt to eradicate CML in animals with established chronic and blast phase disease.

4. Bone marrow transplantation studies will also be carried out. Animals with established chronic phase of CML will have bone marrow removed and treated in vitro with MoAb and C' or MoAb conjugated to drugs and/or toxins. Animals with established chronic and blast phase disease will be lethally treated with irradiation and/or chemotherapy and "rescued" with MoAb treated bone marrow. Reconstitution of bone marrow and recurrence of CML will be carefully monitored. Bone marrow and peripheral blood will be carefully studied for chromosomal abnormality as evidence of recurrent CML.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09236-01 BRTB |
|---|---|---|

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of Human T-T, Monocyte-Macrophage, T-Large Granular Lymphocyte, and Macrophage-Large Granular Lymphocyte Hybridomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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| PI: | Kenneth A. Foon | Acting Head (Proposed) MAHS | BRTB | NCI |
| Others: | Paul Abrams | Expert | BRTB | NCI |
| | James Knost | Expert | BRTB | NCI |
| | John Ortaldo | Biologist | BRTB | NCI |
| | Robert Oldham | Associate Director | BRMP | NCI |
| | Ronald Herberman | Chief, BRTB | BRTB | NCI |
| | Henry Stevenson | Monocyte/Macrophage Section (Proposed) | BRTB | NCI |
| | Eugenie Kleinerman | Senior Investigator | BRTB | NCI |

COOPERATING UNITS (if any)

NCI-FCRF

LAB/BRANCH

Biological Research and Therapy Branch

SECTION

Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION

NCI-FCRF

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | .5 | .5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The human macrophage line U937 and human T lymphoblastoid leukemia line HSB2 have been rendered sensitive to hypoxanthine-aminopterin-thymidine (HAT) culture medium by treatment with 8-azaguanine. The cell lines have been fused to purified T lymphocytes that have been stimulated with concanavalin A, purified large granular lymphocytes, and purified monocytes. Hybridoma cell lines will be selected that retain the characteristics of the human peripheral blood cell but have been immortalized by the cell lines. These hybridomas will be screened for the production of various cytokines and will be studied in functional assays to see if they retain the effector function of the particular cell used for fusion.

OBJECTIVES

1. To develop human natural killer hybridoma cell lines to further study the functional and antigenic characteristics of the natural killer cell. To utilize this cell line to isolate soluble cytotoxic factors, the receptors for target cells, and/or the target cell structures, and characterize them biochemically.

2. To develop human monocyte hybridoma cell lines to study the antigenic and functional characteristics of monocytes and macrophages. To screen the supernatants of these cell lines for the production of various monokines such as interferon and interleukin-1 (lymphocyte activating factor).

3. To develop T lymphocyte hybridomas that secrete various lymphokines including macrophage activating factor, migration inhibition factor, and chemotactic factor.

METHOD EMPLOYED

We have successfully rendered the U937 macrophage cell line and the HSB2 T-ALL cell line sensitive to HAT medium by repeated treatment with 8-azaguanine. Monocytes will be purified by the cell elutriator and fused to the U937 cell line. Large granular lymphocytes (LGL) purified by Dr. John Ortaldo have been fused to both the HSB2 and U937 cell lines. Peripheral blood mononuclear cells have been treated in vitro with the T cell mitogen concanavalin A and after 24 and 48 hours fused to the HSB2 cell line. Fusions have been done by standard techniques using polyethylene glycol. Some of the cultures have been treated with interleukin-2 and colony stimulating factor to see whether these soluble factors enhance growth of the cell lines.

1. LGL Hybridomas

We have recently fused LGL to U937 and HSB2. We will select out the clones that are growing and test for natural killer (NK) activity. Those clones that have NK activity will then be recloned and studied for their antigenic and morphological characteristics. Antigenic characteristics will be studied by a panel of monoclonal antibodies identifying antigens on myeloid and lymphoid cells. Morphology will be studied by light microscopy and electron microscopy. Using LGL hybridomas, Dr. Ortaldo will attempt to isolate and purify soluble cytotoxic factors and the receptors for target cells, and/or the target cell structures recognized by NK cells.

2. T-T Hybridomas

Supernatants from growing hybrids will be screened for macrophage activing factor, chemotactic factor, and migration inhibition factor. Hybrids producing these factors will be cloned and retested and then mass cultured.

3. Macrophage-Monocyte Hybridomas

Supernatants will be tested for interleukin-1 and interferon productivity and hybrids will be tested for cytolytic effect on tumor cells.

MAJOR FINDINGS

This work is very preliminary and to date we have been able to identify what appear to be growing hybrids from macrophage-monocyte fusions. The hybrids will soon be screened for MAF production and cytolytic effect on tumor cells. They will also be antigenically characterized, karyotyped and HIA typed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE BRMP

The function of monocytes and natural killer cells and cytokine production are all major efforts of the Biological Research and Therapy Branch. The proposed hybridomas will be widely used and studied by various Sections of our Branch. Hybridomas producing macrophage activating factor (MAF) in large quantity could be an excellent source of this lymphokine for future clinical trials.

PROPOSED COURSE

As outlined above, we will continue to fuse LGL, T cells and monocytes to the U937 and/or HSB2 cell lines. The addition of interleukin-2 to these cultures may enhance cell growth. Once these hybridomas are established, functional assays will be studied in collaboration with investigators in the relevant Sections.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09237-01 BRTB |
|---|---|---|

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Murine Monoclonal Antibodies to Human Leukocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Kenneth A. Foon | Acting Head (Proposed) MAHS | BRTB | NCI |
| Others: | Henry C. Stevenson | Senior Investigator | BRTB | NCI |
| | John Ortaldo | Biologist | BRTB | NCI |
| | Michael Bernhard | Expert | BRTB | NCI |
| | Eugenie Kleinerman | Senior Investigator | BRTB | NCI |
| | John Harley | Research Associate | LCI | NIAD |

COOPERATING UNITS (if any)

NCI-FCRF; American Red Cross; University of Oregon School of Medicine

LAB/BRANCH
Biological Research & Therapy Branch

SECTION
Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | .5 | .5 |

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed a series of monoclonal antibodies to human granulocytes, monocytes, platelets, and eosinophils. Characterization of the patterns of reactivity of these antibodies and isotyping of these antibodies has been performed by radioimmunoassays, enzyme-linked immunosorption assays (ELISA), immunofluorescence by flow cytometry and cytotoxicity. Characterization of the antigens identified by these monoclonal antibodies will be performed by SDS-PAGE. Patterns of reactivity with bone marrow cells and human stem cells are also under investigation. These antibodies will also be studied for their effect on phagocytosis, chemotaxis, cytotoxic activity against tumor target cells, and natural killer activity. Platelet antibodies have been demonstrated to inhibit aggregation, adherence, and serotonin release of platelets.

PROJECT DESCRIPTION

OBJECTIVES

1. To develop monoclonal antibody reagents to identify differentiation antigens on human monocytes, granulocytes, eosinophils and platelets.

2. To utilize these monoclonal antibodies for the classification, diagnosis and possible therapy of leukemia.

3. To study these monoclonal antibodies' effect on various immune functions including natural killer activity, monocyte cytotoxic activity *in vitro* against tumor target cells, chemotaxis, phagocytosis of latex particles and mitogen and antigen responses.

4. To study the effect of platelet monoclonal antibodies on platelet aggregation, adherence and serotonin release.

METHODS EMPLOYED

Purified populations of human leukocytes and platelets are separated by Dr. Henry Stevenson using the cell elutriator. Eosinophils have been sent by Dr. John Harley from patients with the "hypereosinophilic syndrome." $10^7$ purified cells of each individual population are injected 3 to 5 times intraperitoneally into BALB/c mice spaced approximately one week apart. Four days after the last immunization the animals are sacrificed. The spleens are removed, prepared into a single-cell suspension and fused to a HAT-sensitive murine myeloma cell line. After approximately 3 weeks, hybridoma supernatants are screened against the immunizing cell and either autologous B cells, allogeneic B cells, or B cell lines by radioimmunoassay. Hybridomas producing monoclonal antibodies reacting specifically with the immunizing cell and not with B cells are then cloned. Clones are then screened against large panels of human leukocytes and leukemia cells and cell lines. Hybridomas producing antibodies with specificity for specific cell types are isotypic and molecular characterization of the antigen identified by the monoclonal antibody is performed by SDS-PAGE. Antibodies are also incubated with human bone marrow cells and stained by indirect immunofluorescence using a fluorescent tagged rabbit anti-mouse reagent. Positive cells are separated on the cell sorter in order to specifically identify the level of cell maturation that the monoclonal antibodies react with. This is performed by preparing cell preparations in the CytoSpin and staining with Wright's stain.

*In vitro* assays to study myeloid, erythroid, platelet and eosinophil progenitor cells are available and will be performed by Dr. John Fitchen. The colony-forming unit in culture (CFU-C) is the myelomonocytic progenitor cell, and the erythroid colony forming unit (CFU-E) and the erythroid burst forming unit (BFU-E) are the erythroid progenitors. The platelet progenitor is referred to as the CFU-mega and the eosinophil precursor is the CFU-Eo. These assays are performed on human bone marrow (CFU-C, BFU-E, CFU-mega, CFU-Eo) and peripheral blood (BFU-E) grown in soft agar with cell feeder layers and colony stimulating factor or erythropoietin.

Bone marrow and peripheral blood will be incubated with monoclonal antibody and stained by immunofluorescence and sorted on the cell sorter. One can then study the antigenic characteristics of the stem cells by culturing positive and negative populations for CFU-C, CFU-E, CFU-mega, CFU-Eo, and BFU-E. For monoclonal antibodies that are cytotoxic, a second and easier approach can be used. Bone marrow or peripheral blood is incubated in vitro with monoclonal antibody and complement. Following this incubation the cells are cultured for CFU-C, CFU-E, BFU-E, CFU-mega and CFU-Eo. Growth inhibition is then an indirect way of identifying antigen-positive progenitor cells. MoAbs reactive with leukemia cells and not with progenitor cells have potential application for bone marrow transplantation.

In vitro experiments to study the effect of monoclonal antibodies on various immune functions will be carried out by a number of collaborators. Drs. Stevenson, Kleinerman and Thurman will study the effect of these monoclonal antibodies on monocyte phagocytosis of latex particles, chemotaxis and cytotoxic activity against tumor target cells. Dr. Ortaldo will study the effect on natural killer activity and Dr. Guardinia on platelet function and physiology (aggregation, adherence, serotonin release).

## MAJOR FINDINGS

Monocyte monoclonal antibodies. Three IgG1 monoclonal antibodies raised to monocytes that react with human monocytes, granulocytes, and eosinophils have been developed. Interestingly, these antibodies also react with large granular lymphocytes isolated from human peripheral blood cells. These antibodies have been demonstrated to react with granulocyte and monocyte precursor cells in human bone marrow but not with erythroid precursors or lymphocytes. These antibodies are also being tested against leukemia cells and lymphoblastoid cell lines. Functional studies are currently under way, as is molecular characterization of the antigens.

Granulocyte monoclonal antibodies. Five IgG1 monoclonal antibodies raised to human granulocytes have been identified. Each of these antibodies reacts with granulocytes and not with any other peripheral blood cells including large granular lymphocytes. There was no reactivity demonstrated with granulocyte precursor cells in normal human bone marrow. Further characterization against leukemia cells and lymphoblastoid cell lines, functional assays and characterization of the antigen are underway.

Eosinophil monoclonal antibodies. Eight monoclonal antibodies to human eosinophils have been raised. At least four isotypes have been identified including IgG1, IgG2A, IgG2B and IgM. These antibodies cross-react with granulocytes but do not react with any other peripheral blood leukocytes including large granular lymphocytes. Bone marrow studies demonstrate reactivity with eosinophil and granulocyte precursor cells and no reactivity with erythroid precursors or lymphocytes. Further characterization of these antibodies is underway.

Platelet antibodies. An IgG2 monoclonal antibody that reacts with human platelets has been identified. This monoclonal antibody does not cross-react

with any other peripheral blood cells but does cross-react with common acute lymphoblastic leukemia cells. In the bone marrow it reacts with megakaryocytes and is currently being tested against CFU-megas. Functional assays performed by Dr. Stephen Guardinia have demonstrated that this antibody completely abrogates platelet function including platelet adherence, platelet aggregation, and platelet serotonin release.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE BRMP

The identification of differentiation antigens in human leukocytes is a powerful tool to dissect the immune system and to better understand the mechanisms of cell-cell interaction. In addition differentiation antigens are generally expressed on leukemia and lymphoma cells and may be useful diagnostically and therapeutically in the treatment of leukemia and lymphoma. Therapeutic approaches to leukemia and lymphoma that have been tried include monoclonal antibody serotherapy and monoclonal antibody treatment of leukemic bone marrows prior to bone marrow transplantation.

One of the major directions of this program is investigation of the human cellular immune system. The effect of cell-cell interactions and how they may be compromised or magnified by monoclonal antibodies that specifically react with different human cell populations could be a valuable avenue of investigation. In vitro assays that study monocyte, granulocyte and natural killer activity are extensively studied throughout the Biological Research and Therapy Branch and the effect of monoclonal antibodies on such functions can be readily evaluated. The possible in vitro role of such antibodies could conceivably be extended to in vivo trials utilizing these various monoclonal antibodies either by themselves or mixed with effector cells.

## PROPOSED COURSE

Preliminary characterization of these monoclonal antibodies has already taken place. Further characterization against leukemia and lymphoma cell lines and bone marrow stem cells is currently underway. The effect that these antibodies may have on various functional assays is also under study.

## PUBLICATIONS

Fitchen, J.H., Foon, K.A., and Cline, M.J.: The antigenic characteristics of hematopoietic stem cells. N. Eng. J. Med. 305:17-25, 1981.

Foon, K.A., Schroff, R.W., and Gale, R.P.: Surface markers on leukemia and lymphoma cells: Recent advances. Blood, in press, July, 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09238-01 BRTB |
|---|---|---|

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Murine Monoclonal Antibodies Against Human Bronchogenic Carcinomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
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| PI: | Paul Abrams | Expert | BRTB, NCI |
| Others: | Jeffrey Ochs | Medical Staff Fellow | BRTB, NCI |
| | Edward Kimball | Senior Staff Fellow | BRTB, NCI |
| | Kenneth Foon | Acting Head (Proposed) MAHS | BRTB, NCI |

COOPERATING UNITS (if any)

NCI-FCRF

LAB/BRANCH Biological Research and Therapy Branch

SECTION Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1 | .5 |

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thirteen murine monoclonal antibodies have been produced against non-small cell bronchogenic carcinoma. Monoclonal antibody (MA) 503D8 reacts with adeno and large cell carcinomas of the lung, 1/2 squamous cells and 1/7 small cell carcinomas. MA24X1G9 reacts with large cell (2/12) small cell (3/3), squamous cell (2/2) and adenocarcinomas (2/2). Both antibodies react with adenocarcinomas of the breast and colon and melanoma but not with EBV-transformed B-cells, B-cell lymphomas or T-cell lymphomas. MA1H10 reacts with squamous and large cell carcinomas of the lung but not with any other carcinomas or normal cells. MA503D8 precipitates an antigen of 70,000 daltons from cell lysates which is secreted as a 150,000 dalton antigen in the supernatants of reacting cells. An enzyme-linked immunosorbent assay (ELISA) to test patient urines and sera for circulating antigen will be developed. Immunoperoxidase staining of fresh surgical specimens and washings obtained at bronchoscopy is currently underway. Additional monoclonal antibodies will be produced by using membrane preparations and membrane extracts for immunization of mice.

PROJECT DESCRIPTION

## OBJECTIVES

Development and characterization of murine monoclonal antibodies against the major histologic types of human bronchogenic carcinoma, for application to early diagnosis and treatment.

## METHODS

Murine monoclonal antibodies against human bronchogenic caracinomas have been produced by these investigators by injecting established human bronchogenic carcinoma cell lines into BALB/c and C57BL/6 mice. Splenocytes are harvested and fused with established murine myeloma lines 653 and NS-1. Hybridoma supernatants are tested against the immunizing cell line and at least one B-lymphoblastoid line employing a solid-phase radioimmunoassay. Those hybridomas whose antibodies react with the former, but not the latter, have been cloned, retested, and then subjected to a wide screen against malignant and normal cells and cell lines. Those exhibiting the best selectively for the malignant cells are being grown in ascites to develop high-titer antibody for immunoperoxidase staining against cryostat and fixed sections of fresh bronchogenic carcinomas obtained at surgery.

Development of additional murine monoclonal antibodies against human bronchogenic carcinomas will involve the use of membranes and membrane extracts of freshly obtained tumors or from nude mouse xenografts as the immunizing substance. Screening will be performed as described above, with the addition of the immunizing preparation as a target in the solid-phase radioimmunoassay.

## MAJOR FINDINGS

### I. Adenocarcinoma

Murine monoclonal antibody 503D8 has been developed by injecting human adeno-carcinoma of the lung cell line NCI-H125 into BALB/c mice. This antibody is an IgG2a, and exhibits significant reactivity with adenocarcinoma of the lung (2/2), large cell carcinoma of the lung (2/2), with less significant reactions with squamous cell carcinoma (1/2) and small cell carcinoma (1/7). It also reacts with adenocarcinoma of the colon (2/2) and breast (2/2) and displays a weaker reaction with melanoma (2/2). Dr. Kimball has demonstrated that it precipitates a protein of 70,000 daltons from postiive reacting cell lines and is secreted as a 150,000 dalton protein in the supernatant of NCI-H125. There also exist smaller molecular weight components (15,000 and 18,000 daltons) and the relationship among these constitutents is currently under investigation.

This antibody exhibits no reactivity with B-lymphoblastoid lines and insignificant reactions with cultured skin fibroblasts. It has demosntrated a strong positive reaction with a fresh adenocarcinoma of the lung and a weak reaction with normal lung from the same patient.

II. Large Cell Carcinoma

Using NCI-H157, a large cell carcinoma of the lung cell line, to immunize C57BL/6 mice, Dr. Ochs has produced, cloned, and stabilized a monoclonal antibody 24XG9 which reacts with all four major bronchogenic carcinoma histologies (adeno 2/2; large cell 2/2; squamous 2/2 and small cell 3/3), and exhibits no reactivity with B-lymphoblastoid cell lines, normal hematopoietic cells or hematopoietic malignant cell lines. It does demonstrate reactivity with breast cancer, colon carcinoma and melanoma cell lines. This antibody has been characterized as an IgG1 and is currently being rendered in nude mouse ascites to develop a reagent for immunoprecipitation and immunoperoxidase staining.

III. Squamous Carcinoma

Human squamous cell carcinoma of the lung cell line SK-MES was employed to immunize BALB/c mice. Fusion of the splenocytes with murine non-secretory myeloma 653 has resulted in the production of 10 clones which react with squamous cell carcinoma and large cell carcinoma but not with other malignant or normal cells tested thus far. These antibodies are all IgG1, and are currently being raised in ascites for further testing and screening as described above.

IV. Summary: Monoclonal Antibodies Against Non-Small Cell Bronchogenic Carcinomas

| | Reactions of Monoclonal Antibodies* | | | |
|---|---|---|---|---|
| Immunizing Cell | Adeno | Squamous | Large Cell | Small Cell |
| Adenocarcinoma (2 MoAbs) | + | +/- | + | - |
| Large Cell (1 MoAb) | + | + | + | + |
| Squamous (10 Moabs) | - | + | + | - |

*All screened for relative lack of reactivity against B-lymphoblastoid and other normal or non-malignant cells.

RELEVANCE TO BIOLOGICAL RESPONSE MODIFIERS PROGRAM

Monoclonal antibody technology offers the potential of producing unlimited quantitites of single, pure antibodies. Because of their purity and exquisite specificity, these antibodies promise to be critical reagents in the early diagnosis of malignancy, either by detecting circulating antigen in the sera of asymptomatic individuals, or by imaging with injected antibody in high-risk or clinically suspected patients. Since lung cancer accounts for 25% of the newly diagnosed cases of cancer and for nearly 45% of the deaths due to malignancy in this country annually, and since less than 15% of these patients

are resectable and there exists no other curative therapy (with the exception of a 6-10% longterm disease-free survival after combination chemotherapy in small cell bronchogenic carcinoma), development of a suitably specific monoclonal antibody for this disease entity could have a major impact on cancer mortality. If suitable technologies are developed which can effectively employ these antibodies as carriers of drugs or toxins, a significant advance may be achieved in the treatment of this disease as well. Accordingly, monoclonal antibodies have a high priority in the Biological Response Modifiers Program.

PROPOSED COURSE OF DEVELOPMENT

1. Development of an ELISA assay to test urines and sera of patients and normals for presence of circulating antigen.

2. Immunoperoxidase staining of cryostat and fixed tissues of fresh surgical specimens and autopsy material when available.

3. Immunoperoxidase staining of cytospin preparations of washings obtained at bronchoscopy.

4. Biochemical characterization of the antigens detected by these antibodies.

5. Development of additional monoclonal antibodies as described above.

PUBLICATIONS

Abrams, P.G., Cuttita, F.C., Kimball, E., and Minna, J.D. A monoclonal antibody (503D8) specific for non small cell bronchogenic carcinoma. (Abstract) III World Conference on Lung Cancer, Tokyo, Japan. May 17-20, 1982.

Abrams, P.G., Cuttita, F.C., Rosen, S., and Minna, J.D. A monoclonal antibody (503D8) which reacts with non small cell lung cancer, some other carcinomas, cultured skin fibroblasts but not normal cells. (Abstract) ASCO, Washington, D.C. April, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09239-01 BRTB |
|---|---|---|

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Human Monoclonal Antibodies Against Adenocarcinoma of the Colon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Paul Abrams | Expert | BRTB | NCI |
| Others: | James Knost | Expert | BRTB | NCI |
| | Charles Morgan | Expert | BRTB | NCI |
| | Jeffrey Ochs | Medical Staff Fellow | BRTB | NCI |
| | Kenneth Foon | Acting Head, (Proposed) MAHS | BRTB | NCI |
| | Robert Oldham | Associate Director | BRMP | NCI |

COOPERATING UNITS (if any)

Cancer Metastasis and Treatment Laboratory, NCI-FCRF;
Johns Hopkins University Cancer Center

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 0.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human myeloma cell lines lacking HGPRT have been produced by the principal investigator or obtained from other sources. These lines will be compared for fusion efficiency, growth rate, death in HAT medium, and antibody secretion using leukapheresed cells from a normal donor. The best line(s) shall be chosen for human monoclonal antibody production. Human lymphocytes obtained from the draining lymph nodes of patients with colon carcinoma shall be fused directly or stimulated in vitro with pokeweed mitogen prior to fusion and then fused with the human myeloma cell line. The hybrids produced shall be screened against the patient's tumor, if available, or a human colon cancer cell line. Positive wells shall be further screened against other non-related human tumors and normal cells. Those wells exhibiting selectivity shall be cloned and subjected to a wide screen. In vitro sensitization of lymphocytes has been successfully worked out in a tetanus toxoid system and we will begin to study in vitro sensitization in a melanoma system using the 94K antigen.

PROJECT DESCRIPTION

OBJECTIVES

To develop human monoclonal antibodies against colon carcinoma for diagnosis and treatment.

METHODS EMPLOYED

I. Human Myeloma Line

Successful production of stable human monoclonal antibodies requires the existence of a human myeloma cell line which grows quickly, fuses well, secretes antibody at readily detectable levels (0.1-10 μg/ml) and dies in selective medium (HAT). This has been a significant problem in the human system, as most human myeloma cell lines selected for HAT sensitivity display inhibition of growth but not actual cell death. The spectre of parent and/or revertant myeloma cell growth remains an ever-present threat.

The principal investigator has developed two HGPRT negative human myeloma cell lines, U-266, an IgE secreting myeloma which is inhibited by HAT but which exhibits a high frequency of revertants based upon loss of HAT sensitivity after serial passaging; and AMM2, a lambda light chain secreting myeloma which grows quickly and dies in HAT medium in 7 days based upon exclusion of trypan blue.

In addition, we have obtained GM4672, a B-lymphoblastoid line which secretes IgG and is inhibited by HAT but which grows slowly; LICR-LON-HMy2, an IgG secreting myeloma which is inhibited but not killed in HAT medium, but which grows very quickly, and UC729-6 from the University of California at San Diego, a non-secretory, B-lymphoblastoid line, which fuses well and dies in HAT medium within 10 days.

An experiment using mononuclear cells from leukapheresed patients to compare these lines for fusion efficiency, death in HAT, growth rates, and antibody secretion is currently underway. Based upon the results of this experiment, a single human myeloma line shall be chosen for human X human fusions.

II. Human B Lymphocytes

Attacking the problem of the fusion partner, the antibody-specific lymphocyte has been more difficult. We have used draining lymph nodes of colon tumors as well as peripheral blood from patients immunized with their own irradiated tumor cells as sources of specific antitumor antibody-producing lymphocytes. Most antibody producing hybrids have not been tumor specific. Four hybrids which have been screened had antibodies reactive with the tumor, but not with a fetal fibroblast line, but these hybridomas have been lost either because of instability or overgrowth with revertant parent myelomas.

In vitro sensitization is the most promising solution to this dilemma. This is described in detail by the report of the proposed Lymphokines/Cytokines Section. We are currently repeating experiments in which in vitro sensitization with a known antigen, tetanus toxoid, and fusion with U-266 or a mouse myeloma (NS-1) has produced hybrids secreting specific antibody against this antigen. Once this has been accomplished, then use of known (e.g., 100K), and then unknown, tumor antigens shall be used in the same system. One such experiment is underway at the present time. A second arm of this experiment involves the non-specific stimulation of the lymphocytes in vitro with mitogens prior to fusion. Sources of lymphocytes include the draining lymph nodes and peripheral mononuclear cells from patients immunized with their own irradiated tumor cells on the FCRF-Johns Hopkins protocol.

## RESULTS TO DATE

More than 50 human X human fusions have been performed, most using U-266 and more recently AMM2. Each fusion produced between 0-30% hybrids, and of these 10-15% exhibited evidence of antibody secretion on the initial screening. Four antibodies exhibited some evidence of specificity for the tumor type, but all have been lost (one to fungal contamination and three to instability or growth of a revertant). One of these hybridomas was produced following in vitro sensitization to the 94K antigen. The others were developed against adenocarcinoma of the lung (1) and colon (2).

The in vitro sensitization and subsequent human X human or human X mouse fusions have produced monoclonal antibodies specific for tetanus toxoid. A similar experiment, using CEA, is pending, although the fusion does not appear at 21 days to have been successful.

## RELEVANCE TO THE BIOLOGICAL RESPONSE MODIFIERS PROGRAM

While murine antibodies specific for human tumors offer promise for the early detection and/or treatment of the disease, their repeated use will likely cause problems of anti-globulin and anti-idiotype reactions rendering such use difficult. Human antibodies may alleviate this problem to a large degree and may provide insight into the nature of the humoral response to spontaneous tumor. For both reasons, development of human antibodies is extremely important to the BRMP.

## PROPOSED COURSE OF STUDY

1. Comparison of human myeloma lines for growth rates, fusion efficiency, and antibody secretion (ongoing).

2. Further development of the in vitro sensitization system. (Details in report of the proposed Lymphokine/Cytokine Section.)

3. Fusion of lymphocytes from lymph nodes and peripheral bloods after non-specific stimulation with mitogens.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09240-01 BRTB |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Murine Monoclonal Antibodies to Colon Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | James A. Knost | Expert | BRTB | NCI |
| Others: | Paul Abrams | Expert | BRTB | NCI |
| | Jeffrey Ochs | Medical Staff Fellow | BRTB | NCI |
| | Kenneth Foon | Acting Section Head (Proposed) | BRTB | NCI |
| | Robert Oldham | Associate Director | BRMP | NCI |

COOPERATING UNITS (if any)

Cancer Metastasis and Treatment Laboratory, Frederick Memorial Hospital; NCI-FCRF

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 4.0 | 2.0 | 2.0 |

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To develop murine monoclonal antibodies to colon cancer by immunizing BALB/c mice with whole cells derived from a patient's tumor, whole membranes prepared from a nu/nu BALB/c xenograft of the primary lesion, or protein extracts of tumor membrane derived from the same xenograft. The latter will be linked to lectins to enhance their immunogenicity. It is anticipated that these antibodies will be used therapeutically and diagnostically in patients with colon cancer.

PROJECT DESCRIPTION

OBJECTIVES

1. To develop murine monoclonal antibodies specific for colon cancer or colonic epithelium.

2. To compare the efficacy of whole tumor cells, tumor membrane preparations and tumor extracts, as immunogens in the production of murine hybridomas

3. To determine whether antibodies derived from whole cells, membranes or membrane extracts show specificity for (a) an individual patient's tumor; (b) other patients' tumors; (c) colon cancer cell lines; (d) "normal" colon from the same patient; (e) primary and/or metastatic lesions.

4. To determine whether these antibodies recognize antigens in patient's serum.

5. To determine whether these antibodies localize to a xenograft made from the patient's tumor.

6. To use these antibodies as diagnostic and therapeutic reagents in patient's with colon cancer.

METHODS EMPLOYED

Steplewski and Koprowski have previously demonstrated that monoclonal antibodies reacting with colon carcinoma cell lines can be developed from mice inoculated with cells lines. They also showed that certain monoclonal antibodies produced in this fashion can distinguish between shed and unshed cell surface antigens. The primary thrust of this project is to expand their experience by using whole tumor cells from a primary malignancy or from xenografts of a fresh tumor specimen, passaged a limited number of times in nude mice.

In the first of three groups in this protocol, cells from the primary colon lesion of seven patients were enzymatically dissociated and stored in liquid nitrogen. Each primary lesion yielded between 5 and 7.5 x $10^7$ viable tumor cells (stored at 1 x $10^7$ cells/vial). These cells were used to inoculate 7 groups of mice (3 mice/group). Each mouse received 3.3 x $10^6$ tumor cells per inoculation. Because of the limited number of cells available for immunization the tumor cells were mixed with complete Freund's adjuvant and injected subcutaneously at three sites on the first injection followed by two injections intraperitioneally given on the third and fourth weeks. The inoculation schedule has been completed and as of 5/11/82 all seven fusions have been performed.

Additional tumor cells on each patient are available to use in screening the various hybrids, plus normal colon ($\geq$5 cm from the primary), peripheral lymphocytes and BALB/c nu/nu tumor xenografts from each patient. By using these materials in the appropriate sequence we should have the opportunity to address several questions concerning surface properties of the tumors. By

visual inspection, wells with hybrid growth will be singled out. Supernatants from these wells will be first screened by ELISA to determine which hybrids are producing immunoglobulins (Ig), then the supernatants containing Ig will be screened by RIA to check for reactivity of the Ig with B-cell lines. The search will then be limited to wells with hybrids producing Ig that does not react to B-cells. This subset of hybrids will then be tested for reactivity of the Ig to the patient's tumor cells and two colon cancer lines. Positive wells will be cloned and retested in the same manner. Once a stable hybrid is obtained, then a wide screen will be done, including numerous cell lines, primary tumor cells and the patient's "normal" colon cells. By this process we should be able to make statements about the specificity of a murine antibody developed from a given patient's tumor cells for that tumor, cells from other primary tumors, and various colon cancer cell lines and non-colon cancer cell lines.

The second group in this protocol will consist of hybridomas produced from mice inoculated with whole tumor cell membranes. Over the past nine months, 35 colon carcinoma specimens have been provided by the surgeons at Frederick Memorial Hospital. Eighteen of these have been injected into nude mice and 11/18 have formed tumor xenografts. Five different grafts (FMH59, 42, 67, 84, 89) have been passed on four occasions, and that there are presently ten animals for each type (i.e., 59, 42, 67, 84, 89) in various stages of development. Tumors grow to a diameter of 1-2 cm before gross necrotic areas occur. These five donor patients (59, 42, 67, 84, 89) represent patients in the local community that are alive and potential sources for more materials (i.e., neoplastic, B-cells, serum, etc.) and are candidates for therapy. Whole tumor cell membranes for each patient will be made from the ten tumor xenografts of the respective patient's primary lesion. Whole cell membranes were chosen because of their abundance, ease of storage and utility as screening tools. Whole membranes also have the advantage of containing both protein and glycolipid cell surface structures. The inoculation schedule calls for three mice to be injected on six occasions intraperitioneally. This schedule is patterned after the experience of Reisfeld, Morgan and Bumol who demonstrated that repeated injections over six weeks tended to yield IgG2 antibodies. FMH59 illustrates how this arm will work. Ten mice, each bearing FMH59, were sacrificed, yielding 18 grams of cystic tumor tissue. Membrane preps from this tissue gave a total of 60 mg of protein. Three mice were injected intraperitoneally on six occasions with 2 mg protein/.5 cc. In this manner 36/60 mg of protein was used. The remaining 24 mg will be used as targets for screening. 10 micrograms of cell membrane can be dry fixed to the bottom of a 96-well test plate and used in either the RIA or ELISA test system. FMH59 was fused on 5/4/82 and hybrids should appear two to three weeks from this date. FMH42, 67, 84, 89 will be harvested over the next four weeks. Individual membranes will be prepared and stored at -70°C. The inoculation schedule will be staggered and the fusions shared among the various investigators. Paired B-cell lines are available for 3/5. Also, FMH59 tumor cells from the primary and metastatic sites are stored in liquid nitrogen.

The third arm will study protein extracts of tumor cell membranes. Four xenografts not used in the second arm will be scaled up as outlined above and tumor membrane preps will be made in a similar fashion. The material from all

4 types will be pooled. A lithium salt solution will be used to treat the pooled membranes as a means of extracting peripheral proteins. Lithium was chosen over other agents such as urea or NP40 because it removes surface proteins without denaturization, leaving deeper, transmemberane structures, e.g., HLA, that are often freed with detergents. Also, lithium enhances the immunogenicity of proteins. The proteins will then be linked to one of three lectins (Triticum vulgaris, Lens eulinaris and Bandeiraea simplificfolia). It is projected that one gram of tumor cell membranes will be needed for the LIS extraction. Based on past experience, 10% (or 100 mg) of this protein will be removed by LIS. This would be enough protein to link to the respective lectins. Each protein-lectin group would be injected into 3 mice intraperitoneally on six occasions. These hybrids would then be screened similar to those in the first two arms of the protocol. As stated above, one gram of cell membranes will be used to do the extraction. This gram will be the composite of 250 mg from each of the four xenografts. As of May 11, 1982, there is 250 mg from two of the xenografts and 50 mg and 100 mg from the other two. Enough animals will be sacrificed in the next three weeks to complete production of raw tumor cell membranes. The inoculation schedule will start the following week.

## MAJOR FINDINGS

Seven fusions have been successfully performed. Screening for antibody production will begin by June, 1982.

## RELEVANCE TO BRMP

Colon cancer represents the second most common malignancy in both men and women. Monoclonal antibodies with specificity for colon cancer could be used diagnostically and therapeutically. It is the expressed purpose of the BRMP to develop such biological reagents for cancer therapy.

## PROPOSED COURSE OF DEVELOPMENT

1. Develop monoclonal antibodies with specificity for colon carcinoma.
2. Biochemical characterization of the antigens recognized by these antibodies.
3. Determine whether these antibodies recognize antigens shed in patients' serum.
4. Study localization of these antibody conjugated to $^{125}I$ in tumors growing in nude mice.
5. Expand murine localization studies into patients.

## PUBLICATIONS

None

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09241-01 BRTB |
|---|---|---|

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Monoclonal Antibodies to Idiotypic Determinants on Human B Cell Malignancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Kenneth A. Foon | Acting Head (Proposed) | BRTB | NCI |
| Others: | Jeffrey Ochs | Medical Staff Fellow | BRTB | NCI |
| | Paul Abrams | Expert | BRTB | NCI |
| | James Knost | Expert | BRTB | NCI |
| | Gino Bottino | Medical Staff Fellow | BRTB | NCI |
| | Robert Oldham | Associate Director | BRTB | NCI |

COOPERATING UNITS (if any)

NCI-FCRF

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Monoclonal Antibody/Hybridoma Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 1 | .5 | .5 |

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mice will be immunized with purified monoclonal human immunoglobulin, derived from the peripheral blood of patients with monoclonal gammopathies or from the hybridization of human lymphoma/leukemia cells to murine myeloma cell lines to produce a hybridoma that secretes the human immunoglobulin found in the surface membrane of the leukemia/lymphoma cells. Mice will be immunized with this purified human immunoglobulin and their spleens removed and fused to the murine myeloma cell line P3X63AG8-653. Growing hybridomas will be tested for binding by radioimmunoassay to the particular patient's purified immunoglobulin and a panel of human immunoglobulins. Monoclonal antibodies reacting with only the immunoglobulin from the patient's tumor cells are anti-idiotypic. Monoclonal antiidiotype producing hybridomas will then be grown in the peritoneal cavity of BALB/c mice to produce high titer antibody. This antibody will be precipitated with sodium sulfate twice and dialyzed against normal saline for subsequent use in clinical trials.

PROJECT DESCRIPTION

OBJECTIVES

To develop monoclonal antibodies that recognize the idiotypic determinant unique to a particular patient's leukemia/lymphoma cells, to be used therapeutically to treat that patient.

METHODS EMPLOYED

Malignant cells will be obtained from peripheral blood, bone marrow, lymph nodes or other sites. If there is greater than 80% population of malignant cells in the peripheral blood or bone marrow, this would be the preferable source of malignant cells. In the absence of greater than 80% malignant cells in peripheral blood or bone marrow, an accessible lymph node or other site, i.e., skin, soft tissue, bone, will be biopsied and cells will be prepared into a single-cell suspension. Cells will be tested by immunofluorescence (IF) using flow cytometry against a panel of heteroantisera to immunoglobulin classes (anti-λ, anti-{, anti-κ, anti-ω) and monoclonal antibodies to cell surface antigens (T-101, Leu-1, BA-1, DR). These cells will be fused to the murine HAT-sensitive non-secreting myeloma cell line P3X63AG8-653 (AG8-653). Following a successful fusion, the radioimmunoassay and/or ELISA will be used to screen individual wells for the production of human immunoglobulin. Hybridomas secreting the patient's immunoglobulin, as previously determined by immunofluorescence, will be cloned. The most productive secreting clone will be grown in mass quantity and the immunoglobulin will be purified by affinity chromatography on a Sepharose-linked goat anti-human immunoglobulin column.

Patients with monoclonal gammopathies will not require the above steps as their immunoglobulin can be purified from their peripheral blood. Such purification will be done by affinity chromatography on a Sepharose-linked goat anti-human Ig column.

Immunization of BALB/c mice. BALB/c mice will be immunized with the purified immunoglobulin: 100 {g of immunoglobulin in complete Freund's adjuvant intraperitoneally on day 0; 100 {g in PBS intravenously on day 7. The spleen will be removed on day 10 and fused to AG8-653. Three mice will be initially immunized but only one spleen will be fused at a time. Hybridomas will be screened by radioimmunoassay against a panel of human immunoglobulins in addition to the immunizing immunoglobulin. Immunoglobulins for screening are available commercially from myeloma and Waldenstrom's patients. Monoclonal anti-Idiotype antibodies will be produced in mass quantity by injecting the hybridomas into the peritoneum of BALB/c mice. The subsequent ascites will be precipitated twice with sodium sulfate and dialyzed against normal saline. 2 grams of purified monoclonal anti-idiotype will be aliquoted and frozen for future clinical trials. The aliquots will be ultracentrifuged to remove microaggregates after defrosting. A minimum of 5 monoclonal anti-idiotype antibodies will be prepared.

MAJOR FINDINGS

We have so far successfully fused lymphoma cells from four different patients with the murine myeloma line AG8-653. Each of these human murine hybridomas is producing human immunoglobulin. We have successfully cloned two of these hybridomas to date and are currently producing immunoglobulin for immunization of mice to develop monoclonal anti-idiotype antibodies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE BRMP

A recent study from Stanford has demonstrated that a murine anti-idiotype monoclonal antibody successfully eradicated lymphoma from a patient with a rapidly progressive disease resistant to standard therapies. It is critical that this remarkable experiment be repeated and we are attempting to do this in a number of B cell malignancies. Potential application of this therapy for a large number of human B cell malignancies is great, and must be further pursued. Such therapy using biologics is the express purpose of the Biological Response Modifiers Program.

PROPOSED COURSE

We will attempt to develop murine anti-idiotype monoclonal antibodies to ten patients with B cell malignancies. We expect to be able to successfully develop 5 anti-idiotype monoclonal antibodies and be able to treat these 5 patients. It should take approximately one year to develop 5 monoclonal antibodies and approximately 3-6 months to treat these 5 patients.

PUBLICATIONS

None

## INTRODUCTION

The newly formed Biochemistry Section of the Biological Research and Therapy Branch, BRMP, performs the following functions: 1) Conducts research on the isolation, purification, biochemical and biological characterization of human tumor-associated antigens, soluble mediators of the immune response and cellular growth factors; 2) 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 immuno-diagnostic assays; and 3) Uses purified proteins to assay for amino acid sequence as a first step to determine nucleotide sequence and obtain the cDNA coding for the protein.

The Biochemistry Section is a new section which resulted from the merger of the Laboratory of Immunodiagnosis and the Biological Development Branch of the Biological Response Modifiers Program. Three independent research groups were combined and these consisted of Dr. James Braatz's group from the LID, and Drs. Edward Kimball and Charles Morgan of the BDB, BRMP. The support staff consist of the following individuals: for Dr. Kimball, Mr. Richar Rebar; for Dr. Morgan, Mr. Robert McIntyre and for Dr. Braatz, Dr. David Hua and Mr. Gerald Pincler. Dr. Braatz has been serving as the Acting Section Head.

This report represents not only the first annual report for the Biochemistry Section, but also the first formal annual report of Drs. Kimball and Morgan since joining the program. Nevertheless, significant research contributions have been made by these two new programs during this fiscal year which complement the research efforts of Dr. Braatz's group to the point where it is now obvious that a more concerted effort is being generated to attain similar objectives. Some highlights of this year's research program are briefly summarized here.

A human lung tumor antigen (LTA) has been purified to homogeneity and an improved version of a radioimmunoassay (RIA) for its quantitation has been developed. This RIA was used to measure LTA levels in a panel of 215 sera from patients with lung and other cancers, benign lung disease as well as normal controls. The results obtained were encouraging and suggest the possible usefulness of this technique for the diagnosis or monitoring of lung cancer. More specifically, LTA elevations above an established cutoff value (1.7 g/ml) were found in 2% of the normals, 13% of patients with non-lung malignancies and none of the patients with benign lung disease. Lung cancer patients showed more frequent elevations which was dependent on the histologic type of tumor. Thus, patients with squamous cell and adenocarcinoma tumors were more frequently elevated (42% and 60%, respectively) than those with large cell or small cell tumors (17% and 19%, respectively). To continue these studies, a constant and reproducible source of LTA was identified. Thus the tissue culture lung tumor cell line ChaGo has been maintained for this purpose. A relatively simple purification procedure has been developed which we plan to soon apply to a large batch of cells. This cell line is also being used as a model system for studies of the subcellular localization of LTA and the mechanisms involved in its biosynthesis. Cell-free protein synthesis directed by isolated mRNA from ChaGo is being carried out as an attempt to determine the origin of the multiple size

and charge forms of LTA detected in crude tumor and cultured cell extracts. The isolated and purified mRNA will also serve as a first step toward cloning the gene for LTA.

In an alternate approach to the study of protein or other antigens associated with human lung tumors, a previously described monoclonal antibody (MoAb) 503-D8, with specificity for squamous, adeno, and large cell (but not small cell) lung carcinomas has been extensively used. The antigen recognized by this MoAb is a protein which is associated with the cell membrane, although a component can also be identified in the spent medium of cultured lung tumor cells. Isolated protein subunits of 15,000, 18,000, 70,000 and 150,000 daltons have been identifed using the HUT-125 lung tumor cell line which are immunoprecipitated by 503-D8. The largest form appears only in the supernatant and preliminary evidence suggests it is the only form which is glycosylated. Biosynthetic as well as structural relationships between these forms are being studied.

In a related area, antigens associated with human melanoma cells are being studied. With the aid of a monoclonal antibody, a 250K dalton glycoprotein has been identified which is found primarily associated with the plasma membranes of melanoma cells. Until recently this glycoprotein was thought to be melanoma specific. Previous studies indicated that the antigen was expressed on melanomas of uveal as well as dermal origin but not on normal melanocytes of uveal origin or on normal fetal brain cells. However, recent evidence indicates that this antigen is shared by both cultured and biopsied tumors of glial cell origin. Preliminary studies using fixed sections of melanomas and immunoperoxidase staining has indicated some degree of heterogeneity in number of positive cells and the degree of expression, but ubiquity of expression on cultured melanoma cells. All of these observations suggest that the expression of this glycoprotein is associated with malignant transformation but that there may be heterogeneity in expression and possible molecular forms. These studies have important implications for clinical trials as heterogeneity in antigen expression could result in a lack of therapeutic usefulness of the monoclonal antibody.

A second melanoma-associated antigen is being studied, again with the aid of a monoclonal antibody. This antigen is a 94K dalton glycoprotein which is found primarily in spent culture medium of both melanoma, carcinoma and fetal uveal melanocytes but not of a variety of normal adult tissues and cells. Recently, the 94K oncofetal antigen has been observed in normal human serum. Detectable expression of the antigen has been confined to *in vivo* derived and cultured tumor cells and fetal cells. Detailed analysis of the antigen in human serum indicates that at least some of the antigen circulates complexed with IgG antibody. In addition, free antibody to the 94K antigen can be detected and corresponds in molecular weight to an $F(ab)_2$ fragment.

In addition to these major research areas, studies are also being conducted on human breast tumor-associated gangliosides, a human urinary transforming growth factor, and the development of human-mouse lymphoid chimeras. A unifying objective in all these studies is the molecular characterization of tumor-associated substances and the evaluation of their clinical potential as biological response modifiers or tumor markers.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09210-02 BRTB |
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PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biological and Biochemical Studies with Human Melanoma Associated Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Alton C. Morgan, Jr. Expert BRTB NCI

COOPERATING UNITS (if any)

NCI-FCRF; St. Joseph Laboratories for Cancer Research, Houston, Texas

LAB/BRANCH

Biological Research and Therapy Branch

SECTION

Biochemistry

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 1.0 | 1.0 | |

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on a "melanoma specific" antigen of 250K daltons and a 100K "common" tumor antigen have led to a further understanding of the biology of human melanoma. The 100K antigen was identified in normal human serum and shown to cross-react with C-reactive protein, an acute phase reactant. In addition, serum antigen was shown to circulate as an immune complex with IgG and IgM. Further, the serum form of 100K was found to be evolutionarily restricted to higher apes and man. The 250K antigen was found to be present on other tumors of neural-crest origin, particularly glioblastomas but to differ in molecular form and biosynthesis. As a target for serotherapy with monoclonal antibody, the 250K antigen was found to be ideal because analysis of clones of primary human melanoma cells indicated a lack of qualitative heterogeneity in antigen-expression. Finally, conditions for establishing nude mouse-human lymphoid chimeras have been delineated, enabling us to test the feasibility of this project.

PROJECT DESCRIPTION

## OBJECTIVES

The objectives of this multi-faceted project can be grouped according to the two melanoma associated antigens under study. With reference to the 100K "common" tumor antigen, we wished to 1) determine whether the antigen was present in serum or urine; 2) determine the molecular form in serum or urine; 3) assess the presence and type of immune response directed to the antigen; and 4) develop solid phase ELISA technology for the measure of antigen and antigen-containing immune complexes. With reference to the 250K "melanoma" specific antigen, we wished to 5) characterize the molecular structure in cultured human melanoma cells, both on the cell surface and spent medium; 6) assess the presence and compare the molecular structure of the antigen on cultured neural-crest derived tumor cells; 7) determine the extent of clonal heterogeneity in expression of the antigen. Interrelated with these studies, we wished to create nu/nu mouse-human lymphoid chimeras for the production of human-human hybridomas. In particular, we wanted to 8) determine the conditions necessary for marrow ablation of nu/nu mice; 9) determine conditions necessary for reconstitution and removal of graft versus host potential from grafted human bone marrow cells.

## METHODS EMPLOYED

Serological: Solid phase biotin-Avidin-enzyme-linked immunosorbent assay (ELISA) for antibody. Polyethylene-glycol enhanced inhibition of binding and single determinate reverse sandwich ELISA for detection of antigen. Double determinant, two species antibody, reverse sandwich ELISA for detection of antigen-containing immune complexes.
Radiolabeling: Cell surface lactoperoxidase-$^{125}$I, solid phase chloramine-T $^{125}$I, chloramine-T-$^{125}$I, metabolic labeling with $^{3}$H amino acids and $^{3}$H-sugars
Molecular: Indirect immunoprecipitation and immunodepletion combined with SDS-slab gel electrophoresis. Western blotting and antigen detection combined with SDS-electrophoresis. Chromatofocusing and flat-bed isoelectric focusing. Purification of radiolabeled antigen, digestion with proteolytic enzyme and resolution of peptides by reverse phase high pressure liquid chromatography (HPLC) (comparative tryptic peptide mapping for comparing similarity of molecules).

## MAJOR FINDINGS

### I. Studies on the 100K "Common" Tumor Antigen

We have identified, in normal human serum, material reactive with monoclonal antibody to the 100K antigen. This material, as analyzed by both conventional and HPLC gel sieving on a TSK-4000 column under non-denaturing conditions is heterogenous in molecular weight, ranging from 2 x $10^5$ to 4 x $10^6$ daltons. Similarly 100K antigen from chemically-defined, spent culture medium of melanoma cells is heterogenous in size when analyzed under the same conditions. This heterogenity in size contrasts to antigen from spent culture medium, when analyzed

after treatment with SDS and 2-mercaptoethanol. Under these conditions, antigen reactivity is only 100K daltons. In order to determine the monomeric size of material from normal human serum and preserve antigenicity, HPLC gel sieving was performed on normal serum treated in various ways. To summarize: Treatment with either 2-mercaptoethanol, 1M urea, 2M urea, or 4M urea separately or 4M urea and 2-mercaptoethanol together did not result in reduction of heterogeneity of the antigenic material. Treatment with SDS or acidification to pH 3.0 reduced the serum material to a monomeric size of 20-30K daltons. This contrast in monomeric size of melanoma cell and serum derived antigenic material suggested that serum antigen either represented fragmented 100K antigen or a different molecule cross-reactive with 100K antigen.

To explore these alternatives, we screened monospecific xenoantisera to normal human serum proteins by three methods. The first was by antibody binding to solid phase chemically defined medium from melanoma cells. Antisera which were reactive were screened for ability to immunodeplete antigen reactive with monoclonal antibody to the 100K antigen. The antisera were then further tested by indirect immunoprecipitation and SDS-PAGE to visualize the molecules reactive with the antisera. Out of 37 antisera, only one, an antiserum to C-reactive protein, reacted with the 100K antigen, Subsequent immunodepletion studies showed the antiserum completely removed antigen from spent culture medium reactive with the monoclonal antibody. A number of molecular and biological characteristics are different with the tumor and serum-derived antigens and at the moment, the basis of the serologic cross-reactivity is unresolved.

In addition to antiserum to CRP, antiserum to bovine or human serum albumin could partially deplete spent medium antigen reactive with monoclonal antibody. Furthermore, a comparison of SDS-PAGE profiles of either intrinsically ($^{3}H$ leucine) or extrinsically ($^{125}I$) labeled spent cultured medium derived material, reactive with monoclonal antibody, indicated the presence of albumin, not synthesized by the cell, associated with the 100K antigen. It was concluded that the melanoma monoclonal antibody-defined material in spent medium or serum could associate with albumin, which resulted in at least some of the heterogeneity in antigen size observed by gel sieving.

A further reason for size heterogeneity of melanoma antigen like material in serum was discovered by utilizing reverse sandwich technology. Normal human serum was first adsorbed onto immobilized antibody to the 100K antigen and the presence of antigen detected by subsequent binding of monoclonal antibody to 100K. Binding of monoclonal antibody to IgG and IgM was also detected with serum but not spent culture medium adsorbed antigen. IgM complexed to antigen in serum, was removed by precipitation with 3.5 percent polyethylene glycol and IgG associated with antigen by adsorption on Protein-A Sepharose. Neither IgG or IgM associated with antigen was removed by adsorption on pooled rabbit or human IgG Sepharose indicating the lack of antiglobulin activity. Following elution of complex from immobilized antibody, IgG associated with antigen, could react with spent medium antigen. Thus, it was demonstrated that at least a portion of the melanoma 100K-like material in normal serum, circulated in a immune complex. It is not known yet whether antibody complexed with 100K-like material in normal serum is directed to CRP, albumin, or 100K itself.

Evolutionary restriction of the serum 100K like-antigen was explored by assaying levels of antigen in pooled normal serum from a variety of sources. Antigen was not present in any animal serum, including New World monkeys but was present in pooled Old World monkey sera. Old World monkeys were further tested and in the small number of sera presently available, antigen was found in gorilla, chimpaneeze, but not gibbon sera. These preliminary findings indicated the 100K-like antigen in normal serum is restricted to only higher apes and humans.

In preliminary attempts to determine levels of 100K in patient sera, levels in a group of 20 non-selected breast cancer sera were compared to ten individual normal sera and two pools of AB sera representing over two hundred normal donors. Twelve of twenty patient sera were more than three standard deviations above normal levels.

## II. Studies on the 250K "Melanoma Specific" Antigen

Having established the molecular form of the "melanoma specific" antigen in biosynthetic and pulse chase experiments and its association with proteoglycan on melanoma cells, we sought to determine whether the antigen was present on other neural crest-derived cell lines and if the molecular form differed from that on melanomas. In a preliminary survey 1/1 ocular melanoma, 1/5 neuroblastoma, and 2/2 glioblastomas expressed the antigen. Focusing on glioblastomas, biosynthetically labeled cells demonstrated a 70-80K spent medium form of the antigen as opposed to a 180K spent medium form in melanoma cells. Whereas the melanoma 180K probably represents a proteolytically-derived fragment of the 250K, the 70-80K spent medium form in glioblastomas probably represents a different biosynthetic form of the antigen, since no higher molecular components were detected in cell lysates. Also, in contrast to melanomas, glioblastomas shed more antigen into culture medium than do melanoma cells and express little or no antigen on the cell surface. Studies are presently underway to determine the molecular similarity by tryptic peptide mapping of the 70-80K glioblastoma antigen to the melanoma 250K.

In contrast to this heterogeneity in molecular form of antigen from cells of common neural crest origin, other studies have detected no heterogeneity in qualitative expression on clonal populations of melanoma cells. These studies were initiated to determine whether serotherapy with monoclonal antibody to the 250K antigen would be limited by populations of tumor cells either lacking the antigen or with reduced cell surface expression. For this purpose, five clones of primary melanoma grown in methyl cellulose, and selected for differences in morphology, were assayed by antibody binding and indirect immunoprecipitation and SDS-PAGE. Although a number of glycoproteins differed in the clones and M14 metastatic melanoma for comparison, expression and identical molecular form of the 250K antigen was found amongst all the clones. Presently, we are analyzing the clones and fresh melanoma specimens with the fluorescence activated cell sorter for quantitative differences in antigen expression to determine if this might be a limiting factor for serotherapy.

III. Studies on Human Lymphoid - Nude Mouse Chimeras

To date, various aspects of this study have been addressed. First, in order to determine the state of chimerism in an animal, we made use of the specificity of monoclonal antibody W6/32. The antibody recognizes a β-2 microglobulin-dependent heavy chain determinant on HLA which does not cross-react with mouse H-2. By indirect immunofluorescence on Ficoll-Hypaque mononuclear cells from peripheral blood, 100 percent of human, but 0 percent of mouse cells, are reactive. Thus, the state of chimerism can be easily monitored without sacrificing an animal. The most difficult aspects have been to determine the method of marrow ablation which allows sufficient life span of the animal for donor engraftment. Single or combined marrow toxic drug treatments proved unsuccessful because within the dose range tolerated by the nude mice, peripheral white counts typically rebounded after drug treatment, evidence of survival of mouse stem cells. Total body irradiation has proven to be more tolerated by nude mice, especially when animals are on a oral antibiotic regimen. Genetic background of the nude mice is also important as BALB/c nu/nu survive 5-7 days following marrow ablation whereas outbred Swiss nu/nu survive 12 days. Further conditions are being modified to achieve a life span of 20 days following marrow ablation. This should be sufficient time for donor engraftment.

Following donor engraftment, the most common problem in chimeric animals is graft versus host disease (GVH). GVH has been recently shown by a number of investigators to be due to a small number of T-cells in bone marrow preparations. We have thus treated normal human bone marrow mononuclear preparations with T101 antibody, a pan-T cell monoclonal and adsorbed rabbit complement. As monitored by the fluorescence-activated cell sorter, >90% of T101 reactive cells are removed following a one hour incubation with antibody and complement.

With these studies now mostly complete, we are now attempting engraftment of nude mice which survive 12 days following marrow ablation. Should these prove successful we will immunize animals in a variety of means and fuse splenic and bone marrow mononuclear cells by human-human hybridoma technology presently used in the Hybridoma Section.

SIGNIFICANCE

These studies on a "common" tumor antigen of 100K daltons and a "melanoma specific" antigen have demonstrated considerable potential for clinical usefulness and increased our understanding of the biology of neoplasia.

The potential clinical usefulness is evidenced by upcoming clinical trials involving monoclonal antibody to the 250K antigen. In addition, we are presently engaged in instituting a program for using the antibody in diagnosis of melanoma along with existing histopathological criteria. Also, a screening is underway to determine its usefullness in detection of melanoma or glioblastoma and following disease status. This study was made feasible by the identification of a spent medium form of the 250K antigen.

The 100K antigen, thus far, has been found in spent medium of every cell line of solid tumor origin with the exception of teratocarcinoma. This property, along with its presence in normal and patient sera and its immunogenicity, suggest a possible role in host immune response to tumor cells. Of practical significance in tumor patients serum levels of the antigen, in preliminary studies are elevated in more than sixty percent. Most importantly, the recent finding of cross-reactivity of 100K with CRP, may indicate a possible immunomodulatory role for the antigen in a manner similar to CRP.

## PROPOSED COURSES

### I. 100K "Common" Tumor Antigen

1) The molecular similarity of the serum and tumor cell-produced 100K glycoprotein and CRP will be examined by comparative tryptic peptide mapping. Antigen will be isolated for this purpose by one of two means. The first is affinity chromatography on phosphorylcholine/sepharose. This method is the simplest if both serum and cell form prove to bind phosphorylcholine. Alternatively antigen can be isolated by a combination of chromatofocusing, lectin adsorbtion and elution followed by antibody/Protein-A sepharose. The final product will be labeled with $^{125}I$ by the Bolter-Hunter technique and then subjected to tryptic mapping analysis.

2) The basis for immunological cross-reactivity between the 100K tumor associated antigen and CRP will be further explored. Reciprocal immunodepletion studies with the antisera and labeled antigens will be performed to determine if the antiserum to CRP and monoclonal antibody to 100K are completely or partially cross-reactive. Phosphorylcholine and polycations will be used to inhibit binding of the respective antibodies in the presence and absence of calcium.

3) Based upon the results in the proposed research above, monoclonal antibodies will be elicited to monoclonal antibody-100K immunosorbents and hybridomas screened versus 100K and CRP in order to obtain antibodies reactive with 100K but not CRP. If the normal human serum reactivity of monoclonal antibody to 100K is due to the cross-reaction with CRP, then antibody directed at determinants on 100K not crossreactive with CRP, will give a useful reagent for futher studies in immunodetection of cancer.

4) The cross-reactivity between 100K and CRP, encourages other questions regarding the functional nature of the 100K. Since CRP has a variety of immunoregulatory properties, similar questions can be asked of the 100K molecule. This is especially important for identifying functional significance of tumor associated antigens particularly in host immune response.

### II. 250K "Melanoma" Specific Antigen

1) From our preliminary findings, 250K seems to be expressed by glioblastomas. We will examine, using immunoperoxidase procedures, histological specimens of neural crest-derived tumors. This will be done in collaboration

with Dr. Art Frankel of Cetus Laboratories, Palo Alto, California and in a separate study, Dr. B.C. Giovanella of the St. Joseph Laboratories for Cancer Research, Houston, Texas.

Dr. Giovanella's study will also look for possible differences in antigen expression between primary and metastatic melanoma and metastatic melanoma in different organ sites.

2) Molecular studies on the glioblastoma and melanoma form of the 250K antigen will continue with emphasis on comparative tryptic mapping between the two histological types and between the cell surface and spent medium forms of the antigen. The last comparison is designed to determine possible mechanism of antigen release from cell surface i.e., proteolytic cleavage, if so only the hydrophilic portion of molecule should be found in spent medium.

3) Origin of the 250K antigen will be addressed using cultured fetal uveal melanocytes. The antigen will be assayed on SV40 transformed and non-transformed melanocytes and melanocytes transformed and treated with phorbol esters. Previous data with a non-stable transformed melanocyte strain had indicated a possible synthesis of 250K after SV40 transformation and phorbol ester treatment.

4) We are also presently developing a reverse sandwich ELISA assay for detection of the 250K antigen which will be used to screen patient sera for circulating antigen including sera from patients undergoing serotherapy with the monoclonal antibody. Present studies have indicated the absence of antigen in normal human serum.

PUBLICATIONS

Morgan, A.C., Galloway, D.R., Jensen, F.C., Giovanella, B.C., and Reisfeld, R.A.: Immunochemical delineation of an oncofetal antigen on normal and SV40 transformed human fetal melanocytes. Proc. Nat'l. Acad. Sci. U.S.A. 78:3834, 1981.

Morgan, A.C., Galloway, D.R., and Reisfeld, R.A.: Carbohydrate regulated shedding of immunochemically defined human melanoma antigens. In: Fundamental Mechanisms in Cancer Immunology, Saunders, J.P., Daniels, J.C., Serrou, B., Rosenfeld, C., and Denny, C.B. (eds): Elsevier/North Holland, New York, 1981.

Morgan, A.C., Galloway, D.R., and Reisfeld, R.A.: Production and characterization of monoclonal antibody to a melanoma-specific glycoprotein. Hybridoma 1:17, 1981.

Reisfeld, R.A., Galloway, D.R., Mecake, R.P., and Morgan, A.C.: Molecular and Immunological Characterization of Human Melanoma Associated Antigens. In: Melanoma Antigens and Antibodies. Reisfeld, R.A., and Ferrone, S. (eds): Plenum Press, New York, In press.

Morgan, A.C.: Monoclonal Antibodies to Human Melanoma Associated Antigens: Elicitation and Evaluation with Immunochemically Defined Antigens. In: Melanoma Antigens and Antibodies. Reisfeld, R.A., and Ferrone, S. (eds): Plenum Press, New York, In press.

Morgan, A.C., and Reisfeld, R.A.: Biological Significance of Human Associated Antigens. In: Current Trends in Tumor Immunology. Elsevier/North Holland, New York, In press.

Morgan, A.C., Rossen, R.D., McCormick, K.J., Stehlin, J.S., and Giovanella, B.C.: Circulating Immune Complexes in Normal Donor Sera: Specificity of the Tumor Cytotoxin Component. Cancer Res., In press.

Rossen, R.D., Crane, M.M., Morgan, A.C., Gianninl, E.H., Giovanella, B.C., Tuwomey, J.J., Hersh, E.M.: Circulating Immune Complexes and Tumor Cell Cytotoxins as Prognostic indicatiors in malignant melanoma. A prospective study of 53 patients. Cancer Res., In press.

Reisfeld, R.A., Morgan, A.C., and Bumol, T.F.: Production and characterization of a monoclonal antibody to human melanoma associated antigens. In: Hybridoma in Cancer Diagnosis and Treatment, Mitchell, M.S., and Oettgen, H.F. (eds). Raven Press, New York, 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09213-02 BRTB |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Tumor Antigens and Transforming Growth Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Edward S. Kimball | Senior Staff Fellow | BRTB | NCI |
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| | Steven Sherwin | Expert | BRTB | NCI |

COOPERATING UNITS (if any)

NCI-FCRF

LAB/BRANCH

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SECTION

Biochemistry

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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SUMMARY OF WORK (200 words or less - underline keywords)

We have characterized a lung tumor associated antigen recognized by a monoclonal antibody, 503-D8, that was raised against a human lung adenocarcinoma cell line. Preliminary solid phase serological screening against glutaraldehyde-fixed tissue culture cell lines showed strong reaction with lung adenocarcinoma, large cell carcinoma and squamous cell carcinoma of the lung and little or no reactivity against small cell carcinoma of the lung. No reactivity was found against tumor cell lines of liver, kidney, gastric, or lymphoid origin and only a low degree of activity was noted when melanoma cells were examined. It is being characterized in terms of molecular weight, sub-unit size, nature of association of the antigenic moiety and the degree of glycosylation. Preliminary thin layer chromatography (TLC) studies on a partially purified glycolipid fraction from a human breast tumor cell line reveal the presence of a ganglioside that it is absent from similar preparation from a non-tumorigenic breast cell line. A human transforming growth factor (TGF) found only in cancer patients' urine has been partially purified and characterized with respect to urogastrone and competitive binding to receptors for epidermal growth factor.

PROJECT DESCRIPTION

OBJECTIVES

1) To determine whether 503-D8 will be useful for immunodiagnosis; 2) to examine patient tissues to characterize the antigens present; 3) to develop ELISA assay to accomplish 1 and 2; 4) to identify and biochemically characterize lung tumor antigens on human lung tumor cell lines detected by the monoclonal antibody 503-D8; 5) to determine whether there is biochemical identity between the antigens which exist on tissue culture cell lines and those which may be found on fresh tumor explants, in sera and in urine; 6) to study the immunobiology and biochemistry of these antigens; 7) to develop methodology for using partially purified glycolipid extracts as immunogens; 8) to develop monoclonal antibodies to glycolipid extracts; 9) to determine whether glycolipids exhibit altered expression in, or whether novel glycolipids are expressed by, breast tumors; 10) to purify and biochemically characterize and examine the structure of transforming growth factors (TGF) found in cancer patients urine; and 11) to develop a monoclonal antibody to TGF to provide a more rapid and sensitive means of detecting TGF's.

METHODS EMPLOYED

Surface labelled and biosynthetically labelled cells and their secreted products are being examined via immunoprecipitation with 503-D8. The immunoprecipitated products are analyzed by SDS-PAGE and fluorography techniques. Other standard biochemical techniques include ultraviolet and visible spectroscopy, ion exchange chromatography, affinity chromatography, gel filtration, ultracentrifugation, preparative gel electrophoresis, reverse-phase and size exclusion high performance liquid chromatography (HPLC), peptide mapping by HPLC, ELISA assay and immuno-histochemical techniques. Chloroform-methanol extraction of cell membrane preparations to obtain glycolipid fractions. Folch partitioning of glycolipid extracts to derive gangliosides. Thin-layer chromatography and ELISA assay to examine ganglioside preparations. Carbodiimide-coupling and lectin affinity adsorbtion to render gangliosides immunogenic. Techniques related to production of monoclonal antibodies. Ultrafiltration and reverse phase HPLC to isolate and purify TGF. Soft agar growth assay. Competitive radioimmune binding to EGF receptors.

MAJOR FINDINGS

I. Studies on a Human Lung Tumor-Associated Antigen.

Solid phase screening assay on glutaraldehyde-fixed cells using 503-D8 monoclonal antibody and $^{125}$I-protein A showed that the antigen detected by this antibody is present on all types of lung tumor cell lines except small cell carcinoma of the lung. The antigen is absent from liver, kidney, gastric carcinoma and lymphoid tumor cell lines but is present in varying amounts on some melanoma cell lines. The antigen could also be detected, via a new ELISA assay, on

cell membranes from an adenocarcinoma of the lung taken from a patient. Biochemical characterization studies have concentrated, for the moment, on lung tumor tissue culture cell lines. Immunoprecipitation of detergent-solubilized, trace-labelled cell membrane proteins reveal that the antigens detected by 503-D8 consist of three proteins, one of 15,000 daltons, one of 18,000 daltons and one of 70,000 daltons, when analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions. Non-reducing SDS-PAGE yields the 15,000 and 18,000 daltons chains and a 150,000 dalton chain. Similar results have been obtained from biosynthetically labelled cells. In addition, 3 day spent tissue culture media from the latter experiments have been shown to contain the 15,000, 18,000, and 150,000 dalton chains. In contrast to cell membrane-derived material, the molecular weights of the secreted products are not affected by reducing agents. Studies with oligosaccharide precursors ($^{3}$H-glucosamine and $^{3}$H-mannose) indicate that only the 150,000 dalton chain found in tissue culture media is glycosylated. None of the membrane derived proteins have been seen to incorporate $^{3}$H-glucosamine or $^{3}$H mannose. Attempts to dissociate the subunits and then immunoprecipitate the antigens reveal that the antigenic determinant is destroyed by 6M guanidine HCl but not by treatment with 4M urea or boiling SDS. For example, 4M urea-treated detergent lysates of cell membranes did not ablate immunoprecipitation, by 503-D8, of all three sub-units (15Kd, 18Kd and 70Kd chains). Attempts to re-immunoprecipitate isolated chains after treatment with reducing agents have so far not been successful but re-immunoprecipitation of isolated non-reduced 150Kd chain has been accomplished. Pulse labelling experiments with $^{35}$S-methionine have shown that the 15,000 dalton chain is synthesized earlier than the 18,000 and 70,000 dalton of the former does not decrease as expression of the 18Kd and 70Kd chains increases. The 18Kd and 70Kd chains co-express at the same rate. Preliminary pulse-chase experiments showed that the 18Kd and 70Kd chains' expression is co-dependent but that the 15Kd chain is expressed independently of the rate at which the other 2 chains are produced. These results imply that the 15K protein is associated with the 70K molecule but is not a biosynthetic precursor or sub-unit of the 70K molecule, whereas the 18K molecule may be a sub-unit or biosynthetic precursor of the 70K protein.

## II. Glycolipids as Breast Tumor Antigens

Extraction of human breast tumor cell lines with chloroform-methanol provided glycolipid fractions of each of the cell lines examined. The glycolipid pools were then further fractionated by extraction with distilled water to yield an aqueous ganglioside pool and a neutral glycolipid pool (organic soluble). Analyses on silica gel TLC plates revealed that one of the cell lines examined, MDA MB231, contained ganglioside(s) which was not present in ganglioside preparations from other cell lines.

## III. Studies on Transforming Growth Factors (TGF)

During the past year studies have concentrated on establishing the biochemical identity of TGF's found in the urine of cancer patients. These TGF's promote colony formation of a normal rat kidney fibroblasts in soft agar. The TGF's are acid soluble and have been shown to fall into two molecular weight categories;

30,000 daltons and 6,000 daltons. We have recently worked out a rapid two-step purification procedure in which urine is acidified, concentrated by ultrafiltration and then the TGF's purified by reverse phase HPLC. This procedure has also allowed us to establish that there are 3 different TGF's in the urine, not 2, as previously thought. Each TGF is able to bind to cells bearing epidermal growth factor (EGF) receptors and compete with $^{125}$I-EGF for binding to them. However, authentic EGF (urogastrone) has different HPLC and ion-exchange chromatography elution profiles and does not promote clonal proliferation of normal rat kidney fibroblasts in soft agar. Additionally, HPLC purification and preliminary molecular weight characterization of the 3 TGF's indicates that at least two of them have a 6,000 molecular weight. However a mixture of these TGF's will form a 30,000 dalton complex, suggesting that they are complexed via hydrophobic interactions or via another peptide which is dissociated under HPLC conditions.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH

The lung tumor antigen system described by 503-D8 monoclonal antibody has obvious clinical relevance and could become valuable for patient monitoring and diagnosis. The fact that it is secreted may allow diagnostic studies on blood or urine to be carried out. In terms of basic research, these antigens apparently describe a system of at least two non-covalently associated proteins which occur in highest abundance on lung tumor cells. Many tumor-associated antigens consist of a single polypeptide. Also, the fact that the 150,000 dalton secreted form of the antigen is not reduced to a 70,000 dalton protein, in contrast to the cell surface form suggests that further processing occurs at the cell surface. The finding that the secreted 150,000 dalton chain also labels with $^{3}$H-glucosamine, whereas the cell surface form does not, further suggests that the addition of an oligosaccharide, glycopeptide or glycolipid moiety is the final processing step. This is an atypical processing mechanism and therefore has relevance to glycoprotein biosynthetic pathways.

The finding of a breast tumor-associated ganglioside also has obvious clinical relevance. In addition, the cell lines which did not display the putative unique ganglioside were non-tumorigenic while the one which expressed the ganglioside was tumorigenic. This area obviously needs to be further pursued. The ability to detect and isolate a possibly unique tumor marker also should facilitate development of monoclonal antibodies for immunodiagnosis and immunotherapy studies.

The TGF studies show that another means of cancer diagnosis and prognosis may be available. The rapid purification of TGF by HPLC and the EGF-competing activity they display has two applications. EGF competition assays are rapid overnight assays whereas soft agar growth assays take up to 14 days. Thus, an EGF-competition assay on HPLC fractions of patients' urine could possibly be used as a rapid, indirect diagnostic assay. The second immediate application that comes out of the HPLC purification is that the purified TGF's can be used as pure immunogens for development of monoclonal antibodies that will allow rapid, direct assay for TGF's to be performed. In addition, this will facilitate further biochemical studies on TGF's. For example, the structure of

TGF's could be compared to EGF and other growth factors. We hope that such studies will provide a greater understanding of the structure-activity relationships required for transforming growth ability.

PROPOSED COURSE

During the next year we plan to continue these studies as follows:

I. Human Lung Tumor Antigens

A. Immunodiagnostic feasibility of 503-D8

A sensitive ELISA assay will be used to screen tissues of a wide variety for the presence or absence of reactivity with 503-D8. Tissues to be tested include the following: Normal lung tissue obtained from accident victims, normal and diseased lung tissue from patients with pulmonary pathology, lung tissue from patients who succumbed to other disases, urine and serum from the above. Tissues of histological types other than lung will also be examined as they become available from autopsy, biopsy or surgerical procedures.

The sensitivity of the ELISA assay is such that less than 100 nanograms of cell membrane protein is needed for an assay. Preliminary results on membranes from lung adenocarcinoma indicate the presence of 100 times the amount of antigen as found on normal lung tissue from the same patient.

The present ELISA assay as applied to membrane antigens will first have to be adapted for application to assays on blood or urine, but if successful, serum testing should provide the most relevant results regarding immunodiagnostic feasibility in that serum is easily obtained and can be applied to an ELISA assay in very small amounts, usually less than 10 microliters per well. Negative findings in the sera of lung cancer patients would therefore argue against the 503-D8 antibody as a readily applied immunodiagnostic reagent.

It may also be possible to examine sputum samples, via an immunoperoxidase technique, for the presence of tumor cells and we are actively pursuing this possibility as well.

B. Cytochemical studies

It is not clear which histological cell types in lung tumors express the antigens recognized by 503-D8. An immunohistochemical technique (immunoperoxidase) will be applied to lung tumors and a variety of other types of tissues as they become available.

C. Biochemical studies

We will continue to pursue studies on the characterization of the antigens detected by 503-D8. It is still not clear which protein, the 15K or 70K molecule, bears the epitope identified by the 503-D8 antibody, or whether the association of the two chains is required for antigenic activity. There are some indications that the association of the two chains is nonobligatory. Also, we plan to com-

pare the different sub-units by peptide mapping techniques using HPLC. This is the most sensitive technique for resolving differences and similarities between putatively related molecules. This will also enable us to compare the antigens found on various tissue culture cell lines and on tumor explants. It is not clear what molecular form 503-D8 reactive antigens will take on lung tissue explants, blood or urine, should they be found in the latter two sources. After radioiodination lung tissues can be studied by immunoprecipitation and SDS-PAGE, as can blood and urine. In addition, blood and urine, should they be sources of antigen can be analyzed by size exclusion chromatography and serotyping for the presence of immune complexes or association with other proteins. Finally, the biosynthetic relationships among the 15K, 18K, 70K and 150K proteins will also be further explored, as will the nature of the oligosaccharide moiety found on the secreted 150K protein.

II. Glycolipids as Breast Tumor Antigens

A. Development of monoclonal antibodies to tumor cell gangliosides

Purified gangliosides or ganglioside-rich extracts are less immunogenic than when presented as part of a cell membrane. However, since there is a greater antigenic load involved, the probability of deriving antibodies against novel gangliosides is lessened when whole cells are used as immunogens.

To enhance the probability of deriving monoclonal antibodies to novel gangliosides, three approaches will be attempted. They are:

a. Carbodiimide conjugation to hen egg lysozyme, ovalbumin, or KLH.

A protein that is non-cross reactive with human serum proteins is necessary so that subsequent screening assays are not affected.

b. Covalent coupling to Poly-L-lysine-Sepharose followed by IP injection.

c. Non-covalent coupling to wheat germ agglutinin-Sepharose.

Since most gangliosides contain sialic acid, they should bind to this lectin which can thus be used to present antigen in a multivalent form.

Primary screening for useful hybrids will be by a solid phase assay utilizing cell membranes from the breast tumor or cell line from which the immunogen was derived. Other controls will be cell membranes from a lymphoid tissue culture cell line and a transformed, but non-tumorigenic (e.g., HBL-100), "normal" breast cell tissue culture line. The possibility exists that antibodies that are breast tissue-specific, if not tumor-specific, will be produced. If so, the initial screen can be expanded to include other cell types. At the initial stages of screening, cell membrane preparations, not glycolipid extracts, will be examined. More purified fractions can be used at later stages to help define the nature of any antigens detected.

B. Comparison of gangliosides and neutral glycolipids from breast tumors, breast tumor cell lines, normal breast cell lines (e.g., HBL-100) and cell lines not of breast tissue origin. Also, benign breast tissues, including hyperplastic breast tissue will be examined.

Gangliosides will be obtained by Folch partitioning of chloroform-methanol extracts of cell membranes. Comparison among different gangliosides preparations will be by thin layer chromatography in silica gel G plates using chloroform-methanol-ammonia or propanol-$H_2O$ solvent systems. At the moment, no HPLC system is adequate for resolving ganglioside mixtures. This would also be worth developing.

III. Transforming Growth Factor

A. Biochemical studies

We plan to further characterize TGF in terms of sub-unit structure, degree of association, if any, amino acid composition, amino acid sequence.

B. Clinical Relevance

Using the 2 step purification of TGF's mentioned above we plan to pursue the following course of action.

a. Compare HPLC patterns of soft agar growth activity (TGF activity) and EGF competing activity on a panel of normal and patient urines to determine the clinical relevance of this system.

b. Raise monoclonal antibodies to purified TGF's. This will eventually allow a rapid, highly sensitive, specific assay for TGF's to be performed. Either a radioimmunoassay or ELISA could be utilized.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09242-06 BRTB formerly Z01 CB 08523-05 LID |
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PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical and Serological Studies of Human Lung Tumor-Associated Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | J.A. Braatz | Expert | BRTB | NCI |
| Others: | D.T. Hua | Visiting Fellow | BRTB | NCI |
| | G.L. Princler | Chemist | BRTB | NCI |

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Research and Therapy Branch

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued our studies on a human lung tumor-associated antigen (LTA) which can be demonstrated in 86% of extracts of lung tumors of all major histological types but not in extracts of other tumors, normal adult or fetal lung and other normal tissues. LTA does not appear to be related to other tumor-associated antigens. Efforts during the last year have primarily been directed at improving a radioimmunoassay (RIA) and developing a purification scheme for LTA from a tissue culture cell line (ChaGo). The RIA has been considerably improved by treating radioiodinated LTA with Pansorbin prior to use in the assay. This RIA was used to test a panel of 215 sera. A cutoff of 1.7ng/ml (2 SD above the mean) was selected based on 88 normals, above which 2/88 normals, 0/24 benign lung disease and 3/23 patients with cancer other than lung were found. In the lung cancer group, 13/31 squamous (42%), 9/15 adeno (60%), 3/18 large and 3/16 small cell carcinoma patients were positive. To continue these studies we developed a purification procedure of LTA from ChaGo which yields nearly homogeneous antigen.

## PROJECT DESCRIPTION

### OBJECTIVES

The objectives of this project are: 1) to purify and characterize a human lung tumor-associated antigen (LTA), 2) compare antigens purified from various sources in terms of their physicochemical properties and their structural and immunochemical relationships, 3) develop radioimmunoassays (RIA) for quantitating these antigens, 4) assess the usefulness of the RIA in the diagnosis of lung cancer and for monitoring the course of disease, 5) study the biological role of these antigens in the tumor, and 6) develop monoclonal antibodies against the purified antigens which would be useful in Objectives 1-5.

### METHODS EMPLOYED

Radioiodination of purified protein antigens. Radioimmunoassays using the purified, trace-labeled protein antigens. Standard biochemical isolation and analytical techniques including gel filtration; ion-exchange chromatography; affinity chromatography; acrylamide gel electrophoresis; isoelectric focusing; ultraviolet, visible and fluorescence spectroscopy; ultracentrifugation; lyophilization; etc. High performance liquid chromatography (HPLC) in both the size exclusion and reverse phase modes. Autoradiography. Immunochemical techniques such as double diffusion in gel, radio immunodiffusion and immuno-electrophoresis. Techniques related to cell hybridization studies directed at the hybridization of murine lymphoid cells and long term maintenance of hybrid cells in culture. Monoclonal antibody production by B cell hybridomas and detection by enzyme-linked ammunosorbent assays (ELISA) and RIA. Techniques related to cell-free translation of mRNA including the isolation and characterization of mRNA from tissue culture cell lines and characterization of the products of its translation in vitro. Computerized analysis and graphic presentation of RIA and other results using personal programs as well as public programs, such as MLAB and TELL-A-GRAF which are supported by the NIH computer systems.

### MAJOR FINDINGS

Last fiscal year we reported on the purification and biochemical characterization of a human lung tumor-associated antigen (LTA) which is a) present in 84 of 98 lung tumors of all histologic types, b) undetectable in normal adult and fetal lung, other normal tissues and tumors of organs other than lung (with the possible exception of melanoma), c) unrelated to $_1$-antichymotrypsin and also hLTAA-2a and hLTAA-2b (lung tumor-associated proteins previously characterized in our lab), and d) unrelated to all other tumor-associated antigens we have tested. Briefly, LTA was found to be present in two sizes ($M_r$ = 150,000 and 80,000) and the larger form was comprised of two charge isoforms, labeled LTA-I and LTA-II. These charge forms were found to be equivalent in terms of molecular size (150,000 daltons native, 80,000 daltons in SDS) and their ability to bind antibody. Their electrophoretic mobilities on 7% polyacrylamide gel

electrophoresis (PAGE) differed slightly (LTA-I, Rf = 0.13; LTA-II, Rf = 0.26) which was consistent with the differences in their isoelectric points (LTA-I, pI = 4.7; LTA-II, pI = 3.2). Although LTA could be radioiodinated using Bolton-Hunter reagent, it would not iodinate with the chloramine-T procedure, suggesting absence of tyrosine.

In addition to the biochemical characterization of purified LTA we also reported a first attempt at using a radioimmunoassay (RIA) to measure levels of LTA in the circulation of lung cancer patients. Since the RIA indicated higher serum levels of LTA in lung cancer patients relative to normal serum controls we proceeded with efforts to further evaluate LTA as a lung tumor marker. This report will summary our progress in various aspects of this project.

I. Refinement of the Radioimmunoassay and Testing of a Serum Panel

The RIA previously in use consistently gave high non-specific binding in the range of 7-10% with a maximum binding of about 20% at a final dilution of antibody of 1:10,000. By increasing the antibody concentration to a final dilution of 1:2,000, and by pre-adsorbing the Bolton-Hunter-radioiodinated LTA with Pansorbin, the average non-specific binding was reduced to 2.1 ± 0.4%, while the average maximum binding was increased to 43.9 ± 2.4%. This form of the RIA proved to be remarkably consistent as evidenced by the reproducibility of the RIA parameters (slope and Y-intercept) obtained in 6 separate experiments. Pre-adsorption of the radiolabeled antigen with Pansorbin removed about 8% of the radioactivity which is thought to be immunoglobulin contamination acquired during the affinity immunoadsorption step of the purification procedure. In fact, SDS gel electrophoresis of the purified, radiolabeled LTA preparation indicated two minor peaks of radioactivity at $M_r$=50,000 and 27,700, which probably represent immunoglobulin heavy and light chains. Adsorption with Pansorbin preferentially reduced these two components while not significantly affecting the major antigen peak.

The precision of the RIA was demonstrated by assaying 15 individual normal serum specimens on three separate occasions. The range of values obtained was 0.28 to 1.44 µg/ml. A high degree of consistency was evident when comparing low, medium or high values within the range obtained in the three different assays.

Using this RIA, we then examined a panel of 215 sera for LTA content. All patients' sera were pre-operative, pre-therapy specimens. It was evident by inspection of the results that the lung cancer patients as a group expressed higher levels of LTA in their sera than the other groups. In order to quantitate the results in a comparative sense, we calculated the mean and standard deviation of the normals (0.90 ± 0.4 µg/ml) and arbitrarily selected a value two standard deviations above the mean (1.7 µg/ml) as a cutoff. All values greater than 1.7 µg/ml were then scored as positive for LTA. Using this criterion we determined the number of samples within each group which possessed elevated LTA values. Only 2 of 88 normals (2%) were positive, as were 3 of 23 patients (13%) with non-lung malignancies. None of the 24 patients with benign lung diseases had elevated LTA values. Within the lung cancer group the following positive rates were observed with: squamous cell carcinoma--13 of 31 (42%), adenocarcinoma--9 of 15 (60%), large cell carcinoma--3 of 18 (17%), and small cell

carcinoma--3 of 16 (19%). It is clear that certain histologic types (squamous cell and adenocarcinoma) show more frequent elevations that others (large and small cell carcinomas). A more accurate assessment of these frequencies however must await analysis of a much larger panel of specimens within each group.

A major impediment to the continuation of these studies has been a lack of sufficient quantitites of purified LTA. The few hundred micrograms isolated from a 1.6 kg tumor have all but been exhausted in the characterization and RIA studies reported above. Thus we have examined other sources for LTA production.

## II. Purification of LTA from a Tissue Culture Cell Line

A tissue culture cell line, ChaGo, which originated from a large cell carcinoma of the lung, was the best of seven lung tumor cell lines which have been found to produce LTA. Three lung tumor cell lines were non-producers as were 9 of 18 non-lung cell lines while the remaining 9 non-lung cell lines produced LTA, but at a significantly lower level than the lung tumor cell lines.

Our attempts at devising a purification scheme for the ChaGo LTA have, at this point, provided two very effective steps. The first, ion exchange chromatography on DEAE-cellulose, is almost ideal, since the antigen is recovered in the unbound fraction along with a very small proportion of the bulk protein. Thus this method is equivalent to a filtration step since the column needs no further development. One drawback is the fact that only about 50% of the antigenic activity is unretarded. We plan to increase this yield by altering the ionic strength of the applied fraction to an extent which would maximize recovery of antigen, yet minimize bulk protein recovery.

The second step, chromatofocusing, was initially run with a pH gradient from 10.5 to 7.5. The unretarded fraction from DEAE-cellulose, when chromatofocused under these conditions, gave a single antigenic component with a pI of 9.4. There was gross contamination with other proteins which was greatly improved by applying a shallower gradient, from pH 10.5 to 9.0. Again the antigen eluted with a pI of 9.4. Analysis of this material indicated the need for additional purification to obtain homogeneous antigen. We are exploring molecular sizing by HPLC as a final step and preliminary experiments have been promising. Thus we have been able to isolate two components with demonstrable LTA activity (Ouchterlony analysis) which appear to be quite pure since each gives a single, symmetrical peak on an HPLC sizing column. These peaks correspond to proteins of $M_r$ = 30,000 and 50,000.

In support of these results, an alternate purification attempt utilized antibody affinity chromatography in place of chromatofocusing. Although the yield of LTA was rather low, which was the reason this scheme was not pursued, analysis of the purified preparation by SDS gel electrophoresis under reducing conditions indicated two protein components of 30,000 and 50,000 daltons. Therefore LTA isolated from the cell line, in comparison to primary tumor LTA, is smaller ($M_r$ = 30,000 and 50,000 vs. 150,000) and more basic (pI = 9.4 vs. 3.2-4.7). Nevertheless, the antigenic moiety is identical to the primary form and therefore constitutes a reasonable system for further studies.

III. Biosynthesis and Subcellular Localization of LTA

A. Biosynthesis

As an initial effort directed at understanding the basic biology and function of LTA we are studying the biosynthesis and subcellular localization of the antigen. Biosynthetic studies will hopefully shed some light on the cause or origin of the size/charge heterogeneity we have observed in LTA purified from both a primary tumor and a tissue culture cell line. The basic principle in these studies will be mRNA-directed cell free protein synthesis, and subsequent isolation and characterization of the translation products.

Preliminary attempts at translating mRNA isolated from cultured ChaGo cells has been plagued with high backgrounds but in at least one experiment a translation product, which precipitated with immune serum but not with pre-immune serum, was observed which corresponded in size roughly to the smaller of the two components purified previously from the same cell lines.

In addition to providing biosynthetic information, the isolated mRNA from these studies could also be used to prepare a cDNA copy using avian myeloblastosis reverse transcriptase. This would constitute the first step in the process of cloning the gene for LTA, which in turn would provide a better mechanism for establishing a constant source of the antigen for future studies.

B. Localization

Previous work in this laboratory aimed at LTA isolation has focused on the aqueous-soluble fraction of cell or tissue extracts as starting material in purification attempts. Although LTA is present in the soluble fraction, until recently we had not made an attempt at examining its presence in the particulate fraction. We have recenlty observed that the insoluble cell fraction causes inhibition in the RIA for LTA. In order to study the basic biological properties of LTA, an understanding of its subcellular distribution is essential. In addition, it would be of interest to specifically examine the plasma membrane for the presence of LTA. If a significant proportion were to be found on the external cell surface, this would provide an incentive for future studies concerning LTA-directed therapy.

To this end we have isolated a plasma membrane fraction from ChaGo cells using a published two phase polymer system which has been successfully used in this laboratory in the past. By this method, a sheet of plasma membrane is isolated at the interface of two immiscible layers of polyethylene glycol and dextran. Nuclei and cellular debris collect in the pellet while soluble components are distributed between the two solutions. The plasma membrane fraction when tested in the RIA exhibited inhibitory capacity. Although this suggests that LTA is associated with the plasma membrane, additional controls must be performed before this result can be considered definitive.

As an alternative approach to addressing the same question, we are also preparing to perform a live cell RIA for LTA. Intact and viable ChaGo cells are incubated with antibodies to LTA and the antibodies are then detected with a secondary

reagent. Our first attempt made use of $^{125}$I-Protein A to detect immunoglobulins bound to the surface. The results were encouraging as we found greater than ten times more label associated with the cells after incubation with anti-LTA compared to non-immune serum-treated cells. This suggests the external plasma membrane localization of some form of LTA, a result we hope to expand on.

IV. Evaluation of LTA as a Lung Tumor Marker

Although many laboratories are involved in the search for tumor-related substances which could be useful in the early diagnosis as well as monitoring of various forms of cancer, little progress has been made in the area of lung cancer. The high incidence and mortality rates of this disease justify the search for lung tumor antigens which would serve as indicators of disease status. Our serologic identification of LTA, a human lung tumor-associated antigen, and its subsequent purification provides the incentive for evaluating the clinical usefulness of this protein as a lung tumor marker.

As an extension of our results obtained with a panel of more than 200 serum samples discussed in Section I, we plan to compare serum levels of LTA in lung cancer patients with those in normal individuals (males, females, smokers and non-smokers of various ages), patients with other forms of cancer and patients with non-malignant diseases, particularly those of the lung. Individuals at risk for developing lung cancer, such as heavy smokers and asbestos workers, will also be included in the overall study. Presently we are concerned with isolation of sufficient quantities of LTA to proceed with this study. We are also attempting to develop a solid phase enzyme-linked immunosorbent assay (ELISA) to replace the more technically difficult RIA. Collaborations have been established with commercial firms to assist in the ELISA development, notably with Abbott Laboratories of North Chicago, Illinois and a group there directed by Dr. Joseph Tomita. Abbott, and a number of other companies, have expressed an interest in establishing the commercial market value of a lung cancer diagnostic kit based on LTA. This commercial interest in LTA dictated the need for a patent application based on the description, isolation and quantitation of the antigen. A formal patent application has been submitted during this year.

Another problem we have realized which impacts on the clinical utility of LTA as a marker for lung cancer is its immunochemical cross-reactivity with a normal serum glycoprotein which we have identified as $\alpha_1$-acid glycoprotein (AAG). Although this cross-reactivity is weak (equivalent inhibition in an RIA requires 100-1000 times more AAG than LTA) there is sufficient AAG in normal serum (0.5-1.4 mg/ml) to cause inhibition in the RIA. One possible solution to this problem would be through the use of monoclonal antibodies. A monoclonal antibody to LTA which does not react with AAG would eliminate this difficulty and reduce the basal normal levels in the RIA. Hopefully, this would increase the specificity and sensitivity of the assay.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Studies on LTA are relevant to both clinical and basic science. The significance of the work directed at developing a clinically useful immunoassay for the

detection or monitoring of lung cancer is readily apparent as earlier detection of the disease or a recurrence is essential if survival rates are to be improved. In this regard, our recent results of an evaluation of LTA levels in a panel of more than 200 sera using an improved version of the RIA have been encouraging. In addition to this we have also identified a component of normal serum which produces inhibition in the RIA. Even in the presence of this background noise the assay has performed remarkably well and it is anticipated that our identification of the normal serum inhibitor will enable us to compensate for it and increase the specificity, and hence the clinical usefulness, of the assay for LTA.

In and of itself LTA is an interesting protein. Its restricted expression by lung tumor cells suggests some role in the initiation or propagation of the transformed state. Evaluation of the subcellular localization and the biosynthetic pathways associated with LTA will be the first step in defining its function in the cell. Conceivably, general patterns may emerge which may be applicable to tumor antigens of other systems.

PROPOSED COURSE

During the next fiscal year we plan to continue these studies as follows:

1. Our major emphasis will continue to be isolation and characterization of various forms of the LTA from primary tumors and cell lines. The molecular and immunochemical properties of these forms will be evaluated in order to better understand their relationship. Despite the variety of forms displayed by the LTA with respect to size and charge, we hope to gain some understanding of them by peptide mapping using HPLC reverse phase techniques. Biosynthetic studies of LTA using the ChaGo cell line as a source of mRNA for cell-free translation studies might help to clarify the origin of these various forms.

2. The purification scheme which has been worked out for the ChaGo LTA will be applied to a large batch of ChaGo cells which we plan to acquire from the Fermentation Facility at the NCI-FCRF. This should provide sufficient LTA for its evaluation as a lung tumor marker and for additional biochemical characterization.

3. Evaluation of the clinical usefulness of LTA will be a high priority project in the coming fiscal year. Collaborations with outside, primarily commercial groups, should expedite our assessment of its utility. Development of alternate assays and monoclonal antibodies to LTA are also expected to increase the sensitivity and specificity of its detection. Clearly a substantial effort is being generated which is directed at establishing a useful and needed procedure for the early diagnosis or monitoring of human lung cancer.

4. More basic studies concerning localization and biosynthesis of LTA will be given increased attention. With regard to cell-free translation studies, we will make use of RNA isolation procedures which were obtained from the Laboratory of Viral Carcinogenesis. mRNA is being purified through oligo (dT) columns and then fractionated by sucrose gradient sedimentation according

to their sizes. Each fraction of mRNA will be translated by rabbit reticulocyte lysate, and the products will be precipitated by rabbit and goat anti-LTA sera. Gel electrophoresis and peptide mapping will be applied to compare the molecular weight and charges of LTA present in ChaGo cells and lung tumor. More specifically, $^{35}$S-methionine-labeled translation products precipitated with antisera to LTA will be characterized by SDS gel electrophoresis, and peptide mapping by reverse phase HPLC and the Cleveland technique (SDS gel electrophoresis of proteolytic fragments). These results will be compared to those obtained with purified ChaGo LTA and LTA from metabolically labeled intact ChaGo cells. The LT-120 LTA will be used for comparison.

5. Finally, a serious effort will be made to develop monoclonal antibodies to LTA. These antibodies will be characterized in terms of their affinity for antigen by Scatchard analysis and those with low as well as high affinities will be useful in this program. The specificities of the antibodies will be assessed and in particular, the cross reactivity between LTA and α1-acid glycoprotein will be examined with these reagents. Monoclonals to LTA will be screened for their ability to bind AAG, using $^{125}$I-AAG (purified AAG is available from Sigma and can be radioiodinated using the chloramine-T procedure), and $^{125}$I-LTA for comparison. Antigen-monoclonal antibody complex formation will be determined using either Pansorbin or molecular sizing by HPLC, depending on the immunoglobulin type. The affinity of each monoclonal antibody for LTA and AAG will be determined in a competitive binding assay and the results calculated using the SCAPRE/SCAFIT programs written by P. Munson, NIH, which are available on the DEC-system 10 NIH computer. As stated previously, a monoclonal antibody which recognizes LTA but not AAG would be a highly desirable reagent for use in a clinical assay.

PUBLICATIONS

Braatz, J.A., Scharfe, T.R., Princler, G.L., and McIntire, R.K.: Characterization of a human lung tumor-associated antigen and development of a radioimmunoassay. Cancer Res. 42: 849-855, 1982.

Braatz, J.A., T.R. Scharfe, Princler, G.L., and McIntire, R.K.: Studies on a Purified Human Lung-Tumor Antigen. In Gold, P. (Ed.): Proceedings of the 9th Annual Meeting of the International Society of Oncodevelopmental Biology and Medicine. Amsterdam, Elsevier/North-Holland Biomedical Press, in press.

Jones, C.M., Braatz, J.A., and Herberman, R.B.: Production of MAF/MIF by a murine T-lymphocyte hybridoma. Nature 291: 502-503, 1981.

Jones, C.M., Braatz, J.A., and Herberman, R.B.: A T lymphocyte hybridoma which generates MIF/MAF. In Karnovsky, M. (Ed.): Phagocytosis: Past and Future. Proceedings of the Centennial Conference on the Discovery of the Macrophage. In press.

Princler, G.L., McIntire, K.R., and Braatz, J.A.: Identification and purification of a human lung tumor-associated antigen from a primary lung tumor. Cancer Res. 42: 843-848, 1982.

## INTRODUCTION

The proposed Lymphokines/Cytokines Section was established to meet one aspect of the objectives of the Biological Response Modifiers Program, that is, the investigation of natural products of cells (cytokines), especially the products of lymphocytes (lymphokines) which have potential usefulness in the augmentation and/or restoration of effector mechanisms that are capable of mediating tumor cell destruction *in situ* in cancer patients.

Lymphokines and other cytokines are potent chemical modulators of cell function and are the messengers that orchestrate cellular responses, especially immune responses. Knowledge and utilization of these factors present novel and effective ways of either modifying the growth characteristics of the tumor cells themselves, or controlling and augmenting the function of reactive cell types such as macrophages and lymphocytes which may directly destroy tumor cells through cytotoxic mechanisms. These factors and others, such as thymic hormones, represent powerful tools whereby immune responses can be restored, enhanced, directed or controlled once the knowledge is obtained to intelligently use them.

## FUNCTIONAL STATEMENT AND NAME CHANGE

The Lymphokines Section (HNC 6826) has requested its name be changed to the Lymphokines/Cytokines Section to reflect its expanded role in the Biological Research and Therapy Branch and to include factors of other than lymphoid origin which may modify the biological responses of cancer patients to their tumors. The functional statement has been modified also to reflect this change in scope and now reads:

1. Investigates the mechanism of action and therapeutic usefulness of lymphokines, cytokines and other cellular products in the detection and treatment of cancer;

2. Produces lymphokines, purifies lymphokines, develops and standardizes assays for lymphokines and monitors the effects of treatment with lymphokines on normal immune responses.

3. Extracts and purifies cytokines, develops assays for cytokine activity and monitors cytokine levels in cancer patients.

## PERSONNEL CHANGES

As a result of the merger of the BRMP with the Laboratory of Immunodiagnosis (LID), a variety of personnel changes took place within the proposed Lymphokines/Cytokines Section. Dr. Luigi Varesio's major interest in the macromolecular biology of macrophages led to his reassignment to the Monocyte/Macrophage Section. His work for this year is included in this report. However, his work and physical location will be within the Monocyte/Macrophage Section by the completion of this report.

Dr. Stephen Sherwin, Acting Head of the Clinical Investigations Section, has had his basic research project on tumor-associated transforming growth factor reassigned from the Basic Mechanisms Section to the proposed Lymphokines/Cytokines Section. His report on this project is included within the Section's reports.

Also assigned to the proposed Lymphokines/Cytokines Section folowing the merger of the BRMP with LID was Dr. Guy Bonnard and his project on "Specific Immune T Cell Reactivity to Tumor Associated Antigens in Man." Transferred with Dr. Bonnard were Dr. Jose Alvarez, Visiting Fellow from Spain, and Ms. Randy Zicht, a Biologist.

## CURRENT PERSONNEL

### Scientific

Gary B. Thurman, Ph.D., Acting Head, Lymphokines/Cytokines Section
Stephen A. Sherwin, M.D., Acting Head, Clinical Investigations Section
Guy D. Bonnard, M.D., Visiting Scientist (completed visit 3/21/82)
Jose A. Alvarez, M.D., Ph.D., Visiting Fellow (until 8/30/82)

Also working closely with us and a major contributor to our efforts on these projects is Dr. Jeffrey L. Rossio, Scientist II, Litton Bionetics, Inc.

### Technical

Susan F. Pickeral, Biologist
Harold W. Stull, Bio. Lab. Tech.
Randy Zicht, Biologist
Teresa DeLawter, Stay-in-School Worker

## RESEARCH SUMMARY

### Current Year's Accomplishments

The proposed Lymphokines/Cytokines Section has made significant progress in the development of the capability of production and isolation of lymphokines and cytokines, ranging from macrophage activating factor (MAF) to tumor-associated transforming growth factor. Assays for these and other factors are either under development or are fully developed and in use. Methods of large-scale production of factors such as MAF and macrophage migration inhibitory factor (MIF) are being developed to provide substantial quantities of these lymphokines for experimental purposes. Partial purification of tumor-associated transforming growth factor (TGF) has been accomplished and an animal model that can mimic the observations seen in cancer patients has been developed. A correlation between TGF activity in the urine and tumor burden has been found in this guinea pig mammary carcinoma model. This observation, coupled with the demonstration of TGF activity in the urine of a high percentage of patients with disseminated cancers, suggests that further evaluation of TGF activity in urine of cancer patients may provide information helpful in following a patient's course and response to treatment. Work on a fully purified and biochemically characterized thymic hormone (thymosin $\alpha_1$) has led to designation of a 6 amino acid region

that evidently contains the active region of the molecule when it is included with the rest of the C-terminal portion of the polypeptide. This particular sequence seems to be unique to thymosin $\alpha_1$ and may be a crucial sequence for the development and maturation of lymphocytes.

Work on the effects of lymphokines on macromolecular synthesis by macrophages has shown that activation for cytotoxicity is associated with an inhibition of macrophage RNA synthesis. Compounds that are specific inhibitors of RNA induce cytotoxicity in macrophages, suggesting that a decreased rate of RNA synthesis and the acquisition of cytotoxic activity may be casually related.

A mouse monoclonal antibody (MoAb), produced by injections of human cultured T cells (CTC), was shown to totally abrogate the proliferative responses of CTC to IL-2 and appears to react against the cell-surface receptor for IL-2. Several MoAb against cell surface structures of T lymphocytes or subsets of T cells were screened for their ability to stimulate proliferation of fresh T cells or CTC, or to inhibit the response of $10^5$ CTC to optimal dose of IL-2 in a 3-day microwell $^3$H-thymidine assay (Bonnard et al., in "Immunology 80"). One MoAb reacted with very fresh T cells and with only a fraction (15-80%) of CTC by FACS analysis. However, it completely blocked CTC response to IL-2 in a quantitative manner, between the $10^{-4}$ and $10^{-6}$ dilutions (using ascites fluid). Like the antibody itself, the inhibitory activity could be readily absorbed out by CTC and PHA-blasts ("activated T cells"), but not by fresh T cells or by T lymphoblastoid cell lines, and could not be attributed to contamination by mycoplasma or viruses. Overnight incubation of CTC with MoAb at 37°C capped a receptor of CTC for IL-2, as assessed by the subsequent inability of these CTC to absorb IL-2 or to proliferate with IL-2. CTC similarly treated with OKT4, OKT8 or anti-HLA MoAb were not affected. We conclude that this MoAb may detect the cell-surface receptor for IL-2. The known distribution of the MoAb-defined antigen on "activated" T cells and its preliminary biochemical characteristics are consistent with this antigen being the cell-surface receptor for IL-2.

Very promising results were obtained when human lymphokines were utilized to modulate the sensitization of human peripheral blood lymphocytes to a soluble antigen in vitro. The fusion of these cells to human or murine myeloma cell lines led to the development of hybridomas that make specific antibody against the soluble antigen used for the in vitro sensitization. The numbers of antibody-producing cells produced during the sensitization could be markedly increased with lymphokine preparations in both human and murine systems. In addition, and of particular practical importance, these procedures have led to the production of human hybridomas making high levels of specific antibodies, in contrast to previous results in other laboratories, in which only low levels of antibody secretion were detected. The role of macrophages was shown to be very important in the in vitro immunization procedure and the effects of lymphokines on macrophages during these reactions to antigens appears to be crucial for augmentation or suppression of the antibody response.

Evaluation of various cell lines for lymphokine production has led to the adaptation of a human lymphoblastoid cell line RPMI-1788 as the cell line to be used for the initial isolation of these factors. Our results indicate that MIF, MAF and chemotactic factors are constitutively produced by this cell line, greatly simplifying the production methods.

## Current Goals and Rationale

Our efforts are presently aimed toward obtaining purified preparations of lymphokines and cytokines so that we can define modes of action and cellular targets of these factors. Only with purified materials will we be able to unravel the web of lymphokine and cytokine involvement in cellular maturation, growth and expression of function. A similar and equally important goal of the Section is to determine methods whereby lymphokines and cytokines can be used to manipulate, regulate or monitor cellular reactions. Lymphokines and cytokines will be extremly useful in a variety of clinical settings once detailed information is available on their mode of action and capabilties.

## Future Goals

When purified factors become available and their modes of action and cellular targets are well understood, then protocols for their use in disease states will need to be developed and thoroughly tested in animal models. Our long-range goals include the adaptation of animal models of human disease, especially cancer models, to testing the ability of lymphokines or other cytokines to modulate the ability of the host to respond to his malignant growth in a way to adversely affect its growth. This may be accomplished by a direct effect of the factors on the tumor itself, by augmenting an effect on the cells present that are already reactive to the tumor, or by an increase in the rate of recovery of the immune system depressed due to cytoreductive therapy of the tumor.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09214-02 BRTB |
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PERIOD COVERED: October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Roles of Thymic Hormones in the Maturation of Lymphokine-Producing Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Gary B. Thurman Acting Head BRTB NCI

COOPERATING UNITS (if any)

Genentech, George Washington University Medical Center, Hoffman-La Roche, Inc., NCI-FCRF

LAB/BRANCH: Biological Research and Therapy Branch

SECTION: Lymphokines/Cytokines Section (proposed)

INSTITUTE AND LOCATION: NCI-FCRF, Frederick, Maryland 21701

| TOTAL MANYEARS: 1 | PROFESSIONAL: 0.5 | OTHER: 0.5 |
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thymosin $\alpha_1$ is a biological response modifier polypeptide originally isolated from bovine thymus tissue. Thymosin $\alpha_1$ is presently being tested in a number of clinical trials for its ability to augment the capability or the rate of restoration of immune responses in cancer patients. Thymosin $\alpha_1$ is an acidic polypeptide consisting of 28 amino acid residues with an acetyl group blocking the amino terminus. It has a molecular weight of 3108 daltons and an isoelectric point of 4.2. This project has investigated the activity of thymosin $\alpha_1$ fragments in a thymectomized guinea pig MIF assay using peripheral blood lymphocytes and measuring their reactivity to PPD. A region of the amino acid sequence was found which was necessary for the biological activity of the thymosin $\alpha_1$ fragment to be evident. Amino acids 15-20 were identified as the critical region and were determined to be the active site of the polypeptide for this particular biological effect. Modifications of the N-terminus of thymosin $\alpha_1$ did not affect its biological activity. The amino acid sequence of this particular region of thymosin $\alpha_1$ seems to be unique and may represent the important region of this thymic polypeptide for induction of the capacity of lymphocytes to produce lymphokines.

PROJECT DESCRIPTION

OBJECTIVES

The objectives of this project are: 1) to determine the thymic polypeptides that are actively involved in the maturation and development of lymphokine-producing cells; 2) to determine which areas of the amino acid sequences are the active sites of the thymic polypeptide being studied; 3) to compare various thymic polypeptides, such as thymosin polypeptides, thymopoietin, serum thymic factor and others, for effects on the development and expression of lymphocyte function; and 4) to develop monoclonal antibodies against thymic polypeptides and determine what effects such monoclonal antibodies have on the expression of thymus gland function in normal and tumor-bearing animals.

METHODS EMPLOYED

Thymic polypeptides and amino acid fragments of their structure are prepared and supplied by our listed collaborators. The activities of these polypeptides were measured by a variety of biological assays in vitro, using murine or guinea pig lymphocytes. These techniques included: macrophage migration of guinea pig peritoneal exudate cells from agarose droplets, measured microscopically as areas of migration, using computer assisted analysis of the migration patterns. Techniques are being evaluated for automating the evaluation of the migration patterns, using Image Analysis Technology and computer driven microscopic accessories. Classical techniques of monoclonal antibody production have been used in efforts to develop a hybridoma secreting monoclonal antibody to thymic polypeptides. Techniques of conjugation of the small thymic polypeptides to antigenic carrier molecules such as keyhole limpet hemocyanin have been used to improve the immunogenecity of these weakly immunogenic molecules.

MAJOR FINDINGS

During this past year, the major emphasis in this project was aimed towards determining which area of the molecular structure of thymosin $\alpha_1$ is crucial for the expression of biological activity of this 28 amino acid thymic polypeptide. Previous years of development of this model at another institution had led to development of an assay for thymosin $\alpha_1$ which utilizes thymectomized guinea pigs as a model. In this assay, guinea pigs are sensitized to purified protein derivative (PPD) by intradermal injection of this antigen in complete Freund's Adjuvant. Ten days later the guinea pigs are thymectomized and two days later they are exsanguinated. Peripheral blood lymphocytes (PBL) are recovered by centrifugation of the diluted blood on Ficoll-Hypaque. The PBL are cryopreserved until use. When the PBL are used in an assay, they are mixed with peritoneal exudate cells (PEC) from normal guinea pigs (not sensitized to PPD) at a 9:1 ratio (nine parts of PEC to 1 part of PBL) and suspended in Sea-Plaque agarose. One lambda droplets of this cell-agarose mixture are placed in the bottoms of the wells of a microtiter plate and medium alone or containing thymosin, PPD, or PPD + thymosin is added to the wells. Following 18-24 hours

of incubation, the areas of migration of the macrophages from the PEC are measured and the responsivity of the PBL to PPD is calculated by computing the percent inhibition of the migration of the PEC. The PBL from normal thymus-bearing animals react to PPD in this assay by producing macrophage migration inhibition factor (MIF) which, in turn, limits the migration of the PEC. PBL from thymectomized animals lose this capacity to produce MIF unless thymic hormones are included in cultures. Thymosin $\alpha_1$ is particularly active in this assay at 10-1000 ng/ml, while several other reported thymic factors (serum thymic factor, prealbumin, THF) are not. The active pentapeptide of thymopoietin (TPS) also gives indications of activity in this assay although much higher concentrations are necessary.

Utilizing this assay, we evaluated the activity of a variety of thymosin $\alpha_1$ preparations. We found that biochemically synthesized thymosin $\alpha_1$ and genetically engineered thymosin $\alpha_1$ ($N^{\alpha}$ desacetyl-) were as active as the natural bovine molecule. Because of these observations, we initiated this study into the determination of the crucial amino acid regions in thymosin that are essential for its biological activity in this assay. Fragments of the thymosin $\alpha_1$ molecule were prepared for testing in collaboration with Hoffman-LaRoche and evaluated in the MIF assay. We found that fragments containing the N-14 amino acids did not display biological activity. The C-14 amino acids, however, did, and had as much activity on a molar basis as did the synthetic or the natural thymosin $\alpha_1$ molecule. The C-10 amino acid fragment of thymosin $\alpha_1$ retained its biological activity in the assay, while the C-8 amino acid fragment did not. Fragments smaller than the C-8 fragment did not show any activity at all. These results indicated to us that the crucial area of the thymosin $\alpha_1$ molecule is located in the region of amino acid 15-20 and that the amino acid sequence ASP-LEU-LYS-GLU-LYS-LYS probably contains the active site of thymosin $\alpha_1$. Preliminary testing of this 6 amino acid fragment alone, without the rest of the C-terminal portion of the molecule, has not been active and suggests that perhaps the activity of the thymosin $\alpha_1$ molecule requires more than just the active site of the molecule being present for interaction with its putative receptor or perhaps for internalization of the molecule.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH

Thymosin polypeptides are biological response modifiers that are being evaluated for their ability to increase lymphocyte maturation and function in immunodeficient or immunosuppressed hosts such as cancer patients. Clinical trials are underway in a number of institutions to determine if there is any clinical efficacy in using thymic polypeptides to augment or restore the immunoreactivity of cancer patients. This project is directed towards evaluating the molecular immunobiology of this biochemically well-characterized molecule. Information gained into how thymosin $\alpha_1$ works and what portions of its structure are important will give us added insight into how thymosin $\alpha_1$ might more effectively be used for augmenting the immune system of immunodepressed cancer patients or for hastening the recovery of cancer patients from the immunosuppression caused by their chemotherapy, surgery or radiotherapy. Knowledge of the active site of thymosin $\alpha_1$ gives us a capability to investigate methods of modifying nonessential portions of its structure to help control the cellular or organ sites of localization of injected thymosin $\alpha_1$. We know that

the addition of a tyrosine residue to the N-terminal portion of the molecule should not affect its activity and that is indeed the case. This modification of the structure of thymosin $\alpha_1$ has allowed its radioiodination and the establishment of a radioimmunoassay for measuring thymosin $\alpha_1$ in normal people of varous ages, cancer patients and to follow systemic levels of thymosin $\alpha_1$ in patients receiving the polypeptide as a part of their tumor therapy.

## PROPOSED COURSE

During the next year we plan to continue these studies as follows: 1) We will continue studying the activity of various fragments of thymosin $\alpha_1$ to determine just how much of the C-terminal portion of the molecule is necessary for activity. This will be accomplished by obtaining fragments of thymosin $\alpha_1$ that include amino acids 15-20 plus various segments of the amino acids towards the C-terminal end of the molecule (i.e., amino acids 15-21, 15-23, 15-26, etc.). These fragments will be analyzed for biological activity in the MIF assay as described. 2) We will evaluate synthesized 14 amino acid fragments of thymosin $\alpha_1$ (amino acids 15-28) that have amino acid substitutions in the critical 15-20 amino acid region. This will give us information as to which of these 6 amino acids are really critically involved in the structure and function of the active site of the molecule. 3) We will continue efforts to raise murine monoclonal antibodies against thymic polypeptides to determine if further precise information can be generated on the production of thymosin polypeptides by subpopulations of cells within the thymus, and for use in measuring normal and abnormal levels of thymosin, polypeptides in cancer patients undergoing therapy as an indication of the function of the thymus gland. 4) Further investigations into the role of thymic polypeptides in development of lymphokine secreting cells will be undertaken. Specifically, the thymectomized guinea pig model will be tested in other species such as rat or mouse and efforts will be made to determine if the ability to produce other lymphokines (other than MIF), such as interferon and T cell growth factor are impaired by thymectomy. If that is the case, then investigation into the antigenic markers present on the thymosin sensitive PBL will be initiated.

## PUBLICATIONS

Goldstein, A.L., Low, T.L.K., Thurman, G.B., Zatz, M., Hall, N.R., McClure, J.E., Hu, S.K., and Schulof, R.S.: Thymosins and other Hormonal-like Factors of the Thymus Gland. In Mihich, H. (Ed.): Immunological Aspects of Cancer Therapeutics. New York, John Wiley and Sons, in press.

Goldstein, A.L., Low, T.L.K., Thurman, G.B., Zatz, M.M., McClure, J.E., Hall, N.R., and Hu, S-K.: Recent Developments in the Chemistry, Biology and Clinical Applications of Thymosin. In Scott, W.A., Werner, R., Schultz, J., and Mozes L.W. (Eds.): Cellular Responses to Molecular Modulators. New York, Academic Press, 1981, pp. 237-250.

Hu, S.K., Thurman, G.B., Low, T.L.K., McClure, J.E., and Goldstein, A.L.: Multifaceted role of purified thymosin peptides in differential and function of T cells. New trends in Immunology and Cancer Immunotherapy. In press.

Low, T.L.K., Thurman, G.B., Zatz, M., Hu, S-K. and Goldstein, A.L.: A Multifaceted Role for Thymosin and its Composite Peptides in T-cell Regulation. In Haddon, J. (Ed.): Advances in Immunopharmacology. New York, Pergamon Press, 1981, pp. 67-75.

Thurman, G.B., Low, T.L.K., Rossio, J.L., and Goldstein, A.L.: Chemical Characterization of Thymosin Polypeptides Active in Specific and Nonspecific Macrophage Migration Inhibition. In Goldstein, A.L., and Chirigos, M.A. (Eds.): Lymphokines and Thymic Hormones: Their Potential in Cancer Therapeutics. New York, Raven Press, 1981, pp. 145-147.

Wetzel, R., Heyneker, H.L., Goeddel, D.V., Jhurani, P., Shapiro, J., Crea, R., Low, T.L.K., McClure, J.E., Thurman, G.B., and Goldstein, A.L.: Production of Biologically Active N α-desacetyl Thymosin $\alpha_1$ in Escherichi coli through Expression of a Chemically Synthesized Gene. In Scott, W.A., Werner, R., Schultz, J., and Mozes L.W. (Eds.): Cellular Responses to Molecular Modulators. New York, Academic Press, 1981, pp. 251-270.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09215-02 BRTB |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Production, Purification and Modes of Action of Lymphokines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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| | Randy Zicht | Biologist | BRTB | NCI |
| | Robert Oldham | Associate Director | BRMP | NCI |

COOPERATING UNITS (if any)
NCI-FCRF; Litton Bionetics, Inc.; University of Texas Medical Branch; Hoffmann-La Roche, Inc.; St. Thomas Hospital, London; Organon, Holland.

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Lymphokines/Cytokines Section (proposed)

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 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 (200 words or less - underline keywords)

A variety of cell lines have been evaluated for both constitutive and mitogen induced lymphokine production. Several human T cell hybridomas have also been tested. The human cell line RPMI-1788 has been confirmed to be a producer of macrophage migration inhibitory factor (MIF) and macrophage activation factor (MAF). However, we found some of the preparations contained interferon activity and determined that a portion of the biological activity of the RPMI-1788 produced lymphokines is due to the interferon. One preparation was found which contained no interferon but still had good MIF activity. The assays within the program for measuring MAF activity are being evaluated and standardized as a monitor for MAF production and purification by this project. Our results indicate that interferon may be involved in the mode of action of MIF and MAF and efforts to delineate possible sequential actions of these factors are being pursued.

PROJECT DESCRIPTION

OBJECTIVES

1. To evaluate potential cell sources of human lymphokines for levels of constitutive or inducible production, minimal serum requirements and feasibility for large scale production. Sources presently contemplated for analysis are a) peripheral blood mononuclear cell lines from cytapheresis or thoracic duct drainage of renal transplant candidates; b) lymphoblastoid cell lines such as RPMI-1788; c) established T-cell tumor lines; and d) T-T hybridomas.

2. To evaluate, standardize, and automate several assays for measuring various parameters of the mode of action of lymphokines. This information will be used to determine the most appropriate assays to evaluate lymphokine production.

3. To rapidly produce enough lymphokine-containing supernatant to allow the development of efficient techniques for biochemical separation of specific lymphokines.

4. To obtain partially purified lymphokines for use in production of monoclonal antibodies, for use in further purification and eventual gene cloning.

5. To initiate in vitro studies on the molecular and cellular mechanisms of lymphokine action, and in vivo studies of antitumor efficacy of lymphokines.

6. To initiate the above studies on macrophage activation factor (MAF) and macrophage inhibition factor (MIF) as high-priority lymphokines for development of our mechanisms of production, evaluation and utilization.

METHODS EMPLOYED

Production

Peripheral blood mononuclear cells or thoracic duct lymphocytes are stimulated by a pulse exposure to a mitogenic agent, such as Concanavalin A, and then cultured for two days. Supernatants are rendered cell-free by centrifugation and filtration. This supernatant is concentrated, desalted, ultracentrifuged, aliquoted, and lyophilized for use as the crude material for further purification efforts.

While these efforts are underway, other possible source of MIF and MAF are being evaluated. Among human cell lines reported to be producers of MIF and MAF are the following:

| | | | |
|---|---|---|---|
| RPMI-1788: | lymphoblastoid B cell line | MO: | Hairy cell leukemia |
| CCRF-CEM: | ALL | MOLT-4: | ALL |
| CCRF-HSB-2: | ALL | RPMI-8402: | lymphoblastoid cell line |

The most characterized cell line in terms of lymphokine production is RPMI-1788, and a number of groups have used it as a source of lymphokines. Partially purified MAF has been obtained from this source but not in sufficient quantity for biochemical analysis of the molecule.

Another production methodology underway is the fusion of lymphokine-producing T-cells with a continuous T-cell line and evaluating the hybridomas for MIF and MAF production. We have initiated T-T fusions within our own Section as a possible cell source, because of the retrovirus positivity of existing T-T hybridomas. We have a serum-free $TK^-$ fusion partner that is evidently not a virus-producing line and we have initiated attempts to produce hybridomas.

Assays

A variety of assays for measuring various parameters of macrophage activation are available within the Branch. The feasibility of their utilization in testing the large number of samples projected for this proposal is not evaluated. The assays being considered are a radioisotope release human tumor cell cytotoxicity assay, a plasminogen activator assay, or a phagocytosis assay using Percoll-separated or elutriated monocytes. The positive fractions must be confirmed as being capable of inducing macrophage-mediated tumor cell killing and so the majority of our initial efforts this year have been directed toward development of that assay. Other biochemically oriented short-term assays, measuring parameters such as the oxidative burst, are being evaluated for correlation with the induction of cytolytic capability, to possibly be used for initial screening of samples for MAF activity.

Two different assays are being evaluated for measuring human MIF. The first utilizes human monocytes purified by elutration as indicator cells in an agarose-droplet MIF assay. Techniques for automation of this assay are under development and a method of measuring migration using a computer-assisted Optomax Image Analysis System is being evaluated. The second method utilizes the U-937 human macrophage cell line as the indicator cell and measures migration out of a capillary tube by optical density with a computerized automated reader. The criteria of sensitivity, efficient use of effector cells, variance of replicate samples, assay set-up and evaluation time, accuracy and reproducibility will determine which assay will be routinely used in this project.

Biochemical Separation

The biochemical purification of MIF and MAF is complicated by two major problems: a) the starting material contains relatively small amounts of these lymphokines; b) other lymphokines may be present which mask or augment the action of MIF or MAF; c) contamination with endotoxin may give spurious results; and d) during usual purification steps, much of the material can be lost on membranes, glass or plastic surfaces, on column fittings, or by proteolysis. Therefore the purification steps will need to use novel approaches for concentrating these lymphokines, with techniques such as hydrophobic chromatography at high salt concentration. Separation of MIF and MAF from other contaminating lymphokines can be accomplished using phenyl-Sepharose chromatography. Contaminating interferon levels can be removed by absorbing over a poly (I)-Sepharose column.

MIF is separatable from MAF on an Ultrogel AcA44 column or by electrofocusing. Further purification attempts using HPLC technology should also be initiated.

As purified material becomes available, further analysis into its biochemical structure will be initiated, including amino acid composition and sequence of the peptide components and identification of possible lipid or carbohydrate components.

## MAJOR FINDINGS

### Cell Sources of Lymphokines

A variety of cell lines have been investigated for lymphokine production. Three types of lymphokines have been the major focus of efforts up to this point. They are T-cell growth factor (TCGF) (also called interleukin-2, IL-2), macrophage migration inhibition factor (MIF) and macrophage activation factor (MAF). The following cell lines were evaluated for their production of these three lymphokines: Murine - A-4a lymphoid cell line, the WEHI-3 macrophage-like cell line and EL-4 leukemia cell line; Primate - the MLA-144 gibbon leukemia cell line; Human - the HUT-78 lymphoid cell line (of Sezary syndrome origin) in both serum-containing and serum-free media and the RPMI-1788 lymphoblastoid cell line. We confirmed reports that EL-4 and MLA cell lines make considerable amounts of IL-2 activity and observed that the IL-2 production did not correlate with MIF production. The two types of HUT-78 cells also produced IL-2 activity but at much lower levels in our hands than has been reported. There was no indication that these cell lines also made MIF, and our observations would indicate that IL-2 producing cell lines are not good candidates for MIF production as well.

The RPMI-1788 cell line was found to not be an IL-2 producing cell line. It has been reported to possess MIF and MAF production capability so we evaluated 5 different preparations of material from RPMI-1788 supernatants prepared by Organon in Holland. We found that 4 of the 5 preparations had low but significant levels of alpha interferon present and that in some cases enough interferon was present to account for a significant portion of the biological activity observed in both the MIF and MAF assays. The 5 fractions were all active in the MIF (the most active produced 20% inhibition at concentrations as low as .06 μg/ml) and in the MAF assay (the most active produced 20% kill at concentrations below 100 μg/ml). This cell line also produces chemotactic factors for human monocytes and these factors are potent attractants for human monocytes at less than 1 μg/ml. Analysis of the RPMI-1788 supernatants indicated that only trace amounts of IL-2 could be detected. It is important to have lymphokine supernatants free of IL-2 activity but still containing MAF activity, since IL-2 can act on a small population of NK cells contaminating the macrophages, causing them to expand and increase their spontaneous cytotoxicity, giving spurious results for macrophage killing of tumor target cells. Likewise, we have shown that interferon (of all three types, $\alpha$, $\beta$, $\gamma$) can mimic the effects of MIF and MAF in their respective assays. The fact that one particular preparation of RMPI-1788 supernatants contained both MIF and MAF activity, but had no detectable IL-2 or interferon activity, encourages us to use this cell line as a source of MIF and MAF.

In our hands the HUT-78 cell line produced no MIF activity at all. However, the adaptability of the HUT-78 cell line to serum-free conditions made it a desirable candidate for a T-cell human fusion partner. For this purpose we have devoted a considerable effort into mutating the serum-free HUT-78 cell line to become thymidine kinase negative so it would be a HAT-sensitive partner for fusions. We have succeeded in doing this and the markers of the HUT-78 cell line are being analyzed to determine which markers were lost (if any) during the mutation process.

The MLA-144 cell line is a constitutive producer of primate IL-2 which is also active on human IL-2 dependent cells. This cell line has been evaluated for MIF and MAF activity. There was no evidence of MIF production by the MLA-144 cells, but partially purified IL-2 from the MLA-144 cell line gave some augmentation of killing of tumor targets by human monocytes. Since the augmentation was small, the apparent MAF activity may have been due to effects of the IL-2 on contaminating natural killer cells present in the monocyte preparations. Indeed, we have shown that IL-2 containing preparations can greatly augment the killing of NK-sensitive targets by low numbers of purified large granular lymphocytes (highly enriched for NK activity).

Interferon

Partially purified preparations of human interferon have been obatined from a variety of sources. The types being investigated include human leukocyte, fibroblast and immune interferon as well as human $\alpha$ interferon produced by recombinant DNA technology. Since many of the lymphokine-containing supernatants include interferon, the partially purified interferon preparations were used as controls to evaluate the efect of these potent lymphokines on our assays. We have found that all four preparations of interferon mentioned above have potent effects on the migration of macrophages and can mimic the effects seen with MIF. This has led us to begin investigating the role that interferon may play in the induction of MIF production or the expression of its activity. Methods of interferon removal from lymphokine-containing supernatants are being evaluated and tested to determine optimal ways to remove interferon but retain other lymphokine activities.

Assay System Development

During this past year we have developed or implemented sensitive and reproducible assays for several different lymphokines. We are able to routinely measure IL-1, IL-2, MIF and chemotaxis in our section. The further refinement of the agarose-droplet MIF assay during the last year deserves special comment. This assay has now been adapted to utilize human monocytes as target cells. These elutriator-purified cells from normal donors are an excellent source of MIF sensitive cells and have proven much more reliable and give more consistent results than guinea pig peritoneal exudate cells, used previously. The elutriated human monocytes are extremely sensitive to human immune ($\gamma$) interferon, and their migration can be inhibited by 20% by less than 10 Units/ml. These observations have raised a note of caution in ascribing MIF activity to antigen or mitogen-induced supernatants that may also include interferon or interferon-inducing agents. We have shown that interferon-inducing agents also mimic MIF, presumably

by inducing the production of interferon by the macrophages themselves, which in turn inhibits their migration. We have shown that the MIF assay cannot distinguish between MIF, interferon and interferon inducers and have initiated experiments that will help us distinguish between MIF and MIF-mimicking agents. Our results hav raised the possibility that macrophage MIF may act through the induction of interferon production (perhaps by the macrophages themselves) which, in turn, limits the macrophage capability for migration. To enable us to more precisely measure macrophage migration patterns, we are evaluating the use of an image analysis system that will automate the MIF reading according to stringent image analysis criteria. The technical difficulties with this endeavor are severalfold and are presently being systematically eliminated so that the MIF assay can utilize nonsubjective automated analysis as being used for other assays.

Studies on the development of a reproducible, sensitive MAF assay have recently been initiated. We have found that elutriated monocytes are an excellent source of cytotoxic cells for a variety of human and murine tumor target cells and that their level of spontaneous killing of tumor targets is low and can be induced to significant levels by agents such as interferon, C. parvum and lymphokine-containing supernatants. The further development of this assay for measuring MAF activity is one of the major goals of this project so that human MAF can be purified and isolated. This assay will be the one which will eventually be used as a screening assay when MAF production by the RPMI-1788 cell line is initiated in collaboration with the Fermentation Program at NCI-FCRF.

PROPOSED COURSE

This project will continue to evaluate existing cell lines and produce and evaluate new cell lines (or hybridomas) as potential sources of lymphokine production. Major emphasis will continue to focus on MAF, MIF and IL-2 production and further refinement of the assays to quantitate these factors will be placed on automation, sensitivity, specificity and reproducibility as desirable aspects of the assays. Details of the proposed course of investigation into the MAF assay are listed below.

Initial efforts into the production of MIF and MAF by a known producing cell line, RPMI-1788, will continue in collaboration with the Fermentation Program at NCI-FCRF. Methods of production and purification of these factors will be using the approach given below.

I. Approach for Biochemical Separation of MIF and MAF

The biochemical purification of MIF and MAF is complicated by two major problems: a) the starting material contains relatively small amounts of these lymphokines; b) other lymphokines may be present which mask or augment the action of MIF or MAF; c) contamination with endotoxin may give spurious results; and d) during usual purification steps, much of the material can be lost on membranes, glass or plastic surfaces, on column fittings, or by proteolysis. Therefore the purification steps will need to use novel approaches for concentrating these lymphokines, with techniques such as hydrophobic chromatography at high salt concentrations.

As purified material becomes available, further analysis into its biochemical structure will be initiated, including amino acid composition and sequence of the peptide components and identification of possible lipid or carbohydrate components.

II. Further Development of the MAF Assay

Elutriator-purified monocytes from normal donors will be compared with Percoll-separated monocytes for efficiency as effector cells and for low levels of spontaneous killing and high levels of induced killing. Methods of further purification by adherence will also be tested, especially for the capability for removal of contaminating NK cells. Other methods of NK cell removal, including using anti-NK monoclonal antibodies such as the anti-HNK-1 antibody, will also be tested. We will also test the capability of using cryopreserved monocytes as effector cells in this assay, adding the feature of repeating key experiments with cells from the identical cell population.

The choice of tumor targets to be used is a crucial choice and we will investigate a variety of tumor targets for suitability in this assay. The criteria to be utilized to determine the desirability of tumor cell lines as targets will include: 1) low susceptibility to killing by NK cells; 2) susceptibility to monocyte-mediated killing; 3) low spontaneous release of isotope; and 4) ease of growth and radiolabelling. The above approach should determine which parameters will give the most reliable MAF assay and should shed a great deal of light on the mechanisms and events by which monocytes become activated and capable of tumor cell killing.

III. Investigation into the Possible Roles of Interferon in Macrophage Activation and Function

To approach the question of the possible sequential relationship of IFN and MIF and MAF, experiments will be done by adding anti-IFN antisera into a direct MIF and an MAF assay. If MIF and MAF are independent of IFN, then migration inhibition or activation should be evident in the MIF or MAF driven system in the presence of anti-IFN antibodies. One possibility is that the anti-IFN may actually increase the MAF activity since $\gamma$-IFN has been postulated to have a regulatory role in lymphocyte reactivity. If either/both MIF or MAF activity is deminished by the anti-IFN, it would imply a sequential relationship for IFN with these factors. Whether MIF and MAF act pre- or post-IFN will have to be determined by other appraoches. One method would involve utilizing recombinant IFN as a source of interferon not potentially contaminated with MIF or MAF because of copurification. If this IFN acts by inducing lymphocytes to make MIF and MAF, then ultrapurified macrophages or monocytes or macrophage cell lines should not be affected by IFN as they are with MIF or MAF. This is easily tested and if they are not, then retesting with added purified lymphocytes or NK cells might indicate which cell population is responsible for the factor production following IFN addition.

Although this possibility is not fully explored, preliminary evidence indicates that U-937 cells are inhibited from migrating by IFN to the same degree as macrophages, implying that IFN acts directly on macrophages and not through action on another cell population.

To test whether IFN is an essential factor produced during macrophage migration inhibition or activation necessitates the partial purification of MIF and MAF to a point where interferon activity has been lost. If addition of these partially purified factors to purified monocytes, macrophages, or macrophage cell lines results in a detectable rise in IFN levels, then further experimentation on MIF and MAF as IFN inducers would have to be initiated.

Another test that will be used to shed light on the possible relationship between IFN and MIF and/or MAF utilizes the adsorption of α-IFN by a monoclonal antibody linked to Sepharose. This antibody is known to remove α-IFN subclasses and is easily regenerated for multiple uses (done in collaboration with the Fermentation Program at FCRF). Purified human T cells will be incubated for various periods of time with IFN and the supernatants harvested. The IFN will be removed by adsorption and the supernatants tested for MIF and MAF activity. This test can be done both in the presence and absence of a mitogenic stimulus which is capable of inducing lymphokine production. To further identify the possible sequential action of lymphokines and monokines, other lymphokine agents that do not have MIF or MAF activity can be added. Human IL-1 and IL-2 are two such factors being considered (being obtained from Larry Lachman and ABS, respectively). IL-1 may be necessary for purified T-cells to make MIF and MAF in response to antigen or mitogen, analogous to the necessity of IL-1 for thymocytes to produce IL-2.

IV. Investigations in Animal Tumor Models of Lymphokine Mediated Antitumor Therapy

As various human lymphokines are prepared or received in semi-purified form, protocols will be initiated to evaluate the effects of these lymphokines on tumor growth and survival of tumor-bearing hosts. Existing tumor models presently in use within the BRMP will be used and both systemic and intra-tumor effects of lymphokine treatment will be evaluated. Of particular interest will be the sequential administration of partially purified lymphokines designed to attract and localize macrophages at the tumor site, followed by the administration of activating agents (such as MAF) which will activate the cytotoxic activity of macrophages against tumor cells.

PUBLICATIONS

No publications yet in this recently initiated project.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09216-02 BRTB |
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PERIOD COVERED October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Macromolecular Events Associated with Functional Activities of Macrophages

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | L. Varesio | Visiting Associate | BRTB | NCI |
| Others: | D. Taramelli | Guest Worker | BRTB | NCI |

COOPERATING UNITS (if any)

NCI-FCRF

LAB/BRANCH Biological Research and Therapy Branch

SECTION Lymphokines/Cytokines Section (proposed)

INSTITUTE AND LOCATION NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 1.2 | OTHER: 0 |
|---|---|---|

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Modulation of functional and biochemical activities of macrophages have been examined. We have demonstrated that macrophages become cytolytic upon in vitro and in vivo treatment with lymphokines, endotoxin or poly I:C. However, lymphokine can induce also a suppressor activity, while poly I:C activated macrophages do not alter the lymphocyte reactivity. We showed that activation for cytotoxicity is associated with a reduced rate of $^{3}$H-uridine incorporation by macrophages and we proved that this decrease is due to inhibition of RNA synthesis. Actinomycin D, a specific inhibitor of RNA synthesis, can induce cytotoxic macrophages and at low doses it can synergize with suboptimal amounts of activators to induce cytotoxic activity, suggesting that the decreased role of RNA synthesis and the acquisition of cytotoxic activity may be causally related.

PROJECT DESCRIPTION

OBJECTIVES

The general objectives of this project are to gain insight into the mechanisms by which macrophages become activated and exert functional activities relevant to host defense. The specific purposes of the project performed this year were:

1) To investigate changes in macromolecular synthesis that occur during the activation of macrophages in vitro and in vivo.

2) To establish correlations and cause-effect relationships between changes in macromolecular synthesis and functional activities of macrophages.

3) To analyze the signals which initiate the suppressor and cytotoxic activities of macrophages (MØ).

There has been increasing interest in the study of the macromolecular synthesis during the activation of macrophages. It is likely, in fact, that the phenotypic changes occurring in macrophages during the activation process are due to modulation of the synthesis, processing and translation of the cellular RNA. Therefore, a precise understanding of the RNA metabolism during the MØ activation could lead to relevant insight on the mechanisms by which biological response modifiers can trigger the tumoricidal, bactericidal and immunosuppressive activities of MØ. Moreover, by selectively affecting the RNA synthesis of MØ with appropriate drugs, it might be possible to modulate their effector functions in an attempt to maximize the host's resistance to tumors and infections.

I. Evaluation of $^{3}$H-uridine Uptake into RNA by Macrophages

As a parameter for RNA synthesis we initially studied the incorporation of $^{3}$H-uridine into MØ RNA. However, since no available techniques met our requirements, we developed a new method to harvest labelled RNA from microcultures of macrophages. The technique is based upon solubilization of the macrophage monolayers by guanidine-HCl, followed by TCA precipitation. The recovery of RNA into the precipitate and the reproducibility of the results were strictly dependent on the use of filtered reagents and on incubation of the TCA precipitate for 2 or more hours at 4°C before harvesting. Treatment with guanidine-HCl did not affect the recovery of labeled RNA. Moreover, we observed that the radioactive precipitate had the characeristics of RNA, since its recovery was sensitive to the addition of unlabeled uridine in the culture medium and to the treatment of the macrophages with inhibitors of RNA synthesis, but not of protein synthesis. Tritiated uridine incorporation in microcultures of macrophages can be assessed with this technique, by processing the cells directly in the wells. The main advantages of this procedure are: 1) the radioactivity can be measured by semiautomatic cell harvesters, 2) a small number of macrophages are required, and 3) many samples can be processed simultaneously. Overall, the technique is simple, rapid, and could be successfully adapted to study other metabolic pathways.

II. $^{3}$H-uridine and $^{3}$H-leucine Uptake in Macrophages Activated In Vitro by Lymphokines (LK)

Because of the complex modifications seen in the response of MØ to either in vivo or in vitro stimuli, we asked whether changes in macromolecular synthesis of MØ themselves were associatead with their activation. We analyzed the changes in RNA and protein synthesis of MØ undergoing in vitro stimulation with LK.

RNA synthesis (as measured by $^{3}$H-uridine incorporation) of proteose-peptone induced MØ (pMØ) treated for 18 hr with LK was strongly depressed in comparison with pMØ treated with medium alone. In contrast, there was only a slight decrease in protein synthesis. The LK-treated MØ were also highly cytotoxic against RLol tumor target cells. The range of suppression of RNA synthesis of LK-treated MØ varied among different experiments between 40% and 70%, induced by concentrations of LK optimal for tumoricidal activation. The decrease of RNA synthesis of LK-treated MØ was not evident at 2 hr after treatment with LK, but it was very strong after 16 hr.

Parallel results were obtained when the same MØ population was tested for tumor cytotoxicity. Incubation of MØ with LK for only 2 hr was not sufficient to trigger MØ to become cytotoxic, whereas high levels of cytolytic activity were observed after 18 hr incubation.

III. $^{3}$H-uridine and $^{3}$H-leucine Uptake by Macrophages Activated in vitro by Poly I:C

Proteose-peptone elicited, adherence-purified macrophages from B6 mice were treated in vitro with poly I:C and their activation was evaluated by measuring cytolytic activity and glucose oxidation. Protein and RNA synthesis were measured by the incorporation into acid-insoluble material of $^{3}$H-leucine and $^{3}$H-uridine, respectively. Eight hours after poly I:C treatment, the RNA synthesis of the macrophages began to decrease, and by 24 hr it was 30-40% of the untreatead controls. The decrease in $^{3}$H-uridine incorporation could not be accounted for by a higher turnover of RNA in the activated cells or by increased cell death. Protein synthesis was also inhibited in poly I:C-treated pMØ but only after 20 hr. Despite the decrease in macromolecular synthesis, macrophages at 16 hr after treatment with poly I:C were found to be functionally activated, with high levels of cytotoxic activity against $^{51}$Cr-labeled target cells and a 3- to 4-fold increase in the rate of glucose oxidation. Treatment of resident peritoneal MØ with poly I:C led to the same pattern, a decrease in macromolecular synthesis and high cytolytic activity. We hypothesize that inhibition of macromolecular synthesis, particularly an early inhibition of RNA synthesis, is required for activation of macrophages.

IV. Changes in $^{3}$H-uridine Uptake in in vivo Activated Macrophages

To determine whether the alteration in macromolecular synthesis detected in vitro was a more general phenomenon associated with activation, we studied RNA

and protein synthesis of in vivo activated MØ. B6 were injected with Corynebacterium parvum and the peritoneal MØ (CP-MØ) harvested 8-10 days later. Resident peritoneal MØ (R-MØ) were used as controls. CP-MØ were consistently cytolytic against lymphoma target cells, and had the ability to suppress lymphoproliferation and lymphokine production. To study their macromolecular synthesis, the same number of CP-MØ and R-MØ were purified by adherence to plastic microwells, pulsed for 2 hr with $^{3}$Hleucine or $^{3}$H-uridine and the radioactivity in acid-precipitable material was measured. CP-MØ showed a markedly reduced rate of RNA synthesis, ranging between 20-40% of the R-MØ. The rate, of protein synthesis on the contrary, was not significantly different. A similar pattern was obtained when the isotope incorporation in each well was corrected for the number of adherent cells or the cellular protein content of well. The measurement of the rate of RNA synthesis may represent a quantitative and reliable parameter to determine the degree of macrophage activation.

## V. Relationships Between $^{3}$H-uridine Uptake and RNA Synthesis in RØ

Although $^{3}$H-uridine uptake has been widely used as a parameter for RNA synthesis, a series of factors may affect the interpretation of the results. In particular, changes in the membrane permeability to $^{3}$H-uridine, in the uridinetriphosphate (UTP) pool of the cell may affect the labelling of RNA independently of its rate of synthesis. Therefore, it was of great importance to determine whether or not the decrease of $^{3}$H-uridine incorporation associated with the activation of MØ was accounted for by a decrease in the rate of RNA synthesis.

To determine whether the decrease in $^{3}$H-uridine incorporation was due to a decrease in RNA synthesis or to a dilution of the radioactive tracer by the intracellular pool of UTP we first studied the label of RNA with $^{32}$P-orthophosphate ($^{32}$P). We observed that poly I:C-activated macrophages had a reduced rate of $^{32}$P incorporation into RNA similar to that observed when $^{3}$H-uridine was used as a tracer. In addition, we measured the specific activity of the UTP pool in resting and activated macrophages by separating the acid soluble fraction of $^{3}$H-uridine labelled macrophages by high performance liquid chromatography. Poly I:C activated macrophages showed a 1.5 to 2 fold increase in the specific activity of UTP compared to control macrophages, indicating that we may underestimate the real extent of the inhibition of RNA synthesis.

The same conclusions were reached when LPS plus LK were used as in vitro activators or when LPS and C. parvum were used to activate macrophages in vivo. It was clear, therefore, that the RNA synthesis, rather than the specific activity of the radiolabeled precursor, was generally affected during the activation process.

## VI. Stimulation of Cytotoxic Macrophages by Specific Inhibitors of RNA Synthesis

We have shown that macrophages activated in vitro and in vivo to a cytolytic stage have a reduced rate of $^{3}$H-uridine incorporation into RNA. To examine the possible causal relationship between these events, we investigated the effect of actinomycin D (Dact), a highly specific inhibitor of RNA synthesis, on the

cytolytic activity of murine macrophages. Peritoneal macrophages elicited by proteose peptone injection in C57BL/6 mice were treated in vitro for 45 min with different concentrations of Dact, washed extensively and tested for their cytotoxic activity in an 18 hr $^{51}$Cr-release assay against RL♂1 lymphoma target cells. Concentrations of Dact between 0.2-1 µg/ml induced high levels of cytotoxic activity. Similar results were obtained when Dact was injected i.p. and the peritoneal macrophages were harvested 1 hr later and immediately tested for cytotoxicity. Studies with $^{3}$H-Dact showed that the macrophages incorporated 0.1-0.2% of the drug upon in vitro or in vivo treatment. These low values make it unlikely that all the cytotoxic activity of the Dact-treated macrophages could be attributed to a direct effect on the targets of free Dact release into the medium by the macrophages. Our results suggest that a cause-effect relationship may exist between the decreased rate of RNA synthesis and the activation for cytotoxicity. Moreover they indicate that functionally tumoricidal macrophages can be induced by a short exposure to Dact.

Since both specific RNA inhibitors and stimuli triggering RØ activation were affecting the synthesis of RNA, we reasoned that RNA inhibitors and conventional activators should synergize in triggering the cytolytic activity. To test this hypothesis, we treated pMØ with subthreshold concentrations of Dact or poly I:C, which individually were unable to stimulate cytotoxic activity. However, nanogram amounts of Dact mixed with poly I:C were able to induce significant levels of cytotoxicity. Similar results were obtained when LPS or IFN were mixed with Dact. The fact that Dact could synergize with other MØ activators sustained the hypothesis that the decrease in RNA synthesis could be one step in the activation process. Because of the potential clinical application of these findings, experiments are in progress to verify this hypothesis in vivo.

## VII. Activation of Tumoricidal and/or Suppressor Macrophages

Various signals may trigger cytotoxic and/or suppressor MØ in in vitro studies, indicating that LK as well as other substances such as IFN, LPS or double-stranded RNA can trigger MØ to become tumoricidal. It is not clear, however, whether the same signals could also generate suppressor MØ. Therefore, we decided to investigate the role of LK, IFN, LPS and poly I:C on the in vitro generation of tumoricidal and suppressor MØ.

The MØ-mediated suppression was tested on the production of migration inhibition factor (MIF) and macrophage activation factor (MAF) by activated lymphocytes. Peritoneal exudate cells were elicited in C57BL/6 (B6) mice by injection of pMØ 48 hr earlier. The MØ were purified from the PEC by adherence to plastic. They were then treated for 18 hr with different stimulatory agents. At the end of incubation, their cytotoxic activity was tested in an 18 hr $^{51}$Cr-release assay. Strong cytotoxic activity was obtained by treating MØ monolayers with LK (supernatant of concanavalin A-stimulated normal spleen cells [NSC]), LPS (100 µg/ml) (E. coli 0111:B4), IFN $10^{4}$U/ml (partially purified mouse fibroblast IFN, supplied by Dr. Paucker) or poly I:C, 100 µg/ml. pMØtreated with medium or control supernatants were not tumoricidal.

The same stimulation protocol was used to test whether MØ activated in vitro to a cytotoxic stage were also able to suppress the production of LK by mitogen-triggered lymphocytes.

Different numbers of activated MØ were added to Con A-pulsed NSC, at the time of the mitogenic stimulation. After 24 hr, the supernatants were recovered and tested for the presence of MIF and MAF. MIF activity was assayed using the microdroplet assay technique and MAF activity was tested in an 18 hr $^{51}Cr$-release assay. When 10% of LK-treated MØ were added to the LK-producing system, strong suppression of both MIF and MAF production was observed. The addition of the same number of medium-treated MØ, or MØ incubated with control supernatants, did not have any effect. The suppression was mediated by adherent, Thy 1.2 negative, peroxidase positive cells, with monocyte-macrophage-like morphology. Furthermore, suppressive MØ generated by different LK preparations could inhibit various LK-producing systems.

It seems therefore that the in vitro activation of MØ with LK enables them to inhibit the LK-producing cells, thus providing a feedback control of LK production.

To test whether stimuli other than LK were able to induce suppressors MØ, we treated pMØ for 18 hr with doses of IFN ($5 \times 10^3$ U/ml), poly I:C (100 µg/ml) or LPS (100 µg/ml) optimal for the induction of cytotoxic cells. The addition of 20% or 10% of IFN, LPS or poly I:C-treated MØ did not reduce the amount of MAF produced by Con A-stimulated NSC, while LK-activated MØ were strongly inhibitory. The same pattern of results was obtained when pMØ activated by poly I:C, IFN, LPS and LK were tested for their ability to inhibit MIF production by Con A-stimulated NSC. We concluded that MØ activated by IFN, poly I:C or high doses of LPS did not acquire any suppressive function on LK production despite their cytotoxic activity. This indicates that, depending on the stimuli used, different activities can be generated in vitro within the same MØ population.

Further support for this conclusion came from in vivo experiments in which LPS (20 µg/mouse) or poly I:C (100 µg/mouse) were inoculated i.p. in B6 mice. After 24 hr, peritoneal MØ were recovered and tested for tumoricidal and suppressive functions. The in vivo activated MØ were highly cytotoxic at all the attacker/target ratios tested. However, they were unable to inhibit the production of MAF by Con A-stimulated NSC.

It appears thus that, at least in the conditions used in the present study, poly I:C and LPS can trigger only cytotoxic, and not suppressor, MØ both in vivo and in vitro.

## PROPOSED COURSE OF THE PROJECT

During the next year we will continue to examine the process involved in activation of macrophages and the biology of the macrophage-tumor cell interaction. In addition we will continue to attempt to analyze and possibly identify the signals stimulating immunosuppressive or antitumor activity by macrophages.

The activation of macrophages will be studied by analyzing the expression of the macrophage genome before and after exposure to stimulatory agents in vivo and in vitro. In particular we will investigate the control of activation at the level of transcription of messenger and ribosomal RNA, processing of RNA and translation of RNA into proteins. Attempts will be made to identify specific mRNA coding for proteins selectively synthesized during the activated state.

Based on the information collected from the above studies, we will analyze other drugs affecting RNA synthesis (e.g., picolinic acid, corolycepin, DRB, α-amanitin) for their ability to either boost or inhibit the cytotoxic activity of macrophages. Experiments will be done to look at the effect of drugs and biological response modifiers in vivo for their effect on the activation of macrophages.

It is our intention to verify our results on human cells. Initial experiments will be therefore performed to look at the RNA synthesis in human monocytes and at the effect of biological modifiers on RNA synthesis and monocyte cytotoxicity.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH

The host resistance to neoplasia and conversely the ability of neoplastic cells to grow in a healthy organism are dependent upon a complex series of events involving leukocytes and thier products (cytokines) as well as other cellular and humoral systems not belonging to the immune system. Moreover, it is becoming clear that chemotherapeutic drugs used as antineoplastic agents also affect the normal cell compartment and modify its physiology.

In this context, macrophages may have a major role in modulating the host resistance to tumors either by exerting direct toxic effects on neoplastic cells or by delivering helper or suppressor signals to lymphocytes mediating other types of antitumor activities. Moreover, macrophage activities can, in turn, be modulated by antineoplastic drugs and biological response modifiers. For example, we have shown that in certain conditions lymphokines and actinomycin D can stimulate cytotoxic activity by macrophages. Understanding the network of interactions among macrophages, tumor cells, drugs and biological modifiers will give relevant information for a rational planning of strategies to maximize the antitumor defense in vivo.

In order to pursue this line of research, it is becoming necesary to gain more detailed information on the molecular biology of the cellular response. In fact, on the one hand many chemotherapeutic agents affect the cellular macromolecular synthesis. Therefore, we should know the changes in macromolecular synthesis occurring in macrophages during activation in order to evaluate the possible enhancing or inhibiting activity of a given compound on this function. On the other hand, the macromolecular synthesis of macrophages is clearly determining the expression of cytolytic and suppressor functions. Therefore the study of the changes in RNA and protein synthesis of macrophages exposed to activators, will provide relevant insight on the mechanism of action of these stimulatory agents. This information is definitely needed to optimize the stimulation protocol and/or the activating agents and to identify the macrophage products relevant for their functional activities.

PUBLICATIONS

Taramelli, D., Holden, H.T., and Varesio, L.: In vitro induction of tumoricidal and suppressor macrophages by lymphokines: Possible feedback regulation. J. Immunol. 126:2123-2128, 1981.

Herberman, R.B., Brunda, M.J., Domzig, W., Fagnani, R., Goldfarb, R.H., Holden, H.T., Ortaldo, J.R., Reynolds, C.W., Riccardi, C., Santoni, A., Stadler, B.M., Taramelli, D., Timonen, T., and Varesio, L.: Immunoregulation involving macrophages and natural killer cells. In Gershwin, M.E., and Ruben, L.N. (Eds.): The Biological Significance of Immune Regulation. New York, Marcel Dekker, Inc., in press.

Taramelli, D., and Varesio, L.: Activation of murine macrophages. I. Different pattern of activation by poly I:C than by lymphokine or LPS. J. Immunol. 127: 58-63, 1981.

Varesio, L., Holden, H.T., Taramelli, D.: Macromolecular synthesis in lymphokine-producing and responding cells. In Goldstein, A.L., and Chirigos, M.A. (Eds.): Lymphokines and Thymic Hormones: Their Potential Utilization in Cancer Therapeutics. New York, Raven Press, 1981, pp. 215-225.

Varesio, L., Brunda, M.J., Holden, H.T., Jones, C.M., and Taramelli, D.: Decreased RNA synthesis as an intracellular signal for activation of cytolytic macrophages. Proceedings of the 14th International Leucocyte Culture Conference. Amsterdam, Elsevier/North Holland Biomedical Press, 1981, pp. 319-322.

Varesio, L., Holden, H.T., and Taramelli, D.: Suppression of lymphokine production: II. Macrophage-dependent inhibition of production of macrophage activating factor. Cell Immunol. 63:279-292, 1981.

Jones, C.M., Varesio, L., and Herberman, R.B.: Interferon activates macrophages to produce plasminogen activator. In Dumond, D. (Ed.): Proceedings of the 3rd International Conference on Human Lymphokines. New York, Academic Press, in press.

Brunda, M.J., Varesio, L., Herberman, R.B., and Holden, H.T.: Interferon-independent, lectin-induced augmentation of murine natural killer cell activity. Int. J. Cancer, in press.

Varesio, L., Naglich, J., Brunda, M.J., Taramelli, D., and Eva, A.: Micro-system to evaluate the incorporation of $^{3}$H-uridine in macrophage RNA. Immunol. Commun. 10:577-589, 1981.

Brunda, M.J., Taramelli, D., Holden, H.T., and Varesio, L.: Suppression of murine natural killer cell activity by normal peritoneal macrophages. In Herberman, R.B. (Ed.): Natural Cell-Mediated Immunity, volume 2. New York, Academic Press, in press.

Taramelli, D., Bagley, M.B., Holden, H.T., and Varesio, L.: Activation of tumoricidal and/or suppressor macrophages: Different stimulatory signals trigger either function both in vivo and in vitro. In Sorkin, E., and Normann, S. (Eds.): Macrophage and NK Cell regulation and Function Proceeding of 9th International RES Congress, in press.

Forni, G., Giovarelli, M., Lanfrancone, L., Varesio, L.: Suppressor macrophages in tumor-bearing mice. Inconsistency between *in vivo* and *in vitro* findings? *Int. J. Cancer*, 1982, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09230-02 BRTB |
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PERIOD COVERED: October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tumor-Associated Transforming Growth Factor Activity in the Urine of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
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| Others: | Daniel Twardzik | Staff Fellow | LVC | NCI |
| | Edward Kimball | Staff Fellow | BRTB | NCI |
| | Jack Pearson | Microbiologist | BRTB | NCI |

COOPERATING UNITS (if any)

NCI-FCRF

LAB/BRANCH: Biological Research and Therapy Branch

SECTION: Lymphokines/Cytokines Section (proposed)

INSTITUTE AND LOCATION: NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 2.0 | OTHER: 1.0 |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transforming growth factors (TGFs) are low molecular weight peptides capable of stimulating non-transformed indicator cells to grow as colonies in soft agar. We have obtained the first evidence for the presence of such factors in the urine of patients with a variety of disseminated cancer. Chromatographic analysis of acidified urine aliquots indicates that the majority of cancer patients and only a few non-malignant controls contain a high molecular weight TGF activity with co-elutes with epidermal growth factor (EGF)-competing activity. In contrast, a low molecular weight activity which does not co-elute with EGF competing activity was found in all urines tested. High molecular weight urinary TGF activity is currently being evaluated as a clinically useful biologic marker for disseminated cancer.

PROJECT DESCRIPTION

OBJECTIVES

This project is designed to study the expression of transforming growth factor (TGF) activity in the urine of patients with a variety of disseminated cancers and to determine whether the expression of this activity correlates with extent of tumor or response to therapy. In addition, we will attempt to characterize high molecular weight urinary TGF activity biochemically and will attempt to develop a monoclonal antibody directed against this activity, for purposes of assisting in its purification and developing a competition radioimmunoassay which can be used to assay patient specimens more efficiently. Finally, we will be developing an animal model for the study of urinary TGF activity that can also be used to screen biological response modifiers with antitumor potential based on their ability to interfere with the expression of TGF activity.

METHODS EMPLOYED

Urines analyzed for TGF activity are acidified and chromatographed over Bio-Gel P30 columns. Column fractions are tested for the presence of soft agar growth promoting activity in a bioassay which detects factors capable of supporting the growth of normal rat kidney fibroblasts in 0.3% agar. Column fractions are also assayed on occasion for factors capable of competing with mouse epidermal growth factor (EGF) for binding to EGF receptors on human carcinoma A431 cells. Biochemical analysis of the high molecular weight urinary TGF activity which appears to be tumor-associated has involved the application of high-performance liquid chromatography (HPLC). This system appears to be able to separate the various urinary TGF activities and should be important in furthering its purification. Our attempts to develop a monoclonal antibody to urinary TGF activity will employ standard mouse hybridoma techniques. Finally, the development of an animal model for studying urinary TGF activity will employ a guinea pig mammary carcinoma which spontaneously metastasizes to the lungs. Animals will be inoculated with this tumor subcutaneously, will undergo surgical therapy for their primary tumor and ultimately systemic therapy for metastatic or recurrent disease. Urinary TGF activity will be monitored throughout this period of time.

MAJOR FINDINGS

Urines were collected from patients with a variety of disseminated cancers, with at least 4 weeks elapsing since previous therapy. Eighteen of 22 cancer patients, including patients with cancers of the lung, breast, colon, ovary and melanoma and sarcoma, were found to have a high molecular weight TGF activity (approximately 30-35,000 daltons) in their urine. This activity appeared to co-elute with EGF-competing activity. In contrast, only 5 of 22 nonmalignant control urines were found to contain this high molecular weight TGF activity. In contrast, a low molecular weight TGF activity of approximately 6-8,000 daltons was found in all urines tested. This activity did not appear to coelute

precisely with EGF-competing activity, thus suggesting that the high and low molecular weight urinary TGF activities are unrelated molecules. It is of interest that the 5 nonmalignant control urines which had low levels of high molecular weight TGF activity included 4 patients with various inflammatory or hyperplastic disorders. This suggests that the tumor-associated high molecular weight urinary TGF activity is much like CEA or alpha fetoprotein in terms of being found at low levels in various inflammatory disorders. Unlike CEA and alpha fetoprotein, however, high molecular weight urinary TGF activity appears to have a defined biological activity which is related to the transformed phenotype. The use of this assay as a potential biologic marker for extent of tumor burden or dissemination or response to therapy remains to be defined.

The biochemical characterization of high molecular weight and low molecular weight urinary TGF activities has involved the analysis of these activities using HPLC. These studies have revealed that both high and low molecular weight urinary TGF activity, which can be identified in either the soft agar growth factor assay or the EGF competition assay, are clearly distinguishable from EGF (urogastrone) normally present in urine. This suggests the possibility that HPLC analysis of urines from cancer patients and nonmalignant controls may be quite useful as a rapid assay for TGF in the urine.

Finally, an animal model for studying urinary TGF activity has been successfully established in guinea pigs. Guinea pigs inoculated with cells derived from a spontaneously arising mammary carcinoma of guinea pigs which metastasizes to the lung were found to have a relatively high molecular weight TGF activity in their urine (17-20,000 daltons) that was not present in control animals. Surgical removal of the primary tumor decreased the expression of this activity. When tumors recurred in the abdomen, this activity reappeared. Studies evaluating the response to systemic therapy such as chemotherapy in terms of an alteration in the level of high molecular weight urinary TGF activity are currently in progress. This animal model further supports the notion that urinary TGF activity may be a useful biologic marker for malignancy. The model will also afford the opportunity to screen biologic response modifiers with potential as anticancer agents by way of inhibition of TGF activity.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH

High molecular weight urinary TGF activity may be a useful biologic marker for patients with disseminated cancer. Many of these patients have tumors for which no other markers are available. Moreover, the fact that the biologic activity of TGF is related to the transformed state may mean that this substance is useful to monitor the activity of a patient's tumor as well as its location and size. The development of an animal model for urinary TGF activity which appears to be tumor-associated could allow for screening biological response modifiers that have potential as anticancer agents. New classes of agents with antitumor activity which act through inhibition of TGF activity could conceivably be identified in this manner.

PROPOSED COURSE

Future experiments in this area will involve testing the urine of cancer patients before and after surgery and before and after chemotherapy to determine whether high molecular weight urinary TGF activity correlates with extent of tumor and response to treatment. The animal model for urinary TGF activity employing a guinea pig mammary carcinoma will be tested for its ability to identify biological response modifiers with antitumor effect. Further biochemical purification of urinary TGF activity using HPLC will also be carried out in the next year. Work toward the development of a stable hybridoma producing a monoclonal antibody capable of inhibiting urinary TGF activity in the soft agar growth factor assay will continue.

PUBLICATIONS

Twardzik, D., Sherwin, S., Ranchalis, J., and Todaro, G.: The urine of pregnant and tumor-bearing human contains transforming growth factor-like activities. J. Natl. Cancer Inst. In press.

Sherwin, S., and Todaro, G.: Transforming growth factors in human lung cancer. In Greco, F. (Ed.): Lung Cancer, (Volume 2). In press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09243-01 BRTB |
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PERIOD COVERED
October 1, 1982 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Lymphokine Effects on Murine and Human In Vitro Immune Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
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| Others: | S. Pickeral | Biologist | BRTB | NCI |
| | J. Knost | Expert | BRTB | NCI |

COOPERATING UNITS (if any)
NCI-FCRF

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Lymphokines/Cytokines Section (proposed)

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Murine or rat crude lymphokine (LK) preparations were produced by Con A stimulation of splenocytes and were partially purified. Addition of these lymphokines to in vitro immunization cultures of murine splenocytes and the antigen sheep erythrocytes (SRBC) resulted in modulation of anti-SRBC antibody production during the in vitro immunization period. Moderate LK concentrations resulted in strong stimulation of antibody production. High LK amounts significantly suppressed the antibody production, but not through a cytotoxic mechanism. Depletion of splenic macrophages from the SRBC in vitro sensitization cultures eliminated normal antibody responses. LK could restore the response to normal levels but, over quite high concentrations, was not suppressive. Reconstitution of cultures with syngeneic, but not allogeneic, plastic-adherent splenic cells restored the ability of LK to suppress antibody production. Human LK preparations could, at moderate concentrations, stimulate the production of anti-tetanus toxoid antibody by human peripheral blood mononuclear cells during in vitro immunization.

PROJECT DESCRIPTION

OBJECTIVES

The objectives of this project are 1) to investigate the nature of the inhibitory effects which high lymphokine concentrations exhibit on murine in vitro sensitization reactions; 2) to produce and partially purify lymphokines for use in the investigation of their effects on in vitro immune responses; 3) to investigate whether the stimulatory effects of moderate lymphokine concentrations on in vitro immune responses in murine systems are parallelled by increased in vitro responses in sensitization of human peripheral blood lymphoid cells; 4) to determine whether lymphokine-induced stimulation can be used to enhance the production of useful antibody-producing cells in such reactions; and 5) to delineate the contributions of various cell types to the in vitro immune response in terms of effects of specific lymphokines on these cell subpopulations.

METHODS EMPLOYED

In vitro cell culture. Production of lymphokines by mitogen or other (e.g., PMA) stimulation of lymphocytes and/or macrophages. Purification of lymphokines by salt precipitation, ion exchange, hydrophobic and affinity chromatography and electrophoresis. Immunoassays for antibody production including radioimmunoassay (RIA) and enzyme-linked immunoassay (ELISA). Isolation of mononuclear cell subpopulations, depletion of cell subpopulations by physical or mechanical means. Assays for lymphokines (IL-2, IL-1).

MAJOR FINDINGS

In experiments using the model antigen, sheep erythrocytes (SRBC), it was found that C57Bl/6 mouse splenic lymphoid cells responded in vitro to antigen during primary in vitro culture by the production of IgM antibody. Antibody was detected by a plaque-forming cell assay utilizing localized hemolysis in gel. Addition of lymphokine produced through Concanavalin A stimulation of murine or rat spleen cells caused different effects, depending on concentration and time of addition of the lymphokine. Small amounts of lymphokine added at the initiation of the 5-day culture period resulted in a significant increase in the number of antibody-producing cells. This increase was often two to three times the control value. However, addition of higher amounts of lymphokine resulted in a marked depression of antibody production, even though the lymphokine was not cytotoxic to the cells. To further investigate the nature of this inhibition, the spleen cell population was depleted of macrophages by carbonyl iron ingestion and passage over a column of small magnets. This depletion abrogated antibody response in the control cultures; addition of lymphokine could reconstitute the response to normal, but not higher than normal, levels. Addition of syngeneic plastic-adherent cells to the reaction reconstituted the baseline antibody response, the ability of lymphokine at moderate doses to enhance the response and the suppressive effects of high

lymphokine concentration. Addition of allogeneic macrophages could not restore the response of macrophage depleted cultures, and LK preparations could only restore the normal stimulation seen in unmanipulated cultures. Also, in these cultures there was no inhibition by high LK doses. Thus, there is an apparent requirement for syngeneic macrophages in this sytem for the production of inhibitory effects by high LK concentrations.

LK produced by stimulation of spleen cells with Con A was further purified by precipitation with ammonium sulfate (50-80% precipitate used), followed by dialysis to remove the salt. In addition, this step resulted in removal of residual mitogen in the LK supernatant. These supernatants were monitored for the presence of interleukin-2 (IL-2) or T cell growth factor, by their ability to support DNA synthesis in an IL-2 dependent murine cell line.

Assay for IL-2 activity of this purified preparation on an IL-2 dependent murine cell line showed it to contain approximately 25-fold more IL-2 than the crude material. The purified LK was much more potent in suppressing in vitro SRBC responses than the crude material.

The effects of LK preparations of human peripheral blood origin were studied in an in vitro immunization system using the model soluble antigen tetanus toxoid. In this system, it was found that, as in the murine system, lymphokine addition in moderate amounts at the initiation of culture could result in an increased yield of antibody in the culture supernatants. This antibody was measured by an ELISA assay, which gives a quantitative reading of antigen-specific antibody.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH

Further development of our understanding of the immune response can come from models which allow detailed study of the contributions of various cell types and cellular products; the systems described above offer this capability. Relatively little is known about the potential for controlling immune response through the use of soluble products, lymphokines and cytokines, produced during immune reactions. The in vitro systems allow manipulation of cell inputs and soluble products, as well as investigation of timing considerations.

Specifically, using the LK data presented above, we have been successful in increasing human antibody production levels in vitro, and have used these sensitized cells as hybridoma fusion partners, as described in an accompanying report. Detailed knowledge of the ability of LK to suppress or enhance immune responses in vitro will be important in designing LK-based therapies in cancer patients, and potentially in the development of hybridomas and/or cell lines of immune cells for therapeutic applications.

These systems also offer the potential to be of value in the preliminary screening of lymphokines or other substances proposed as biological response modifiers for use in cancer therapy.

PROPOSED COURSE

We propose to continue these investigations and to concentrate on 1) the specific role(s) of individual lymphokines in controlling augmentation and suppression of in vitro immune responses; 2) continued application of the knowledge gained in these experiments to the production of appropriately activated human lymphocytes for use in B- or T-cell hybridoma fusions; 3) isolating and purifying LK so that they may be individually studied in these in vitro systems; and 4) investigating the effector roles of the various cell types in the in vitro system (e.g., macrophages, T cell subpopulations) with the aim of optimizing the sensitization protocols. The overall long-term goal is to develop information applicable to producing immunized cells of desired anti-tumor specificity and immune reactivity for direct therapy or hybridoma fusion uses.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09244-01 BRTB |
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PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Production of Human Monoclonal Antibodies by Use of Human In Vitro Immunized Lymphocytes as Hybridoma Fusion Sources

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| OTHERS: | J. Knost | Expert | BRTB | NCI |
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COOPERATING UNITS (if any)

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LAB/BRANCH

Biological Research and Therapy Branch

SECTION

Lymphokines/Cytokines Section (proposed)

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Normal human peripheral blood mononuclear cells were immunized in vitro to the soluble antigen tetanus toxoid. Antibody production of IgG and IgM was monitored by ELISA assay. Time, antigen dosage and various culture conditions were evaluated to optimize the antibody response. Cells immunized in vitro were used to make human-human hybridoma fusions and human-mouse hybridoma fusions. Fusions of a human plasma cell tumor with B cells from cultures immunized for 6 to 11 days in vitro resulted in the production of antigen-specific human monoclonal antibodies, mostly of the IgG class. The peak time for fusion slightly preceded the time of maximal antibody production (11 days). Hybrids were stable and continued to produce for at least 60 days. No antibody to a non-crossreacting antigen, keyhole limpet hemocyanin, was detected. Human-mouse hybrids which produced specific human immunoglobulin against tetanus toxoid were also obtained using in vitro immunized human cells as fusion partners. Addition of semi-purified lymphokine preparations could enhance the numbers of antibody-producing cells during sensitization in both the human and murine systems.

PROJECT DESCRIPTION

OBJECTIVES

The objectives of this project are to: 1) develop and optimize methods for in vitro sensitization of human lymphoid cells; 2) examine effects of lymphokines related to their ability to enhance in vitro sensitization; 3) test in vitro sensitized lymphoid cells as fusion sources in human/human and human/mouse hybridoma formation; and 4) examine the time course and/or methods of in vitro sensitization which are most suitable for efficient hybridoma production. The long-range objective of this project is to utilize the methodology developed in this model system for production of human monoclonal antibodies against human tumor associated antigens.

METHODS EMPLOYED

In vitro cell culture. Immunoassays for antibody production include radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). Hybridoma production, culture, screening, cloning. Long-term cell line maintenance. Computerized data analysis. Cell sorting and analysis using fluorescence-activated cell sorter (FACS)

MAJOR FINDINGS

I. In Vitro Sensitization of Human Peripheral Blood Lymphoid Cells

The first experiments in this project were performed in January, 1982. A number of experiments have been completed defining the in vitro sensitization system for the production of antibody by human peripheral blood lymphoid cells. Cells were collected and the mononuclear cells isolated by Ficoll-Hypaque gradient centrifugation. These cells were incubated with optimal amounts (empirically determined) of the antigen tetanus toxoid in vitro in 1 ml culture volumes. At various times following the initiation of the cultures, samples of the supernatant fluids were taken and analyzed for the presence of antibody to tetanus toxoid, or the unrelated antigen, keyhole limpet hemocyanin (KLH). The antibody was detected using an ELISA system. In the ELISA, tetanus toxoid was adsorbed to polystyrene plates. The test samples were placed into wells on the plate, and any antibody present in the supernatants adhered to the antigen. Non-antibody material, and non-specific antibody was washed away, and anti-human immunoglobulin (anti-IgM or anti-IgG) labelled with the enzyme alkaline phosphatase was added. After incubation and washing, enzyme-labelled second antibody was detected through hydrolysis of the substrate p-nitro phenyl phosphate. Enzymatic activity was monitored at 405 nm using an automated transmittance spectrophotometer. The amount of enzyme breakdown product present could be directly correlated with the amount of antibody to tetanus toxoid present in the test supernatant. Results showed that antibody could be detected in most cases beginning at day 6, and reached a peak by about day 11 of culture. A slow decline followed, returning to baseline levels by day 18. Of seven

different donors tested, IgG was the predominant antibody class detected. Only two experiments, both from the cells of a single individual, showed any IgM production. In no case was any significant level of antibody production against the non-specific antigen KLH seen. This is an important control, since the cultures contain fetal calf serum as a supplement, and this material has been implicated as a polyclonal activator of human B cells in some reports.

## II. Hybridoma Production using Human In Vitro Sensitized Lymphoid Cells

The cells which were sensitized in vitro to the model soluble antigen tetanus toxoid as described above were used as partners in both human-human and human-mouse hybridoma fusions, with the intention of investigating the suitability of in vitro methods in producing stable and useful hybrids. The fusions were performed after varying periods of in vitro immunization, ranging from 5 to 11 days, since it was hypothesized that the optimal time of fusion might precede the time of maximal plasma cell antibody secretion. It was found that human-human hybridomas producing tetanus toxoid-specific antibody could be produced from cultures immunized in vitro for as little as 6 days, but that the optimum time for hybridization appeared to be at 10 days of in vitro culture. Only a small percentage of the cultures yielded hybridomas on day 6, 7 and 8, but 12-34 percent of the cultures taken at days 9 and 10 yielded producing hybrids. The hybridoma fusions were relatively stable, maintaining specific antibody production for at least 60 days without reduction in titer. Different fusions produced different amounts of antibody; the highest amounts of antibody were produced by hybrids of 10 day cultures with the human myeloma parent. In preliminary determinations, the cloned hybrids appear to produce about 5 ng/ml/week specific antibody, from an estimated clone size of 100 to 500 cells. Extrapolated, this would be approximately 10 µg/1-2 x $10^6$ cells after optimal culture, which is in the range of that produced by typical mouse-mouse fusions. About 80% of the hybridomas which produced anti-tetanus toxoid antibody produced IgG, about 20% produced IgM. It should be noted that other antibody classes were not measured in these experiments. Determination of the subclasses, binding affinities, and complete characterizations of the hybridoma monoclonal antibody products is ongoing in our laboratories. In one experiment, sensitization in vitro was performed against carcinoembryonic antigen (CEA). In this case, the peak antibody production appeared earlier than in the tetanus system, at about 6-7 days. Five and 6-day cultures did produce specific antibody as detected by competition radioimmunoassay, but the cumbersome nature of this assay led us to abandon this antigen model. However, this work did point out the individualized nature of these in vitro responses; timing, dosage, etc. have to be empirically determined for each antigen used.

Preliminary trials have indicated that in vitro immunization in the presence of crude human lymphokine preparations yields 2-4 times the amount of antibody of control cultures. It is not known presently whether this represents additional B cell clonal expansion, or an increase in antibody produced per activated B cell.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

This work has significant implications in both clinical and basic realms. The major goal of this research program is to develop human hybridomas which produce useful monoclonal antibodies directed against human tumor-associated antigens. Human monoclonal antibodies, as compared to mouse or rat antibodies, would, potentially have two major advantages: avoidance of likely sensitization to heterologous proteins if used in clinical diagnostic or therapy trials; and the likelihood of recognition of finer tumor-associated specificities rather than broad specificities or tissue antigens. It has been difficult to obtain highly immunologically reactive cells against such antigens since the use of active immunization in normal humans is contraindicated. However, by using in vitro immunization, one can produce a source of cells for hybridoma fusion. In addition, this work shows that the efficiency of hybridoma formation using in vitro sensitized cells is much increased compared to cells from immunized donors. The in vitro reaction may be preferred on the grounds of increased efficiency.

In the basic research realm, these systems give us the opportunity to examine the contributions of various cell types (e.g., lymphocytes, macrophages) to the human antibody response. We are particularly interested in defining the role of lymphokines and cytokines in the sensitization and effector phases of the human in vitro immunization reaction. It offers a way to test various immune modulators for their effects in terms of either enhancement of inhibition of antibody production.

PROPOSED COURSE

In the near future we will pursue this research in several relevant and promising directions. These include: 1) further study of the optimal conditions for in vitro antibody production by human cells and for using these cells as fusion sources, with particular emphasis on the role of specific lymphokines to enhance the sensitization response; 2) extension of the assay into a tumor-associated antigen system, such as the 94K melanoma antigen system available within our Branch; and 3) complete characterization of the monoclonal antibodies already produced.

Since this research is in its early stages, it is difficult to project too far into the future, but the melanoma antigen model, if successful, would certainly lead to attempts to develop hybridomas that would produce clinically useful anti-tumor monoclonal antibody by these techniques.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09245-08 BRTB formerly Z01 CB 08500-07 LID |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Specific Immune T Cell Reactivity to Tumor-Associated Antigens in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Lymphokines/Cytokines (proposed)

INSTITUTE AND LOCATION

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 3 | 2 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We studied human T cell activation and regulation in the system of continued cultures of T cells that respond to T cell growth factor (TCGF). T cells cooperate with monocytes and with B cells during lymphoproliferation to the lectin phytohemagglutinin. Monocytes and a few myeloid and monocytic leukemia cell lines function as accessory cells and produce interleukin-1 (IL-1). We studied the TCGF-producing cells, and cells responding to TCGF, by means of anti-T cell sera, Percoll gradients. Cultured T cells (CTC) are cytotoxic in antibody-dependent cell-mediated cytotoxicity, natural cytotoxicity and specific immune cytolysis. We studied a mouse monoclonal antibody against cultured human T cells and determined that the antibody appears to block the postulated receptor for IL-2 on activated human T cells. We have also tested monoclonal antibodies against the non-polymorphic framework of several molecules within the MHC and shown that portions of HLA-D/DR, HLA-A,B,C and $\beta_2$m are probably involved in regulating proliferative responses to PWM and OKT3.

PROJECT DESCRIPTION

OBJECTIVES

This group has concentrated on work with continued cultures of human T cells. These cultured T cells (CTC) have been derived from fresh peripheral mononuclear leukocytes (PBL) or from cultures of in vivo sensitized lymphocytes, with the aid of conditioned media from phytohemagglutinin (PHA)-stimulated PBL, which contain T-cell growth factor (TCGF). We have developed this technology to achieve one major, long-term goal: the development of cellular reagents specific for tumor-associated transplantation antigens (TATA) in man and specific for cells which react to TATA. To achieve this goal, it is necessary to first obtain more basic information on the cellular requirements, and the soluble factors, involved in T cell immune responses in man.

Our present priorities are mainly to dissect the heterogeneity of T cell subsets found in PBL and CTC, particularly as they relate to the mechanisms of T cell activation and regulation: 1) a study of T cell-monocyte and T cell-B cell collaborations during stimulation with lectins, in terms of the resulting proliferation, production of mediators, and cooperation between structures at the cell surfaces; 2) an examination of the TCGF-producing T cells (Tp) and the T cells responding to TCGF (Tr), with regard to their maintenance in cultures; also by means of heterologous and monoclonal antibodies to T cell subsets and other cell separation procedures; 3) a study of whether CTC contain cells bearing receptors for the Fc portion of immunoglobuln G (Fc R), that may be regulated by immune complexes, and may have regulatory functions on other T cells; 4) a study of whether CTC contain natural killer (NK) and killer (K) cells, raising the question of their contribution to cytotoxicity by CTC against human tumors; and 5) attempts to clone CTC with one or more of the above characteristics. This detailed view on the heterogeneity and functions of cultured T cells will allow us to adequately vary the conditions of our in vitro cultures, to enhance or eliminate certain T cell subsets and their functions, when we generate specific immune T cells against human TATA.

Our feasibility study on the development of cellular reagents helpful in investigating cellular reactions to TATA in man is as follows: 1) improve culture conditions for CTC such as to achieve better cell yields, longer cell survival, and allow cloning of effector CTC; 2) analyze the relative contribution of various types of cytotoxicity by CTC against tumor cell lines and fresh solid tumors; 3) utilize proliferative responses and $^{3}$HTdR-incorporation as an alternate means to detect CTC sensitized against TATA; and 4) develop reagents that will help delineate the role of reactive and accessory cells in reactions to tumor cells.

MAJOR FINDINGS

I. Introduction

Because deficient responses to tumor antigens, as well as other antigens, could be due to the lack of malfunction of one of several cell types participating in

the response, it is important to detect all the cells involved in responses to antigens, and to design new methodologies to assay their functions individually. Our demonstration of a role for TCGF-producing cells (Tp); and cells responding to TCGF (Tr), and also for monocytes in TCGF production, has opened up the possibility to test the function of these cells and their secreted products, separately, by appropriate protocols.

There has been considerable interest over the past few years in the role that immune complexes (IC) may play in enhancing tumor growth. In fact, several empirical protocols have been developed to attempt to remove, by plasmapheresis, immune complexes found in certain cancer patients. So far, there has been little in depth biological information to support these clinical trials. Very little is known about the influences that immune complexes exert on human T cell responses to antigens. One well-documented finding has been their ability to abolish the lymphoproliferative responses of isolated T cells bearing receptors for Fc R. We have continued to explore the suppressive effects of IC on T cell lymphoproliferation.

One of the major problems with the development of unique cellular reagents against TATA in man, using the CTC technology, has been the limited expansion and life-span of these cultures. Human CTC usually undergo a crisis at 6-8 weeks and die. In experiments starting the cloning from a single cell, this means that less than $10^7$ cells can be generated by the time of the crisis in most instances, which is not enough for extensive testing. It is thought that improvement of the level and quality of TCGF production could alleviate these problems.

## II. Biological Studies on T Cell Heterogeneity, Activation and Regulation

### A. Studies of T Cell-Monocyte and T Cell-B Cell Collaborations During Stimulation with Lectins

We have demonstrated that Tp, whose fate was hitherto unknown, grow out with CTC. When CTC were adequately stimulated with PHA in the presence of purified monocytes as accessory cells, they produced TCGF in the supernatant, and they proliferated to this endogenously-produced TCGF. It could be that Tp and Tr are the very same cells, and this has important implications for our model of T cells activation. Also, the possibility exists that Tp cells could be cloned, thus providing monoclonal sources of TCGF. Examination of the activity of TCGF from different clones may provide evidence for heterogeneity of TCGF, a much debated question. The presence in CTC of the right kind of Tp cells may be important for the long-term survival of the culture.

As controls for the blood monocytes in these experiments, we used cells from a variety of cell lines, and most did not have the same accessory function. However, K-562, a cell line derived from a patient with chronic myeloid leukemia in blast crisis, was as effective as monocytes in their accessory function for TCGF production by CTC. A systematic study of the available myelomonocyte human cell lines revealed that U-937 also had a similar accessory function, whereas HL-60, KG-1, and the pre-B cell line NALM-1 did not. We find it of considerable interest that K-562 lacks HLA-D,DR antigens, yet is able to cooperate with CTC.

This may indicate that antigens other than those commonly detected by anti-HLA-D, DR sera may be important for macrophage-T cell cooperation. The availability of monoclonal antibodies to HLA-D,DR subregions and to K-562 cells should enable us to make progress in this sera. We found that K-562 and U-937 produced moderate to high levels of IL-1 upon stimulation with the tumor promoter phorbol myristate acetate (PMA). IL-1 in these supernatants were measured on C3H/HeJ mouse lymphocytes, in the presence of PHA. C3H/HeJ thymocytes did not respond to PHA alone, or to PMA alone. The material from these cell lines may be suitable for extensive biochemical characterization, which has not been readily possible with material derived from blood monocytes. The availability of two cell lines that produce IL-1, and cooperate with T cells, inducing their TCGF production, may represent an easy approach towards the analysis of the requirements for cell surface structures and mediators that are involved in such cooperation. In particular, clones of K-562 or U-937, or hybrids made with K-562 or U-937, could be analyzed for their cell surface markers, their production of IL-1, and their ability to cooperate events between normal T cells and monocytes or macrophages during the immune response.

During our study of the K-562 cell line, known to represent precursor cells of the myeloid and erythroid series, we also examined human granulocytes for their ability to cooperate with CTC in our system. We found that our granulocyte preparations worked as well as monocytes, provided we used higher cell numbers. However, we are not sure, at this point, whether this activity is mediated by contaminating macrophages, some special activity of the contaminating erythrocytes, or the granulocytes themselves. This deserves further study.

Our new system with CTC stimulated with PHA and monocytes also represents a very practical test of monocyte accessory function, and could be applied to testing monocytes from patients with a variety of diseases.

We previoulsy described the enhancement of TCGF production by PBL when cells from B lymphoblastoid cell lines (B-LCL) were added to the mixtures of PBL and PHA. Only B-LCL and not T lymphoblastoid cell lines had the effect, which excluded a role for HLA-A,B or C alloantigens. Furthermore, Daudi, a B-LCL lacking these antigens, enhanced well. HLA-D,DR antigens did not seem to be involed either, since an HLA-D,DR negative mutant cell line and its parental line, kindly provided by Dr. D. Pious, both enhanced to the same level. We are presently studying the possible roles of EBV antigens, FcγR, and of soluble products released specifically by the B-LCL. It will be important, also, to use normal peripheral B cells to conclude whether this reaction indicates a T cell-B cell collaboration, that takes place normally.

B. Studies of T Cell Subsets, Particularly Tp and Tr

According to our previous model of T cell-activation (Bonnard et al, J. Immunol., 123, 2704, 1979), the subset of Tp may be different from a subset of Tr, which grow as CTC. The experiments described above on the production of TCGF by CTC suggest, on the other hand, that Tp and Tr could be the same cells. Alternatively the possibility exists that CTC contain separate populations, Tp and Tr. This was studied further in collaboration with seversl members of LID. First, they looked at subsets of freshly isolated PBL for TCGF production. The

recently developed technique of Percoll gradients allowed the separation of PBL into LGL, mostly NK cells, 50% of which form rosettes with SRBC at 4°C, and into small lymphocytes, mostly typical T cells. Further SRBC-rosetting of the LGL fraction at 29°C yielded greater than 95% pure LGL preparations. Examination of the TCGF production by LGL and T cells showed T cells to be much better producers than LGL. The LGL preparations produced low levels of TCGF, but it is unclear as yet whether this level of TCGF was due to contamination with minute numbers of T cells. In any event, this was extended by the demonstration that both highly purified LGL preparation, as well as highly purified T cells, grew on exogenous TCGF. These results were not simply due to a failure to separate these cells properly, since LGL in culture kept their typical morphology, and characteristic pattern of cytotoxicity, quite distinct from that of cultured T cells. Thus, both the LGL fractions and the T cell fractions contained Tr cells.

Leukemia T cells might also fall into the categories of Tp and Tr. Therefore, during the past year we examined the blasts of two patients with T cell malignancies for their ability to produce TCGF and to respond to exogenous TCGF (Tr function). These results, however, were difficult to interpret due to potential contamination with normal PBL. Typically, when we were able to grow CTC from the cells of a patient with a monoclonal OKT8+ chronic T cell leukemia, after 14 days the CTC had the same cell surface markers as normal control CTC. However, with other T cell malignancies, it may be possible to grow the clone of malignant cells with TCGF, or to show that the cells produce TCGF. Therefore, this study is being pursued.

C. A mouse monoclonal antibody (MoAb), produced by injections of human cultured T cells (CTC), was shown to totally abrogate the proliferative responses of CTC to IL-2 and appears to react against the cell-surface receptor for IL-2. Several MoAb against cell surface structures of T lymphocytes or subsets of T cells were screened for their ability to stimulate proliferation of fresh T cells or CTC, or to inhibit the response of $10^5$ CTC to optimal dose of IL-2 in a 3-day microwell $^3$H-thymidine assay (Bonnard et al., in "Immunology 80"). One MoAb reacted with very fresh T cells and with only a fraction (15-80%) of CTC by FACS analysis. However, it completely blocked CTC response to IL-2 in a quantitative manner, between the $10^{-4}$ and $10^{-6}$ dilutions (using ascites fluid). Cell viability, by trypan blue or analysis with the OKT reagents on a FACS, was not affected. Like the antibody itself, the inhibitory activity could be readily absorbed out by CTC and PHA-blasts ("activated T cells"), but not by fresh T cells or by T lymphoblastoid cell lines, and could not be attributed to contamination by mycoplasma or viruses. We were not able to saturate the IL-2 receptor with this MoAb at 4°C, such as to block absorption of IL-2 from a sample (see Bonnard et al., J. Immunol. 123, 2704, 1979). However, overnight incubation of CTC with MoAb at 37°C capped a receptor of CTC for IL-2, as assessed by the subsequent inability of these CTC to absorb IL-2 or to proliferate with IL-2. CTC similarly treated with OKT4, OKT8 or anti-HLA MoAb were not affected. We conclude that this MoAb may detect the cell-surface receptor for IL-2. The known distribution of the MoAb-defined antigen on "activated" T cells and its preliminary biochemical characteristics are consistent with this antigen being the cell-surface receptor for IL-2.

D. We have found that inhibition of proliferative responses of human mononuclear cells to polyclonal activators could be induced by several monoclonal antibodies against the major histocompatibility determinants. We found that biochemically well-characterized antibodies against the non-polymorphic framework of several molecules within the major histocompatibility complex (MHC) strongly blocked the proliferation of mononuclear cells (MC) to PWM or OKT3, but only weakly inhibited proliferation to PHA or Con A. The cooperating cell populations were MC from peripheral blood ($10^5$/microtiter well) or were cultured T lymphocytes (CTC, $10^5$) to which adherent accessory cells (AC, 5 x $10^4$) were added (Fagnani and Bonnard, Clin. Res. 28, 345A, 1980). Eleven mouse IgG2a monoclonals which reacted with either one class of HLA-D/DR, HLA-A,B,C, or B2m molecules were added during the 3-day culture with either PHA, Con A, PWM or the monoclonal OKT3. Under these conditions the reagents were non-cytotoxic. Only the highest concentration of some antibodies inhibited PHA and Con A responses (1/8 supernate of clones), whereas much lower concentrations (around 1/128) often blocked 50% of the PWM and OKT3 responses, as assessed by $^3$H-thymidine uptake. The pattern of inhibition was reproducible with different MC preparations or combinations of CTC and AC. Thus, the antibodies did not profoundly affect PHA and Con A responses, contrary to what has been reported for polymorphic alloantisera to HLA-D/DR and some polyclonal xenoantisera. On the other hand, we conclude that portions of the framework of each of HLA-D/DR, HLA-A,B,C and B2m are probably involved in regulating proliferative responses to PWM and OKT3. This could happen on either T cells or AC. These experiments may help with the characterization of these and similar monoclonals, since the blocking of responses to certain lectins or antigens could conceivably reveal a reaction with a give portion of the framework of one MHC molecule.

## III. Clinically Oriented Studies on Cellular Reagents That Might Detect Tumor-Associated Transplantation Antigens in Man

### A. Improved Culture Conditions for CTC

We have expanded our efforts to produce good quality TCGF preparations from lymphoid cells obtained from thoracic duct lymphocytes. Certain patients with prospective kidney graft undergo intense lymphoid cell depletion by thoracic duct drainage, and massive numbers of cells become available for TCGF production. Since these protocols are being performed all over the country, our new method may be generally applicable.

We have also made progress in the maintenance and long-term culture of CTC by eliminating infection with mycoplasma. Mycoplasma have been known for quite some time to be toxic to cell lines, and in particular to malignant T cell lines in culture. Our experience this year extended these findings to CTC which seem extremely susceptible to mycoplasma infection. This was noticed during our above studies on the potential accessory role of various cell lines for T cell proliferation to PHA. A control in these experiments include CTC and TCGF with the cell line. A number of cell lines proved to inhibit CTC responses to TCGF, and these results were attributable to infection with mycoplasma. Our findings is of considerable practical importance, because the commercial laboratories that prepare TCGF have not been aware of the deleterious role of mycoplasma. They will now be able to take specific steps to avoid such contamination.

B. Cytotoxicity Mediated by CTC Against Autologous Fresh and Cryopreserved Tumors

In order to get specific immune CTC against human tumors, one would have to distinguish these cells from effector cells which mediate other types of cytotoxicity (due to polyclonal activation, lectin-induced cytotoxicity, and NK and K cell activity).

During this year, we were able to examine the cytotoxicity mediated by CTC from patients with solid tumors. The patients' T cells, purified by nylon column passage, were cultured with single cell suspensions of the tumor for six days. The blasts were then separated and expanded with TCGF-containing conditioned media, and the help of feeder cells. In several instances the CTC from the MLTR were found to be much more cytotoxic against the autologous, cryopreserved, $^{51}Cr$-labeled solid tumor target cells than were any of several control CTC. Because this pattern of reactivity was of considerable interest and might indicate anti-tumor reactivity in these CTC populations, attempts have recently begun to clone these effector cells by limiting dilution, in the presence of feeder layers and TCGF. As mentioned above, however, there are still considerable technical problems with the maintenance of these clones in culture in order to reach sufficient cell numbers for more detailed studies.

C. Secondary Proliferative Response of CTC as Assay for Recognition of TATA

Using the CTC derived from autologous MLTR of patients with solid tumors, we have been able to demonstrate, on the first few experiments done, the capacity of the original stimulating tumor cells, and some but not all allogeneic tumor cells, to elicit a secondary proliferative response. The antigens responsible for such stimulation are under study: it will be necessary to distinguish recognition of TATA from responses to normal auto-antigens.

SIGNIFICANCE FOR BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

The capability to generate large numbers of pure and functional T cells from any given individual or population of lymphocytes opens considerable avenues for use of these cells in in vitro assays of cell-mediated immunity. The technique may turn out to be the cleanest approach to the purification of considerable numbers of T cells reactive against human TATA, and this will certainly hold a very high priority in many cancer research laboratories for years to come. These studies are also potentially useful for the immunodiagnosis of cancer and in measuring changes of immunity in response to chemotherapy or immunotherapy. This would fall under Objective V, Approach 4 and Objective VI, Approaches 1 and 4 of the National Cancer Plan.

Detection of tumor antigens and of immune reactions to human tumor-associated antigens is a major goal in tumor immunology. The development and standardization of in vitro assays for cell-mediated immunity is one of the most important aspects. Because immunogenicity cannot be studied directly in the human, the evaluation of immunologic reactivity and the antigenicity of tumor cells by in vitro assays is of paramount importance. This would fall under Objective V, Approach 4 of the National Cancer Plan.

The question of whether immunosuppressive factors play a role in interfering with effective immunity is also very important. It appears that the stimulation of the host immune system may be useless, unless one can first rid the body of the inhibitory phenomena related to tumor cell growth. Investigation in this area would fall under Objective IV, Approach 4 and Objective VI, Approaches 1, 2, and 4 of the National Cancer Plan.

The capability to distinuish between different T cell subsets and their functions would represent very significant progress towards the identification of T cells immune to tumor-associated antigens in cancer patients. This may lead to a better understanding of reactions against the tumor that may be important in vivo. The findings of our laboratory that T cells with various functions can be adequately studied in continued cultures open major avenues of research. Further, techniques are now available to study the factors that mediate the needed cellular collaborations during immune responses to tumors. It is possible that large quantities of T cells immune to the tumor or of factors that enhance responses to the tumor could be used in immunotherapy. This would fall under Objective V, Approach 4 and Objective VI, Approaches 1, 2, and 4 of the National Cancer Plan.

PROPOSED COURSE

With the conclusion of Dr. Bonnard's term as a Visiting Scientist at NCI, and his acceptance of a position at the Institute for Clinical Immunology at the University of Bern Medical Center in Switzerland, this project is being terminated as a separate entity. This work will be pursued by Dr. Bonnard in Bern and further studies will be done in close collaboration with scientists of the Biological Research and Therapy Branch of the NCI. Portions of this project are being continued independently within the Branch under other project titles and the efforts and progress made in this project will be of great advantage to other projects within the Branch.

PUBLICATIONS

Uchiyama, T., Broder, S., Bonnard, G.D., and Waldmann, T.A.: Immunoregulatory functions of cultured human T lymphocytes. Trans. Assoc. Am. Physicians, 93: 251-262, 1980.

Bonnard, G.D.: Long-term cultures of immunocompetent T lymphocytes. In Sell, K.W., and Miller, W.V. (Eds.): The Lymphocyte. Progress in Clinical and Biological Research, Volume 58. New York, Alan R. Liss, Inc., 1981, pp. 45-56.

Slease, R.B., Strong, D.M., Gawith, K.E., and Bonnard, G.D.: Clinical effects of infusions into chimpanzees of primed autologous cultured T-cells. J. Natl. Cancer Inst., 67:258-264, 1981.

Ortaldo, J.R., Timonen, T., and Bonnard, G.D.: Natural killing and antibody-dependent cell-mediated cytotoxicity by human cultured T cells. Behring Inst. Mitt., 67:258-264, 1980.

Herberman, R.B., Timonen, T., Ortaldo, J.R., Bonnard, G.D., Kedar, E., and Gorelik, E.: Characteristics of NK cells and their possible role in resistance against tumor growth. In Saunders, J.P., et al. (Eds.): Fundamental Mechanisms in Human Cancer Immunology. New York, Elsevier/North Holland, 1981, pp. 499-510.

Kedar, E., Herberman, R.B., Gorelik, E., Sredni, B., Bonnard, G.D, and Navarro, N.: Antitumor reactivity in vitro and in vivo of mouse and human lymphoid cells cultured with T cell growth factor. In Fefer, A. (Ed.): The Potential Role of T Cell Subpopulations in Cancer Therapy. New York, Raven Press, in press.

Silva, A.G., Alvarez, J.M., Bonnard, G.D., and de Landazuri, M.O.: Human IL-2: Production by both $T_G$ cells and other T cells. Scand. J. Immunol., in press.

Vose, B.M., and Bonnard, G.D.: Specific cytotoxicity against autologous tumour and proliferative responses of human lymphocytes grown in interleukin 2. Int. J. Cancer, 29: 33-39, 1982.

Gramatzki, M., Dolan, M.F., Fauci, A.S., Maples, J.A., Bonnard, G.D., and Strong, D.M.: Immunological characterization of a helper T cell lymphoma. Blood, in press.

Vose, B.M., and Bonnard, G.D.: Antigens of human tumors defined by cytotoxicity and proliferative responses of cultured lymphoid cells. Nature 296: 359-361, 1982.

Vose, B.M., Riccardi, C., Marchmont, R.J., and Bonnard, G.D.: Clonal analysis of human natural killer cells. In Herberman, R.B. (Ed.): Natural Cell-Mediated Immunity, Vol. 2. New York, Academic Press, in press.

The research activities of the National Immunity Section are primarily focused on the role of natural effector cells in resistance against cancer. There have been extensive studies on natural killer (NK) cells, with emphasis on the characteristics of these cells, the factors regulating their activity, and their in vivo role in resistance against tumor growth. Several major new findings have resulted from the ability to isolate highly purified populations of human and rat large granular lymphocytes (LGL), a small subpopulation of cells which we have shown to be responsible for NK and antibody-dependent cell-mediated cytotoxic activity. LGL from these species have been extensively characterized and shown to share features with T cells and also monocytes. During the past year, it has also been possible to enrich for mouse LGL and show their association with mouse NK activity. Cytotoxic studies with the LGL have led to new insights into the mechanisms of their reactivity and of augmentation of their function by interferon (IFN) and other lymphokines. In addition to their potent cytotoxic activity, LGL have been found to also produce IFN and IL-2. The potential for characterization and practical utilization of LGL has been expanded greatly by development of procedures to propagate these cells in culture and even to clone them. This has already provided an increased ability to discriminate between NK cells and immune cytotoxic T cells with anti-tumor activity. A further, major advance in our ability to obtain large homogeneous populations of LGL for detailed studies has come from the observation that aged F344 rats have a high incidence of functionally active LGL tumors. Studies with immunotherapy of rat tumors with LGL are providing essential information needed for the design of clinical therapy trials with human LGL. Further evidence has also accumulated in support of in vivo roles of NK cells, including their participation in immune surveillance against the development of chemically or radiation-induced tumors, in reactivity against primary autologous human tumors, and in resistance against metastases.

## SOME HIGHLIGHTS OF ACHIEVEMENTS IN THE LAST YEAR

### I. Characterization of NK Cells and Other Natural Effector Cells

A major advance in the characterization of human NK cells has come from the finding of their close association with LGL, that possess a distinct morphology with Giemsa staining, containing azurophilic granules, with pale, blue-staining cytoplasm. More than half of these LGL form lytic conjugates with NK-susceptible targets. These cells have been isolated from peripheral blood lymphocytes using discontinuous Percoll density gradients and subsequent further purification by rosette formation with sheep erythrocytes at 29°C, to minimize contamination with small typical T lymphocytes. Using these procedures, we have been able to reproducibly obtain fractions containing >90% LGL by morphological analysis and containing <1% classical T cells as determined by morphology and monoclonal antibody analysis. Virtually all of the LGL have Fc receptors for IgG and they account for most, if not all, of the NK cell activity against NK-susceptible targets. We have extended our previous results by examining in detail a large variety of NK-susceptible targets, including both lymphoid and anchorage-dependent lines. High density fractions of cells, which were enriched for

classical T cells but devoid of LGL, could not be induced to become cytotoxic by the addition of activating agents such as IFN or IFN-inducers. Coupling our purification procedure with a single cell cytotoxicity assay, we have estimated the >70% of the LGL are functionally active NK cells. We have examined the blood or spleen cells of a variety of animal species for the presence of LGL and for the parallel expression of NK activity or antibody-dependent cell-mediated cytototxicity (ADCC) activity. LGL and NK and/or ADCC activities have been detected in most of the species examined, including rats, mice, nonhuman primates, dogs, cats, sheep, cattle, pigs and chickens. In all of the species studied, the frequency of LGL among peripheral blood mononuclear cells varied between 1 and 10%. In parallel with the high NK activity in the blood or spleen of nude, athymic rats, LGL frequency was shown to be increased 2-5 fold in these rats.

Because of the ability to obtain highly purified human and rat LGL and their strong association with human NK cell activity, we were able to perform detailed phenotypic studies with these effector cells and compare them to the phenotype of typical small, classical T lymphocytes. This has been performed with a series of monoclonal antibodies, using the fluorescence activated cell sorter. A considerable proportion of human LGL reacted with antibodies OKM1 (60-80%) and anti-Ia (15-30%). 25% reacted with OKT8, which is similar to its expression on T cells. In contrast, however, very few LGL reacted with OKT3 or OKT4 antibodies, which react with a high proportion of mature T lymphocytes. OKT11A and 9.6 antibodies, which react with the sheep erythrocyte receptor, also reacted with approximately 50% of the LGL. OKT10 (which as been found to react mainly on pro- or early thymocytes) reacted with a high proportion of LGL, whereas none of the other peripheral blood lymphoid cell populations were positive. By depletion on monolayers of the cells coated with mouse monoclonal antibodies, we were able to determine that most, if not all, of the active NK cells were OKT10 positive and the majority were also OKM1 positive. Only a small proportion of the functional NK cells possessed the OKT8 or Ia antigens.

As in the human studies, the rat LGL were found to be an antigenically distinct population of cells which share some characteristics with monocytes, T cells, and granulocytes. Essentially all of the LGL expressed the W3/13, OX-8, leukocyte-common (L-C), and asialo GM1 antigens. A portion of these cells were also positive for ART-$1^a$. In contrast, few LGL expressed Ia, W3/25, surface immunoglobulin, or Thy 1.1 antigens. Monocytes had a similar pattern but were OX-8 and ART-$1^a$ negative, and T cells expressed ART-$1^a$, W3/25 and/or OX-8.

Determination of the possible association of mouse NK cells with LGL has been considerably more difficult. However, during the past year, by modification of the conditions used for Percoll density gradient centrifugation, it has been possible to enrich fairly well for LGL in low density fractions and to obtain high density fractions of lymphocytes that are virtually devoid of LGL. The LGL-enriched subpopulations have been shown to be enriched for NK activity and the LGL-depleted subpopulations have had no NK activity.

Natural cytotoxic (NC) cells have been described as mouse natural effector cells that are responsible for natural cytotoxic reactivity against certain solid tumor target cells. To determine the relationship between NK and NC

cells in mice, we have performed detailed studies with target cells which have been shown to be highly sensitive to lysis by NC cells but resistant to lysis by NK cells. We have found that the LGL fractions possess NC as well as NK activity, and the fractions depleted of LGL are devvoid of NC as well as NK activity. Thus, is appears that both NK and NC activities are included within the small subpopulation of LGL. Studies have also been performed in vivo to determine the characteristics of the natural effector cells reacting against such solid tumor targets. Rather unexpectedly, we have found that several of the characteristics of the effector cells involved in rapid in vivo elimination of intravenously inoculated radiolabelled-tumor cells are considerably more compatible with those of NK cells than of NC cells.

A major limitation for the detailed analysis and characterization of NK cells is that they represent a small proportion of the lymphoid cells in the blood or peripheral organs. A potential solution to this problem was suggested by the observation that human peripheral blood lymphocytes can be cultured in the presence of partially purified T cell growth factor (interleukin 2 or IL-2) and these continuously growing cell lines have two distinct cytotoxic capabilities: 1) an NK-like and 2) a polyclonally activated T cell-like activity. These cultures have been shown to be cytotoxic against a variety of NK-sensitive targets and against alloblasts. Highly purified populations of LGL, which were essentially devoid of mature typical T lymphocytes, as well as classical T cells, maintained rapid growth when cultured in the presence of IL-2. The T cells maintained relative stability in culture, not expressing new antigens as determined by a battery of monoclonal reagents, with the exception of the expression of Ia, which has been previously reported on activated T lymphocytes. In contrast, the LGL cultures demonstrated some very significant changes in phenotype. OKT10, OKM1, and Fc receptors were much diminished or absent by 10 days. Conversely, the appearance of Ia antigens and OKT3 antigens as well as the ability of a subset of cultured LGL to form rosettes with sheep erythrocytes at 29°C became quite apparent at about the same time in culture. The cultured LGL demonstrated a pattern of cytotoxicity that was similar to that of fresh LGL, reacting mainly against NK-susceptible targets, and both fresh and cultured NK cells exhibited antibody-dependent and lectin-induced cytotoxicity. In contrast, the fresh and cultured T cells only demonstrated lectin-induced cytotoxicity and did not demonstrate NK-like killing against the panel of NK targets. Polyclonal activation against alloblasts as well as some activation of reactivity against anchorage-dependent target cells appeared in the cultured T cell fractions.

It has been possible to also maintain the proliferation of mouse cytotoxic cultured lymphoid cells (CLC) for 1-12 months in the presence of IL-2. Clones from these cultured lymphoid cells were established by either limiting dilution or soft agar techniques. Most of the clones had strong cytotoxic activity against a variety of syngeneic and allogeneic tumor target cells. The clonal populations generally exhibited a more restricted pattern of cytotoxic activity than the parental CLC and the pattern varied among the clones. One group of clones reacted preferentially against lymphoid tumor target cells and the others reacted strongly against both lymphoid and solid tumor targets. Some clones were isolated which failed to react with the classical NK target cell, YAC-1, but reacted strongly against some solid tumor targets. The clones expressed some markers associated with NK cells (asialo GM1, NK2, T200) and also some characteristic T cell markers (Thy 1.2, Lyt 2).

Another approach to isolate a large number of higly enriched LGL would be to find naturally occurring LGL tumors which maintain their morphological and functional characteristics. These cells would be available in essentially unlimited quantities and could be used for those studies requiring a large number of cells for detailed analysis. Recently, we have demonstrated that there are LGL tumors in a high percentage of aged (greater than 24 months old) F344 rats. These tumor cell lines are obtained from animals with spontaneous mononuclear cell leukemia. A significant percentage (30-40%) of these cell lines demonstrate very high cytolytic potential. These tumors have been readily transplanted and some of these tumor lines have consistently shown levels of cytotoxicity which approach 200 times the level of activity seen in normal, age-matched controls. Antigenically, most of these cell lines are similar to normal LGL, but considerable variation among tumors in the expression in certain markers has been seen. Analysis of these cell lines histochemically has also shown much similarity to normal LGL. We have recently begun to examine the cytotoxic specificity of these cell lines and it has become clear that those lines with cytotoxic activity kill NK-susceptible tagets but do not kill NK-resistant target cells. Most of these cytotoxic tumor cell lines have avid Fc receptors and can mediate antibody-dependent cell mediated cytotoxicity (ADCC). Interestingly, these tumors do not arise in splenectomized animals. These data are consistent with our hypothesis that the spleen may be a major site for LGL development and differentiation. Ongoing studies with these NK (LGL) tumors should provide an excellent source of cells for biochemical studies on the mechanisms involved in NK activity.

## II. Regulation of the Activity of NK Cells

IFN has been shown to have a variety of effects on immune reactivity, including the ability to rapidly augment cell-mediated cytotoxic responses such as the reactivity of NK cells, and macrophages, or monocytes. Some of these effector mechanisms may have in vivo importance in the resistance against tumor growth or against infections by various microbial agents. To obtain better insight into the nature of the diversity of such effects by IFN, more than 12 preparations of human leukocyte IFN (homogenous or partially purified species of natural recombinant and hybridized recombinant) as well as highly purified beta (fibroblast) and immune (gamma) IFN were tested for their ability to augment the reactivity of NK cells and monocytes.

All of the IFN species tested were shown to be able to significantly augment the cytotoxic reactivity of both NK cells and monocytes. However, when low levels of IFN were imployed, appreciable quantitative differences among the various species were seen. Work in progress with restriction enzyme-derived recombinant hybrid IFN molecules have been examined for similar biological variations. These pure hybrids of recombinant A and D IFN have been shown to vary in their antiviral activity and their efficacy in boosting NK activity. In our previous experiments, the species specificity of recombinant interferon was maintained with the leukocyte species and recombinant A IFN. However, recombinant D IFN has demonstrated biological cross-reactivity with murine NK cells. The examination of A/D hybrids may enable the determination of valuable information regarding the location of the active sites for the antiviral and immunomodulatory functions on the interferon molecule. The substantial results demonstrated substantial quantitative differences in the ability of the various

species of human leukocyte IFN to significantly augment levels of cell-mediated functions and such information should have a significant impact in choosing the IFN species for appropriate clinical trials. In addition, this demonstration that homogeneous natural and recombinant IFNs have potent effects on effector mechanisms provides a rational basis for developing new protocols for clinical therapy with IFN.

Detailed analysis of treatment of human LGL with IFN, which causes augmentation of NK activity, has indicated that IFN has multiple effects on NK cells, depending on the target cell tested. The effects include 1) an increase in the rate of the reaction, 2) an increase in the recycling of the NK cells, i.e., the ability of NK cells to interact with and lyse multiple targets, 3) an increase in the number of lytically active NK cells, and 4) an increase in the number of effector cells able to recognize and bind to certain targets (especially seen with anchorage-dependent target cells). By using a single cell agarose cytotoxicity assay, virtually all of the human LGL which bind to NK-susceptible targets in the presence of IFN have been shown to be capable of killing their target, especially when the assay time is extended to between 4-24 hours.

Studies have been performed to determine the mechanism of IFN effects on NK cells. The IFN-induced increase in human NK activity was shown to be paralleled by an increase in (2'-5')oligo A synthetase, a major pathway involved in the antiviral action of IFN. By introducing the product of the enzyme, (2'-5')pppApApA, into human NK cells by the calcium phosphate precipitation method, the effect of IFN was mimicked. The mechanism by which the (2'-5')pppApApA increased the NK activity is presently not clear. It was shown that when this molecule is introduced into non-lymphoid cells it activates a (2'-5')oligo A-dependent endonuclease, which results in the degradation of RNA in the cells. It is also possible that the introduction of the (2'-5')pppApApA into NK cells results in a limited inhibition of synthesis of certain proteins.

We have found that partially purified preparations of IL-2, when present in the cytotoxicity assay, or after pretreatment of the effector cells, could substantially augment NK activity, to levels at least as high as that induced by IFN. The possibility was then considered that IL-2 might be an important factor for the spontaneous reactivity of human NK cells. To explore this possibility, we preincubated human LGL for various periods of time with monoclonal antibodies against human IL-2. Preincubation for 1-4 hours caused a significant decrease in spontaneous NK activity, and after 20 hours of preincubation with these antibodies, virtual elimination of reactivity was observed. Reactivity could be partially restored by further culture of the cells in the absence of the antibody, and could be rapidly and fully restored by treatment of the effector cells with IL-2. Thus, it appears that endogenous production of IL-2 within an LGL population may be responsible for the spontaneous activation of human NK cells.

We have found that natural suppressor cells for NK activity account, at least in large measure, for the low NK activity in some mice. Addition of spleen cells from infant mice to those of normal high NK-reactive mice resulted in a substantial inhibition of activity. This inhibitory activity was associated with both adherent and nonadherent cells. In studies of strains of mice with low NK activity, particularly SJL and A strain mice, we have been able to associate low reactivity with naturally occurring suppressor cells, capable of

inhibiting the effector phase of NK activity. Adherent spleen cells from the low NK-reactive strains caused a substantial inhibition of NK activity, when mixed with spleen cells from high reactive mice. In contrast, adherent spleen cells from high NK-reactive mice had no inhibitory activity. Characterization studies indicated that the suppressor cells in SJL and A mice were macrophages.

Prostaglandins, particularly of the E series (PGE), have been shown to be potent inhibitors of mouse and human NK activity. It has been suggested that this inhibition is mediated by induction of elevated levels of cyclic AMP. However, no direct evidence for this possibility has been reported. We therefore performed a study with human LGL, to examine the possible role of cyclic AMP in mediating the PGE-induced suppression of NK activity. With LGL, PGE suppressed NK activity by 75% and markedly enhanced cellular cyclic AMP, with a mean increase of 600%. In contrast, in T lymphocytes, PGE caused very small increases in cyclic AMP (only 20%). Phosphodiesterase inhibitors such as isobutylmethylxanthine and theophylline also increased cyclic AMP and suppressed NK activity in LGL. The combined effect of PGE plus these phosphodiesterase inhibitors on cellular cyclic AMP and on NK activity, was considerably greater than the effect of either agent alone. These results indicate that in LGL, increased cellular cyclic AMP mediates the action of PGE on the suppression of NK activity.

Another type of negative regulation was found in studies of short-term incubation of human peripheral blood mononuclear cells. After incubation for 1-2 hours in medium lacking human serum, increased NK activity was seen. This seemed to be due to release from inhibition by human serum factors, since incubation in autologous or allogeneic serum prevented the augmentation and at high concentrations produced inhibition of activity. The serum-mediated effect appeared attributable to the degree of binding of cytophilic monomeric IgG to the cells. The overall pattern of results suggested that this mechanism may play a role in negative regulation of NK activity in vivo.

## III. Specificity of NK Cells

We have begun a detailed biochemical analysis of initial binding steps of purified LGL to NK-susceptible targets. We have found that solubilized material from the membranes of K562 target cells, inserted into lipid vesicles, can efficiently inhibit effector-target cell interactions, but does not inhibit the subsequent cytolytic reaction. Target cell structures, isolated from K562 (a myeloid leukemia cell line) has been demonstrated to be a glycoprotein (sensitive to 65°C, sensitive to trypsin, adherent to Con-A columns) of 100-150,000 MW. The specific activity of the material (based on 50% inhibition of binding) can be increased 25 to 100 fold by lectin column purification and elution with alpha-methylmannoside.

The cytotoxic activity of NK cells against primary tumors remains an important issue. Because of the ability to isolate highly purified populations of LGL and T cells, we have begun to examine NK activity of patients against autologous and allogeneic primary human tumors. Carcinomas of the lung, colon, breast, or neck; and melanoma have been dispersed in collagenase, separated in discontinuous Ficoll gradients, and used as $^{51}Cr$-labeled targets in assays with PBL and

purified subpopulations of cells from allogeneic cancer patients. Adherent cells (AC), LGL and T cells (isolated from low and high density fractions of a discontinuous Percoll gradient) have been isolated from patients' PBL, and tested in an 18 hr $^{51}$Cr release assay. Using PBL, significant cytotoxicity ($p < 0.05$) has been seen against the majority of primary tumors. In addition, LGL from the same donors had significantly higher reactivity against primary tumors. In contrast, no cytotoxicity was mediated by T cell or adherent cell fractions against either primary tumors or tumor cell lines. Furthermore, the addition of T cells (50%) or adherent cells (10%) to LGL-containing (>60%) effector cells, did not cause a significant decrease in the reactivity when tested against primary tumors. These results indicate that the lysis of primary tumors by PBL of normal donors or allogeneic cancer patients is strongly associated with LGL. Thus, LGL may be involved in natural resistance against primary human tumors as well as tumor cell lines. Studies are currently being performed with patient PBL against autologous primary tumors in order to determine the role of LGL (NK) in host resistance against human tumors.

## IV. Role of NK Cells in Resistance Against Tumor Growth

With the ability to depress NK activity in vivo and specifically reconstitute animals with highly enriched populations of LGL, the rat provides a very useful model system for investigating the role of NK cells in the natural resistance to tumors. We have recently shown that it is possible to specifically reconstitute the cytolytic function of animals treated by agents which depress NK activity (X-irradiation or anti-asialo GM1) by the transfer of 3-6 x $10^6$ LGL but not T cells or monocytes. These recipients demonstrated normal levels of cytotoxic activity when their spleen or blood was tested in vitro. To further test the ability of LGLs to function when tranferred in vivo, we have utilized an in vivo tumor cell clearance assay, in which the levels of NK activity are reflected by the rate at which radiolabelled tumor cells are cleared from the lungs. Using this technique we have shown a significant reduction in the clearance of tumor cells from the lungs of animals treated with either X-irradiation or anti-asialo GM1, and the reactivity of these animals could be returned to near normal levels by the in vivo transfer of LGL but not with T cells or monocytes. These results demonstrate that it is possible to specifically reconstitute both the in vitro and in vivo NK activity of animals with depressed reactivity by in vivo transfer of highly enriched LGL. Our results using the MADB106 mammary adenocarcinoma line have shown that IV injected tumor cells will colonize the lungs and grow at an increased rate in rats with depressed NK activity (anti-asialo GM1 treated). Preliminary results with LGL transfer indicates that LGL can significantly reduce the number of lung colonies and the rate of tumor growth in reconstituted animals. These results, although preliminary, are the first direct evidence for a role of LGL (NK cells) in control of metastasis. Studies are now underway using this system to examine the role of NK cells in the development of primary tumors following administration of various carcinogens (NMU, DMN-AOC). Our results, at present, indicate that at least one effect of these agents is to depress NK activity, which is consistent with the hypothesis that succesful carcinogenesis may be dependent on interference with natural host resistance mechanisms as well as on malignant transformation of cells.

The findings that in vivo administration of anti-asialo GM1 could selectively inhibit the cytotoxic activity of mouse NK cells without detectable inhibition of T cell-mediated immunity, also provided a good approach to further study the role of NK cells in the control of metastatic spread and growth of tumors. Treatment of mice with such antiserum made them considerably more susceptible to the development of lung metastases from intravenously inoculated B16 melanoma cells. Similar results to those observed with the antiserum treatment were obtained when B16 cells were inoculated intravenously into beige mice. In both situations, there was a more than 10-fold increase in the number of lung metastases and also the appearance of metastases in the liver, where metastases are normally not detectable. We also observed a dramatic acceleration of the development of postoperative pulmonary metastases from the Lewis lung carcinoma, in mice pretreated with anti-asialo GM1. Thus, these data indicate that NK cells play an important role in the control of metastatic spread and growth of various transplantable tumors.

Studies have also been continued to explore the possible importance of NK cells in immune surveillance. As one model of primary carcinogenesis in mice, we have injected urethane into young mice. Strains of mice susceptible to development of pulmonary tumors after urethane showed an early, profound depression in NK activity after receiving urethane. In contrast, urethane treatment of strains of mice that are resistant to pulmonary carcinogenesis did not show depression of NK activity. The number of detectable tumor nodules in the lungs of mice transplanted with normal syngeneic bone marrow or spleen cells was significantly less than in control urethane-treated mice. Also, young mice of susceptible strains have been found to be more sensitive to the carcinogenic effects of urethane than older mice, and this was paralleled by greater sensitivity of the young mice to the NK-suppressive action of urethane.

As a second model of primary carcinogenesis, C57BL/6 mice were treated with a schedule of multiple, low doses of x-irradiation. Such treatment was found to result in a substantial deficit in NK activity and this depressed NK activity could be restored by transfer of normal bone marrow cells, a procedure which has been shown to interfere with radiation-induced carcinogenesis. As a further indication for the importance of NK depression in carcinogenesis in this model, mice of different ages were irradiated. It was previously demonstrated that susceptibility to carcinogenesis decreases rapidly after one month of age. In parallel, we have found that older mice do not have as long-lasting or profound a depression in NK activity after the treatment. Thus, in both of the primary carcinogenesis models studied to date, it appears that NK cells play an important role in resistance against development of tumors.

The recent results demonstrating the feasibility of transferring resistance to tumor growth by transfer of NK cell-enriched lymphocyte populations has supported the contention that NK cells are an important mechanism of defense against the growth and/or spread of tumors. In the human, large numbers of LGL can be obtained by Percoll gradients and expanded in vitro in IL-2 containing media. With these observations in mind, one can envision the potential therapeutic value of these cells in adoptive immunotherapy. However, very little is known about alterations which occur in this population during in vitro culture, in their circulation and homing patterns, in vivo persistance, or function. Some of our present studies in rats have therefore been concerned with establishing and

the circulation parameters for rat LGL, T cells and monocytes. These results demonstrated that isolated LGL and T cells can be adoptively transferred into normal recipients and that their distribution pattern is consistent with their organ distribution. In addition, we have also attempted to determine whether these cells can in fact, migrate to and enter a tumor site. In preliminary experiments with rats bearing a small subcutaneous MADB106 adenocarcinoma, it has been possible to show localization of up to 3% of LGL at the tumor site, which increased over the first 24 hrs and then appeared to remain stable. Experiments are now underway to determine if this limited infiltration is sufficient to provide objective therapeutic effect or if agents can be used which will alter the migration of LGL into tumors.

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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Natural Cell-Mediated Immunity in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LCC, NCI-FCRF; NCI; Bureau of Biologics; Univ. of Genova, Italy; Pfizer Central Research Labs

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have shown rat natural killer (NK) cells to be large granular lymphocytes (LGL). Analysis of the cell surface phenotype of these cells using monoclonal antibodies has demonstrated a sharing of antigens with both T cells and monocytes. The in vivo administration of these antibodies, X-irradiation, or diethylstilbesterol (DES) caused a marked depression in NK activity. The in vivo and in vitro reactivity of these animals, however, could be returned to near normal levels by the in vivo transfer of LGL but not with T cells or monocytes. Studies investigating the homing patterns of LGL and T cells have demonstrated a pattern of migration consistent with the organ distribution of these cells. A low percentage (1-3%) of LGL migrated to the site of a growing tumor. Another cell in the spleen which is morphologically similar to LGL is the large agranular lymphocyte (LAL). Our recent studies have shown that LAL are 1) found in the spleen and bone marrow, 2) antigenically very similar to LGL and 3) isolate with LGL but do not bind or kill tumor targets. We have also examined the appearance of spontaneous LGL leukemias in F344 rats. Analysis of these tumor lines indicate a great deal of morphological, histochemical, functional and antigenic similarity to LGL.

PROJECT DESCRIPTION

The objectives of this project are: 1) to further study the developmental, morphological and functional characterisitics of the effector cells in natural cell-mediated immunity in rats; 2) to evaluate their role in the mechanisms of in vivo resistance to tumors; 3) to examine the characteristics of spontaneous NK cell tumors in rats; and 4) to investigate in detail the effects of various in vivo manipulations on the levels of natural cytotoxicity.

MAJOR FINDINGS

I. Natural Cell-Mediated Immunity Against Tumor Cells

A. Characteristics of natural killer (NK) cells

It has previously been demonstrated that both human and rat NK cells are large granular lymphocytes (LGL). This observation, and the ability to isolate these cells on discontinous gradients of Percoll, have provided a much needed procedure for analyzing highly enriched populations of NK cells. Using this technique, we have shown that rat LGL's are an antigenically distinct population of lymphocytes which share some characteristics with both mature T cells (W3/13, OX-1, OX-8, ART-1[a]) and macrophages (W3/13, OX-1). LGL also express asialo-GM1, a marker found to be on human, rat and mouse NK cells. We have also examined LGL by histochemistry and have found them to be acid phosphatase and beta-glucuronidase positive; alkaline phosphatase, esterase, peroxidase and lysozyme negative. As with the antigenic profile, this enzyme pattern identifies a distinct population of peripheral blood lymphocytes. We have also begun to examine the ultrastructural morphology of LGL by use of electron microscopy. The results demonstrate a clear difference between LGL and mature T cells. LGL contain an active Golgi apparatus and number of vacuoles and granules, all of which suggest a possible secretory nature for these cells.

B. Mechanisms of in vivo suppression of NK activity

The ability to morphologically identify and separate LGL has provided us with the means to examine the mechanism(s) by which various agents can modulate in vivo levels of NK activity. We have been specifically interested in using three NK-suppressive treatments: 1) X-irradiation; 2) antibodies against LGL; and 3) diethylstilbesterol (DES). Animals given high doses of X-irradiation (900R) demonstrate a precipitous drop in their total leukocyte counts but a significant increase in the % LGL in the blood and spleen. However, even with this increase in % LGL, there was a strong depression in cytotoxicity in these organs. Interestingly, the cytolytic activity from spleen cells could be restored, or even augmented above normal levels, by separation of the cells on Ficoll-Hypaque gradients. Preliminary evidence suggests that there is a separation of LGL from a radiation-induced suppressor cell, probably a polymorphonuclear leukocyte (PMN). These results demonstate that LGL are relatively radioresistent and suggests that the reduction in activity in X-irradiated animals is due, at least in part, to the activation of a suppressor cell.

Animals given antibodies against LGL also have depressed levels of NK activity. The mechanism for this depression seems to depend on the antibody used. Rats given a single injection of rabbit antibody to asialo-GM1 had depressed NK activity by day 1 which persists until day 5-6. The blood from these animals had a significantly lower % LGL which corresponded with the depression in NK activity. Animals given a mouse monoclonal antibody (OX-8) reactive with rat LGL also have depressed cytotoxicity. These animals, however, did not show signs of a significant reduction in % LGL. The results demonstrate that the in vivo administration of antibodies to LGL cell-surface antigens can cause a depression of NK activity; however, the mechanisms by which different antibodies function appear quite different.

DES is another agent found to cause an in vivo reduction in NK activity. Dose response experiments have suggested that low doses of DES (0.3-3.0 mg/rat) will cause a depression in cytotoxicity, whereas, higher dose (10.0-30.0 mg/rat) have no effect on NK activity.

C. In vivo relevance of LGL in the mediation of natural resistanct to tumors

With the ability to depress NK activity in vivo and specifically reconstitute animals with highly enriched populations of LGL, the rat provides a very useful model system for investigating the role of NK cells in the natural resistance to tumors. We have recently shown that it is possible to specifically reconstitute the cytolytic function of animals treated with agents which depress NK activity (X-irradiation, antiasialo-GM1) by the transfer of 3-6 x $10^6$ LGL but not T cells or monocytes. These animals demonstrated near normal levels of cytotoxic activity when their spleen or blood was tested in vitro. To further test the ability of LGLs to function when transferred in vivo, we have utilized an in vivo tumor cell clearance assay in which the levels of NK activity are reflected by the rate at which radiolabelled tumor cells are cleared from the lungs. Using this technique, we have shown a significant reduction in the rate of clearance of tumor cells from the lungs of animals treated with either X-irradiation or antiasialo-GM1. The reactivity of these animals could be returned to near normal levels by the in vivo transfer of LGL but not with T cells or monocytes. These results demonstrated that it is possible to specifically reconstitute both the in vitro and in vivo NK activity of depressed animals by in vivo transfer of highly enriched LGL.

The next step in further investigating the role of LGL in tumor resistance was to examine the growth of tumor cells in NK-depressed animals. Our results using the MADB106 mammary adenocarcinoma line have shown that IV injected tumor cells will colonize the lungs and grow at an increased rate in rats with depressed NK activity (anti-asialo GM1-treated). Preliminary results with LGL transfer, indicate that LGL can significantly reduce the number of lung colonies and the rate of tumor growth in reconstituted animals. These results, although preliminary, are the first direct evidence for a role of LGL (NK cells) in the control of metastasis. Studies are now underway using a similar system to examine the role of NK cells in the development of primary tumors following carcinogen administration (NMU, DMN-AOC). Our results, at present, indicate that at least one effect of these agents is to depress NK activity. This

depression may be an important mechanism by which these agents work. Experiments are now in progress with LGL to specifically reconstitute the NK activity of carcinogen-treated animals, and examine whether NK cells are an important factor in the development of carcinogen induced tumors.

D. Distribution and persistance of lymphocyte and monocyte populations in normal and tumor-bearing animals

The recent results demonstrating the feasibility of transferring resistance to tumor growth by transfer of NK cell-enriched lymphocyte populations has supported the contention that NK cells are an important mechanism of defense against the growth and/or spread of tumors. Previous results have also shown that large numbers of human LGL can be obtained by Percoll gradients and expanded in vitro in IL-2 containing media. With these observations in mind, one can envision the therapeutic value of these cells in adoptive immunotherapy. However, very little is known about alterations which occur in this population during in vitro culture, in their circulation and homing patterns, in vivo persistance, or function. Our present studies in rats have therefore been concerned with establishing and comparing the circulation parameters for rat LGL, T cells and monocytes. In addition, we have also attempted to determine whether these cells can, in fact, migrate to and enter a tumor site. Our results have shown that Indium-111 labelled LGL, when injected IV, arrest in the lungs, the first capilary bed encountered. During the following 24 hrs, the cells migrate predominantly into the blood, liver, and spleen. In comparison, T cells remain in the lineup for a shorter period of time and migrate to a greater extent into the spleen and lymph nodes. This pattern of migration is consistent with that previously reported distribution of LGL, in which we found large numbers of LGL in the lungs, and blood but far in the T cell-rich spleen and lymph nodes.

Similar experiments were conducted in animals carrying a small subcutaneous, MADB106 adenocarcinoma. The results with LGL and T cells in these animals were similar to our previous experiments. In addition, however, 2-4% of the radiolabelled LGL migrated to the tumor site. Experiments are now underway to determine if this limited infiltration is sufficient to provide an objective therapeutic effect or if agents can be used which will alter the migration of LGL into tumors.

We have also initiated in vitro experiments to culture LGL for use in in vivo transfer studies. Our results have demonstrated that cultures from highly enriched LGL fractions can be grown in IL-2 containing media. These cells can be initiated without the addition of exogenous lectin but do grow best in the presence of irradiated feeder cells. These cells maintain an LGL-like morphology and remain highly cytotoxic in vitro. We are now in the process of growing sufficient cells for distribution and adoptive immunotherapy studies.

E. Relationship between LGL and LAL in the spleen

Although we have recently made significant advancements in examining the detailed morphology and function of NK cells, very little is known about the ontogeny of

these cells. Previous studies have clearly shown that the precursors for NK cells are present in the bone marrow of both rats and mice. We have recently shown that congenitally athymic rats have higher NK levels and an increased percentage of LGL in their lymphoid organs. These results are consistent with our recent data demonstrating that splenecomized rats have decreased levels of NK. Together these data suggest that bone marrow stem cells for LGL do not require the thymus for differentiation but instead may to a large extent differentiate in the spleen.

Another cell in the spleen which is morphologically very similar to the LGL is the LAL (large agranular lymphocyte). This cell is distinguished from LGL in Giemsa-stained cytocentrifuge preparations only by their conspicuous absence of azurophilic granules. The nature, source or function of these cells, however, is unknown. Our recent studies have shown that: 1) LAL are found in bone marrow and spleen of euthymic and athymic rats but not other lymphoid organs; 2) LAL are morphologically and antigenically similar to LGL; and 3) LAL separate together with LGL on Percoll density gradients but do not bind or kill NK cell targets. These results demonstrate that LAL are not typical B cells, mature T cells, or macrophages. They are most similar to LGL but lack functional cytolytic activity. The results from these studies have clearly demonstrated the thymic-independent nature of LGL and LAL and have suggested that the process of LGL differentiation in the spleen and/or bone marrow may involve an intermediate step of differentiation through an LAL precursor.

F. Immunological characterization of LGL tumors in rats

It has been very difficult to examine the detailed nature of NK cells since it is virtually impossible to isolate a large number of highly enriched LGL. One means to overcome this difficulty would be to find naturally occurring LGL tumors which maintain their morphological and functional characteristics. These cells would be available in essentially unlimited quantities and could be used for those studies requiring a large number of cells for detailed analysis. Recently, we have demonstrated that there are LGL tumors in a high percentage of aged (greater than 24 months old) F344 rats. These are from animals with spontaneous mononuclear cell leukemia, and it has been possible to transplant most of these tumors. A significant percentage (30-40%) of these cell lines demonstrate very high cytolytic potential. Some of these tumor lines have consistently shown levels of cytotoxicity which approach 200 times the level of activity seen in normal, age-matched controls. Antigenically and morphologically, these cell lines are similar to normal LGL, but considerable variation among tumors has been seen. Analysis of these cell lines by histochemistry has also shown a great deal of similarity to LGL. We have recently begun to examine the cytotoxic specificity of these cell lines and it has become clear that those lines with cytotoxic activity kill NK-susceptible targets but do not kill NK-resistent target cells. Most of the cytotoxic tumor cell lines have avid Fc receptors and can mediate also antibody-dependent cell-mediated cytotoxicity (ADCC). Interestingly, these tumor lines do not appear in splenectomized animals. These data are consistent with our hypothesis that the spleen may provide a major site for LGL development and differentiation. Ongoing studies with these NK (LGL) tumors should provide an excellent source of cells for many studies which were heretofore impossible because of the limited availability of highly purified normal NK cells.

Significance to Biomedical Research and the Program of the Institute

A large amount of data in experimental animal systems now supports the notion that natural cell-mediated immunity may play a significant role in immune surveillance against tumors. The further understanding of this system in experimental animal systems should greatly facilitate the understanding of the significance of natural cell-mediated immunity in man. The recent results regarding the morphology and histochemistry of NK cells have already provided a mechanism for enumerating human NK cells. Our observations regarding the mechanisms of NK suppression should lead to a better understanding of how these agents affect NK activity and how their effects might be avoided in patients undergoing radiation or estrogen therapy. The results obtained regarding the distribution and persistance of passively transferred LGL are an important prerequisite to the development of clinical protocols involving the passive immunotherapy of cancer patients with activated or cultured LGL. The finding that LGL and LAL share many morphological and antigenic characteristics has provided a novel approach towards the ontogeny of NK cells. Alterations within this developmental pathway may be an important factor in determining an individual's ability to resist the development of spontaneous neoplasms. In addition, our finding that LGL tumors can be identified in aged rats provides us with a large source of highly active cells for the isolation and analysis of target receptors, cytoplasmic granules, and lytic machinery. These cell lines will also provide us with enough material to examine mitogen and/or antigen stimulation and lymphokine production by NK cells. These results should greatly facilitate studies to examine the overall function and relevance of NK cells in human tumor systems.

Proposed Course of Project

Extensive studies on natural cell-mediated immunity in rats will be continued. The rat provides an ideal system for the isolation of highly purified LGL and the specific reconstitution of recipient animals with these cells. Specifically, our efforts will be directed towards: 1) a more detailed characterization of the NK cell system; 2) a better understanding of the in vivo role of NK cells in reactivity against transplanted and carcinogen-induced primary tumors; and 3) a further examination of the functional and morphological composition of LGL tumors.

A more detailed study of the NK cell system will involve the further characterization of LGL cell surface antigens, ultrastructural morphology, ontogeny and homing and recirculation patterns. We are presently attempting to produce new monoclonal antibodies against LGL/LAL specific cell surface antigens. In addition a number of existing antibodies to human or mouse NK cells (HNK-1, NK-1, NK-2) are being examined for their ability to specifically identify rat LGL. Electron microscopy studies are underway to examine the formation and composition of the characteristic granules in these lymphocytes. In vivo and in vitro experiments have also recently begun which will examine the precursor frequency of LGL in the bone marrow, blood and spleen. In addition we are planning to examine the effects of various biological response modifiers (IFN, IL-2, IL-3, colony stimulating factor) on the differentiation and maturation

of splenic LGL and LAL. These agents should provide a more detailed understanding of factors regulating the number and activity of rat NK cells. Such experiments, however, might also be quite useful in providing further insight into the lineage of NK cells. Experiments will also continue to examine the in vivo fate of passively transferred LGL, T cell and monocyte populations. These experiments will include studies in additional tumor systems, intraperitoneal transfer of cells into normal and tumor-bearing animals and the transfer of in vitro cultured LGL and T cell populations.

To better understand the in vivo role of NK cells, we will continue to examine the effect of NK-suppression and specific LGL reconstitution on the growth of transplanted mammary tumors, the development of spontaneous and artificially induced metastases and on the production of carcinogen-induced tumors (NMU, DMN-OAC). A particular focus will be on determining whether there are critical times at which LGL seems to play an important role in the development of tumors in these systems. Previous studies have also indicated an in vivo role for NK cells in the mediation of allogeneic bone marrow graft rejection. We will therefore use a similar system of selective depression/reconstitution of NK activity, to examine the ability of highly enriched LGL populations to affect the in vivo or in vitro growth of syngeneic and allogeneic bone marrow cells.

The further examination of LGL tumors will include a number of morphological, biochemical and functional studies. Specifically we will continue to examine these cell lines for enzyme markers, ultrastructural morphology and histochemistry. In all cases we will compare these characteristics with the results of normal leukocyte counterparts (LGL, T cells, monocytes).

We have also begun studies to disrupt these tumor cells and to isolate cytoplasmic granules, membrane target receptors and cytolytic molecules. These biochemical studies require a large amount of material and can only be done with cytolytically active LGL tumors. A further examination of the functional nature of these cells will include experiments to determine if they proliferate in response to mitogens, antigens, or other BRM's and to investigate whether these tumor lines produce significant quantities of lymphokines reported to be the product of normal LGL (IFN, IL-2).

PUBLICATIONS

Reynolds, C.W., Timonen, T., and Herberman, R.B.: Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cells. J. Immunol., 127:282-287, 1981.

Reynolds, C.W., Sharrow, S.O., Ortaldo, J.R., and Herberman, R.B.: Natural killer activity in the rat. II. Analysis of surface antigens on LGL by flow cytometry. J. Immunol., 127:2204-2208, 1981.

Reynolds, C.W., Ortaldo, J.R., Denn, A.C., III, Barlozzari, T., Herberman, R.B., Sharrow, S.O., Ramsey, K.M., Okumura, Ko. and Habu, S.: Cell surface antigenic characteristics of rat large granular lymphocytes. In NK Cells and Other Natural Effector Cells, R.B. Herberman (Ed.), Academic Press, New York, in press.

Reynolds, C.W., and Holden, H.T.: Genetic variation in natural killer (NK) activity in the rat. In NK Cells and Other Natural Efector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Reynolds, C.W., Timonen, T., and Herberman, R.B.: Pleiotropic effects of interferon (IFN) on the augmentation of rat natural killer (NK) cell activity. In NK Cells and Other Natural Effector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Reynolds, C.W., Rees, R., Timonen, T., and Herberman, R.B.: Identification and character of the natural killer (NK) cells in rats. In NK Cells and Other Natural Effector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Reynolds, C.W., Ward, J.M., Denn, A.C., III, and Bere, E.W., Jr.: Identification and characterization of large granular lymphocyte (LGL) leukemias in F344 rats. In NK Cells and Other Natural Effector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Reynolds, C.W., Timonen, T.T., Holden, H.T., Hansen, C.T., and Herberman, R.B.: Natural killer (NK) cell activity in the rat analysis of effector cell morphology and effects of interferon on NK cell function in the athymic (nude) rat. Eur. J. Immunol., in press.

Greiner, D.L., Reynolds, C.W., and Lubaroff, D.M.: Maturation of functional and antigenically distinct lymphocyte subpopulations in the rat. Thymus, 4:77-90, 1982.

Reynolds, C.W., Ward, J.M., Denn, A.C., III, and Bere, E.W., Jr.: Characterization of large granular lymphocyte (LGL) tumors in the rat. In B and T Cell Tumors: Biological and Clinical Aspects. E. Vietta and C.F. Fox (Eds.), Academic Press, New York, in press.

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TITLE OF PROJECT (80 characters or less)

Natural Cell-Mediated Immunity: Characteristics, Regulation and In Vivo Relevance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mouse natural killer (NK) cells and natural cytotoxic (NC) cells have been shown to be closely associated with large granular lymphocytes (LGL), as has been found previously for man and rats. In in vivo studies, natural effector cells reacting with certain solid tumors were found to share some characteristics of both NK and NC cells. Natural effector cells with the characteristics of NK cells, in both mice and humans, were found to frequently be reactive against primary carcinomas, and such reactivity was augmented by interferon (IFN) and by interleukin-2 (IL-2). Detailed studies have been performed on the conditions for in vitro growth and differentiation of mouse and human NK cells, using limiting dilution assays. IL-2 was shown to be an important stimulus for both growth and activity of NK cells, and IFN was shown to be able to both stimulate and inhibit in vitro growth of NK cells, depending on culture conditions. Negative regulation of NK activity has been studied, with emphasis on the nature of suppressor cells and the mechanism of inhibition by prostaglandins. Increased evidence has been obtained for an in vivo role of NK cells, in resistance against metastases and in immune surveillance.

PROJECT DESCRIPTION

OBJECTIVES

The objectives of this project are: 1) to determine the characteristics of natural effector cells in mice and in man; 2) to determine the factors regulating the levels of activity; and 3) to evaluate the role of natural cell-mediated immunity in resistance against tumor growth.

MAJOR FINDINGS

I. Characterization of Natural Effector Cells in Mice

In previous studies in this laboratory, it was clearly shown that cells with a characteristic morphology, termed large granular lymphocytes (LGL), were responsible for human and rat natural killer (NK) activity. This association, coupled with the ability to purify LGL by centrifugation on discontinuous density gradients of Percoll, has provided the basis for a large series of detailed studies on the characteristics of NK cells in these species. In contrast, determination of the possible association of mouse NK cells with LGL has been considerably more difficult. However, during the past year, by modification of the conditions used for Percoll density gradient centrifugation, it has been possible to enrich fairly well for LGL in low density fractions and to obtain high density fractions of lymphocytes that are virtually devoid of LGL. The LGL-enriched subpopulations have been shown to be enriched for NK activity and the LGL-depleted subpopulations have had no NK activity. A continuing problem, in studies with mouse spleen cells, has been the rather high frequency in the LGL-enriched fractions of other cells, morphologically very similar to LGL but lacking the characteristic cytoplasmic granules and therefore termed, large agranular lymphocytes (LAL). Studies are in progress to determine the relationship between LGL and LAL and the possible cytotoxic reactivity of LAL. Studies are also in progress to determine the cell surface phenotype of the LGL-LAL fractions, particularly after depletion of any residual typical T cells, by treatment with anti-T cell antibodies plus complement.

Natural cytotoxic (NC) cells have been described by both Stutman and Burton as being somewhat different mouse natural effector cells, that are responsible for natural cytotoxic reactivity against certain solid tumor target cells. However, the actual relationship between NK and NC cells in mice has not been well defined. We have therefore been performing detailed studies on this issue, using target cells which have been shown to be highly sensitive to lysis by NC cells but resistant to lysis by NK cells. Such target cells have been tested for susceptibility to lysis by LGL-LAL enriched and depleted subpopulations. We have found that the LGL-LAL fractions possess NC as well as NK activity, and the fractions depleted of LGL-LAL are devoid of NC as well as NK activity. Thus, it appears that both NK and NC activities are included within the small subpopulation of LGL and/or LAL. Studies have also been performed *in vivo* to determine the characteristics of the natural effector cells reacting against such solid tumor targets. Rather unexpectedly, we have found that several of the characteristics of the effector cells involved in rapid *in vivo* elimination of intravenously inoculated radiolabelled-tumor cells are

considerably more compatible with those of NK cells than of NC cells. The rapid clearance was strongly impaired by pretreatment of mice with cyclophosphamide, whereas NC activity in vitro has been found to be quite resistant to this drug. Treatment of mice with antibodies to the glycolipid, asialo GM1, a marker for NK cells but not for NC cells, was shown to strongly interfere with the rapid clearance in vivo. Further, the levels of in vivo reactivity among various strains of mice followed closely the pattern of strain distribution for NK activity but did not fit that observed for NC cells. However, beige mice, which have a considerable deficit in NK activity, had unimpaired in vivo reactivity against most of these solid tumor target cells. Also, old mice, greater than 1 year of age, which have very low levels of NK activity but have similar NC activity as young mice, reacted strongly in the in vivo tumor clearance assay. Thus, it appears that the natural effector cells for in vivo reactivity against these solid tumor cells, at least in the lungs, share some characteristics with both NK and NC cells. Further studies will be needed to determine the basis for the considerable discrepancies between the in vitro and in vivo findings.

We have continued our studies on the susceptibility of primary tumors in mice and in humans to lysis by natural effector cells. Last year, we observed that the majority of freshly harvested, spontaneous mammary tumors of C3H mice had significant, although usually quite low, susceptibility to lysis by normal syngeneic as well as allogeneic spleen cells, in an extended, 18-hr $^{51}Cr$ release cytotoxicity assay. We have now performed detailed studies on the nature of these natural effector cells. Reactivity was shown to be considerably enriched in Percoll fractions containing LGL and LAL and was depleted from fractions devoid of such cells. The natural effectors against these primary tumors were also shown to have cell surface markers very similar to those of mouse NK cells, lacking high density of Thy 1, and expressed Ly5 and asialo GM1. Thus, NK cells rather than NC cells seem to be the effector cells. Studies have been continued to determine the factors regulating the reactivity of the NK cells against these targets, and to determine the basis for the degree of susceptibility of the primary tumor cells to lysis. As with NK cells that are reactive against cell lines, reactivity against the primary tumors could be substantially increased by pretreatment of effector cells with either IFN or IL-2. The primary tumor cells, after growth in vitro for a period of 2-3 weeks, became considerably more susceptible to lysis. In contrast, transplantation of the primary tumors in mice resulted in a decrease or elimination of susceptibility to lysis. In order to study in more detail the nature of the alterations in susceptibility upon in vitro vs. in vivo growth, we have performed studies with a cloned cell line of a mouse mammary tumor. This cloned line showed similar variations in susceptibility upon in vivo growth, changing from its high susceptibility as a culture cell line to high resistance in vivo. Thus, it appears that the variation in susceptibility is due to modulation of some characteristics of the tumor cells, probably at the cell surface, rather than due to selection of different subpopulations of cells under the varying growth conditions.

Similar studies have been initiated to characterize the natural effector cells reactive against the primary, freshly harvested human carcinomas. Reactivity against the majority of tumors has been observed with lymphoid cells from normal donors and also from cancer patients. In both normal donors and in allogeneic

cancer patients, the effector reactivity has been closely associated with LGL and fractions enriched for typical T cells have lacked activity. We have been particularly interested in studying the nature of the reactivity of autologous peripheral blood mononuclear cells of cancer patients against their primary tumor cells. With several patients, it has been possible to show that reactivity against autologous tumors was present and that LGL were the predominant or exclusive effector cells. However, with a few patients, small T cells also appeared to have cytotoxic reactivity against the autologous tumors. Thus, for autologous cytotoxic reactivity, both NK cells and immune cytotoxic T cells might play a role.

## II. Regulation of Development and Reactivity of Natural Killer Cells

### A. Activating factors

From extensive studies in this and many other laboratories, it has become clear that IFN is a major positive signal for the reactivity of NK cells. It appears, however, that other activating factors may exist. During the past year, investigators in the laboratory of Christopher Henney reported that IL-2 could substantially augment NK activity in mice. We were therefore very interested in examining the possible role of IL-2 in regulating the reactivity of human NK cells. We have found that partially purified preparations of IL-2, when present in the cytotoxicity assay, or after pretreatment of the effector cells, could substantially augment NK activity, to levels at least as high as that induced by IFN. The possibility was then considered that IL-2 might be an important factor for the spontaneous reactivity of human NK cells. To explore this possibility, we preincubated human LGL for various periods of time with monoclonal antibodies against human IL-2. Preincubation for 1-4 hours caused a significant decrease in spontaneous NK activity, and after 20 hours of preincubation with these antibodies, virtual elimination of reactivity was observed. Reactivity could be partially restored by further culture of the cells in the absence of the antibody, and could be rapidly and fully restored by treatment of the effector cells with IL-2. Thus, it appears that endogenous production of IL-2 within an LGL population may be responsible for the spontaneous activation of human NK cells. Studies are in progress to determine whether the release of IL-2 causes a subsequent production of IFN, which then is the more immediate signal for activation of the effector cells.

### B. Negative regulation of NK activity

We have continued our studies to determine the mechanisms involved in the spontaneous or treatment-induced depression of NK activity. Infant mice of all strains and young mice of certain strains have been shown to have very low or undetectable levels of NK activity. We have found that natural suppressor cells for NK activity account, at least in large measure, for the low NK activity in such animals. Addition of spleen cells from infant mice to those of normal high NK-reactive mice resulted in a substantial inhibition of activity. This inhibitory activity was associated with both adherent and nonadherent cells. In studies of strains of mice with low NK activity, particularly SJL and A strain mice, we have been able to associate low reactivity with naturally occurring suppressor cells, capable of inhibiting the

effector phase of NK activity. Adherent pleen cells from the low NK-reactive strains caused a substantial inhibition of NK activity, when mixed with spleen cells from high NK-reactive mice. In contrast, adherent spleen cells from high NK-reactive mice had no inhibitory activity. Characterization studies indicated that the suppressor cells in SJL and A mice were macrophages, since adherence procedures substantially enriched for inhibitory activity, whereas removal of phagocytic cells by treatment with iron and magnet removed most of the inhibitory activity. Further, a possible role for suppressor T cells was virtually ruled out by the resistance of suppressor cells to treatment with monoclonal anti-Thy 1.2 plus complement. The inhibition of NK activity by SJL macrophages was found to be mediated by production or release of soluble factors. The nature of the soluble factors is still unknown and experiments are in progress to characterize these factors. Since NK activity has a characteristic tissue distribution, we were interested in determining whether macrophages could also be responsible in part for the low levels of NK activity exhibited by peritoneal exudate cells (PEC). Adherent cells with the characteristics of macrophages capable of suppressing NK activity were found in the peritoneal cavity, but not in the spleen of high NK-reactive mice as well as low NK-reactive mice. The adherent PEC of athymic nude mice also had suppressor activity, indicating that the thymus may not play a role in the generation of naturally occurring peritoneal suppressor cells. Suppressor activity could be modulated by either in vivo or in vitro treatment with interferon or interferon inducers. These agents led to a substantial reduction in suppressor activity by the peritoneal macrophages.

The immunoadjuvant, Corynebacterium parvum (Cp) has been studied as a model agent for the induction of suppression of NK activity. It was previously shown that administration of Cp to mice produced a profound depression in splenic NK activity. The spleen cells from mice treated 10 days earlier with Cp were shown to be able to inhibit NK activity and this inhibitory activity was associated with adherent cells and was not affected by treatment with anti-Thy 1.2 plus complement. In contrast, removal of phagocytic cells by treatment with iron and magnet removed most activity, suggesting that the suppressor cells were macrophages. It was therefore of interest to determine whether the appearance of such suppressor cells in the spleen of treated mice could account for the low NK activity in such animals. If so, one would predict that removal of the suppressor cells by an adherence procedure would reverse the depressed NK activity. However, after such treatment the levels of cytotoxic reactivity remained quite low, suggesting that the presence of adherent suppressor cells is not the only mechanism responsible for Cp-induced depression of NK activity. It was possible to show that nonadherent spleen cells from Cp-treated mice were also capable of inhibiting the effector phase of NK activity. These nonadherent suppressor cells were resistant to treatment with anti-Thy 1.2 or anti-asialo GM1 antisera plus complement, indicating they were neither mature T cells nor mature, inactive NK cells. Further support for the conclusion that the suppressor cells were not mature NK cells was obtained by the finding of nonadherent suppressor cells in the spleen of Cp-treated beige mice, which are deficient in NK activity. In contrast to the depression induced by Cp in euthymic mice, the depressed levels of NK activity in the spleen of Cp-treated nude mice were not associated with detectable adherent suppressor cells. However, these mice did have some evidence of nonadherent suppressor

cells in their spleens. As with the suppressor cells studied in normal mice, it was possible to show that suppression could be mediated by release of soluble factors from these cells. The nature of these inhibitory factors remains to be determined, but no evidence for a role of prostaglandins could be obtained, with the addition of endomethacin not reversing the inhibition.

Prostaglandins, particularly of the E series (PGE), have been shown to be potent inhibitors of mouse and human NK activity. It has been suggested that this inhibition is mediated by induction of elevated levels of cyclic AMP. However, no direct evidence for this possibility has been reported. We therefore performed a study with human LGL, to examine the possible role of cyclic AMP in mediating the PGE-induced suppression of NK activity. With LGL, PGE suppressed NK activity by 75% and markedly enhanced cellular cyclic AMP, with a mean increase of 600%. In contrast, in T lymphocytes, PGE caused very small increases in cyclic AMP (only 20%). Phosphodiesterase inhibitors such as isobutyl-methylxanthine and theophylline also increased cyclic AMP and suppressed NK activity in LGL. The combined effect of PGE plus these phosphodiesterase inhibitors, on cellular cyclic AMP and on NK activity, was considerably greater than the effect of either agent alone. Exogenous derivatives of cyclic AMP also suppressed NK activity. These results indicate that in LGL, increased cellular cyclic AMP mediates the suppressive action of PGE on NK activity.

C. Regulation of the in vitro growth and differentiation of NK cells

Recent studies have demonstrated that IFN can also influence the growth and differentiation of NK cells from their progenitors. It has been possible to culture normal BALB/c spleen cells in the presence of IL-2 and to determine the frequency of proliferative and cytotoxic progenitors by limiting dilution assays. Approximately 1 in 1000 spleen cells were found to proliferate in the presence of lectin-free IL-2 and if the spleen cells were pretreated with IFN, the proliferative frequency was substantially increased. Approximately 1 in 20 proliferating cells were found to have NK activity and when IFN-pretreated spleen cells were tested, the frequency of cytotoxic progenitors was appreciably increased. In contrast to these results, when cultures of normal BALB/c spleen cells were cultured in the presence of IFN, an appreciable decrease in the frequency of proliferative and cytotoxic cells was seen. Thus, depending on the nature of the exposure of the cells to IFN, either augmentation or inhibition of growth and differentiation of NK cells from their progenitors could be seen. Some insight into these conflicting results was obtained when we studied cultures of spleen cells from nude, athymic mice. With these spleen cells, either pretreatment with IFN or the presence of IFN in the cultures resulted in appreciable augmentation of proliferation and cytotoxicity. This suggested that inhibition of growth and differentiation by IFN in the cultures was dependent on the presence of T cells. We were able to directly examine this hypothesis by varying the feeder cells used for the culture of nude spleen cells. Ordinarily, irradiated feeder cells of the same type as the responder cells were used. However, when nude spleen cells were cultured in the presence of irradiated spleen cells from euthymic BALB/c mice, a considerable increase in the frequency of proliferative and cytotoxic cells was seen. When IFN was added to such cultures, this augmentation of proliferation was entirely

reversed. Thus, it appears that IFN can affect the growth and differentiation of NK cells in two, opposite, ways. First, it can directly interact with NK cell progenitors, to promote their growth and differentiation. In addition, it can stimulate T cells to act as inhibitors of the growth and differentiation of NK cells. Overall, it has become clear that the effects of IFN on NK cells are quite complex, with the possibility of affecting NK activity at several different levels or phases or differentiation or interaction with target cells. Such complex interactions may have considerable potential impact on the strategies and results related to in vivo administration of IFN.
T cells have also been found to have an important positive effect on the growth and differentiation of mouse NK cells. In contrast to the high NK activity which has been associated with nude mice, cultures of nude spleen cells in the presence of IL-2 and syngeneic feeder cells showed a quite low frequency of proliferative and cytotoxic progenitors. This frequency of progenitors in nude spleens could be markedly increased by culturing the spleen cells in the presence of irradiated feeder cells from spleens or thymusus of normal mice. Further, elimination of mature T cells by pretreatment with anti-Thy 1.2 plus complement eliminated this augmentation in the frequency of nude progenitors. Thus, it appears that the in vitro growth of NK progenitors in the presence of IL-2 depends considerably on the presence of some accessory T cells. Further studies are in progress to determine the nature of these accessory T cells and the mechanism for their augmentation of proliferation.

Similar limiting dilution assays have been performed with highly purified populations of human LGL. Human LGL were found to have a frequency of proliferation of approximately 1 in 200 cells, when lectin-free IL-2 was used. Addition of small amounts of PHA (0.1 μg/ml) was sufficient to increase the frequency of proliferating LGL to 1 in 70 cells. A high proportion of the proliferating LGL had NK activity. As in the studies with mouse spleen cells, pretreatment of human LGL with either alpha or beta IFN increased the frequency of cells proliferating in the presence of IL-2. The increased proliferative frequency was paralleled by increased frequency of NK activity. In contrast, again as in the mouse, growth stimulation by IFN was not seen when it was present throughout the culture. Rather, proliferative and NK activities were reduced in a dose-dependent manner by both types of IFN. This inhibitory activity of IFN was detectable only when T cells were present in the cultures. Thus, with LGL as responder cells, and irradiated monocytes as feeders, no IFN-induced growth suppression was observed. Separation of T cells into OKT4 and OKT8 positive populations suggested that both cell types were essential for maximum suppression. Thus, as in the mouse, IFN can induce T cell-dependent suppression of IL-2 dependent proliferation of NK cells.

## III. In Vivo Relevance of NK Cells

A major emphasis has continued to be placed on the evaluation of the possible role of NK cells in in vivo resistance to tumor growth. These studies have focused on the role of NK cells in resistance against metastatic spread of tumors, and in resistance to carcinogenesis by urethane or x-irradiation.

A. Role of NK cells in resistance against metastases

From our previous studies and those of other laboratories, it has been suggested that NK cells may participate in the control of metastatic spread by the elimination of tumor cells entering the bloodstream. This possibility was supported by studies indicating that NK cells are primarily responsible for the initial in vivo elimination of tumor cells, since suppression of activation of NK activity was paralleled by an increase or decrease in the number of surviving tumor cells in the lungs after intravenous inoculation of radiolabelled tumor cells. The findings of Kasai, Okumura and Habu that in vivo administration of anti-asialo GM1 could selectively inhibit the cytotoxic activity of NK cells without detectable inhibition of T cell-mediated immunity provided a good approach to further study the role of NK cells in the control of metastatic spread and growth of tumors. Treatment of mice with such antiserum made them considerably more susceptible to the development of lung metastases from intravenously inoculated B16 melanoma cells. Similar results to those observed with the antiserum treatment were obtained when B16 cells were inoculated intravenously into beige mice. In both situations, there was a more than 10-fold increase in the number of lung metastases and also the appearance of metastases in the liver, where metastases are normally not detectable. Since these studies were performed with an artificial model of intravenous inoculation, we also studied the development of spontaneous metastases in mice with suppressed NK cell function. In these studies, B16 tumor cells were inoculated subcutaneously into the footpads and after local tumor growth to a moderate size, the tumors were excised and the mice followed for development of spontaneous metastases. The number of metastases in mice inoculated with antiserum was significantly higher than in mice treated with normal serum, and beige mice also had more metastases in the lungs. We also observed a dramatic accelration of the development of postoperative pulmonary metastases from the Lewis lung carcinoma, in mice pretreated with anti-asialo GM1. Thus, these data indicate that NK cells play an important role in the control and metastatic spread and growth of various transplantable tumors.

Studies have also been continued to explore the possible importance of NK cells in immune surveillance. As one model of primary carcinogenesis in mice, we have injected urethane into young mice. We previously demonstrated that strains of mice susceptible to development of pulmonary tumors after urethane showed an early, profound depression in NK activity after receiving urethane. In contrast, urethane treatment of strains of mice that are resistant to pulmonary carcinogenesis did not show depression of NK activity. To obtain further evidence for the importance of NK activity in resistance to development of the lung tumors, we studied the influence of transplanted normal lymphoid cells in susceptible mice treated with urethane. The number of detectable tumor nodules in the lungs of mice transplanted with normal syngeneic bone marrow or spleen cells was significantly less than in control urethane-treated mice. This inhibition of urethane carcinogenesis was more profound in mice transplanted with bone marrow cells than in mice inoculated with normal spleen cells. It was of note that spleen cells of donors pretreated with urethane lost their ability to transfer the anti-carcinogenic effect. Also, young mice of susceptible strains have been found to be more sensitive to the carcinogenic effects of urethane than older mice, and this was paralleled by greater sensitivity of the young mice to the NK-suppressive action of urethane.

As a second model of primary carcinogenesis, C57BL/6 mice were treated with a schedule of multiple, low doses of x-irradiation, which is known to be highly effective in inducing thymic lymphomas in this strain. Such treatment was found to result in a substantial deficit in NK activity and this depressed NK activity could be restored by transfer of normal bone marrow cells, a procedure which has been shown to interfere with radiation-induced carcinogenesis. Transfer of bone marrow cells from normal mice has also decreased the subsequent development of lymphomas. As a further indication for the importance of NK depression in carcinogenesis in this model, mice of different ages were irradiated. It was previously demonstrated that susceptibility to carcinogenesis decreases rapidly after one month of age. In parallel, we have found that older mice do not have as long-lasting or profound a depression in NK activity after the treatment,. Thus, in both of the primary carcinogenesis models studied to date, it appears that NK cells play an important role in resistance against development of tumors.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

Natural cell-mediated immunity may play an important role in immune surveillance against tumors. Understanding of this phenomenon and determination of its in vivo role in mice should be very helpful for the understanding of the significance of human natural cell-mediated immunity. The recent findings of a morphologic counterpart to the functional activity of mouse as well as rat and human NK cells should greatly facilitate studies on the frequency of these cells under various situations, their in vivo circulation patterns, their differentiation, their mechanisms of action, and their relationship to other cell types.

The new insights into the factors affecting the regulation of the activity and differentiation of NK cells should be particularly helpful in the development of rational protocols for optimal and persistent augmentation of NK activity, which may result in more effective therapy of cancer patients.

## PUBLICATIONS

Herberman, R.B.: Natural killer (NK) cells and their possible roles in resistance against disease. Clin. Immunol. Rev., 1:1-65, 1981.

Gorelik, E., Kedar, E., Sredni, B., and Herberman, R.: In vivo antitumor effects of local adoptive transfer of mouse and human cultured lymphoid cells. Int. J. Cancer, 28:157-164, 1981.

Korec, S., Herberman, R.B., Cannon, G.B., Reid, J., and Braatz, J.A.: Cytostasis of tumor cell lines by granulocytes from cancer patients and normal human donors. Int. J. Cancer, 28:119-124, 1981.

Herberman, R.B.: Possible roles of natural killer (NK) cells. In Immunobiology of transplantation, Cancer and Pregnancy. P.K. Ray (Ed.), Pergamon Press, Elmsford, NY, in press.

Herberman, R.B.: Immunologic defenses against cancer. In The Pathophysiology of Human Immunologic Disorders. J.J. Twomey (Ed.), in press.

Herberman, R.B.: Effector cells and mechanisms in cell-mediated immunity to tumors. Chemioterapia Oncologica, 1:25-30, 1981.

Kedar, E., Herberman, R.B., Gorelik, E., Sredni, B., Bonnard, G.D., and Navarro, N.: Antitumor reactivity in vitro and in vivo of mouse and human lymphoid cells cultured with T cell growth factor. In The Potential Role of T Cell Subpopulations in Cancer Therapy. A. Fefer (Ed), Raven Press, New York, in press.

Cannon, G.B., Reid, J., Herberman, R.B., Connor, R., Dean, J.H., Jerrells, T., and Perlin E.: Rapid effects of BCG on T cell function in cancer patients. Cancer Immunol. Immunother., 11:265-276.

Riccardi, C., Santoni, A., Barlozzari, T., Cesarini, C., and Herberman, R.B.: Suppression of natural killer (NK) activity by splenic adherent cells of low NK-reactive mice. Int. J. Cancer, 28:811-818, 1981.

Riccardi, C., Santoni, A., Barlozzari, T., Cesarini, C., and Herberman, R.B.: Suppression of natural killer (NK) activity by splenic adherent cells of low NK-reactive mice. Int. J. Cancer, 28:811-818, 1981.

Herberman, R.B.: Possible effects of central nervous system on natural killer (NK) cell activity. In Biological Mediators of Behavior and Disease: Neoplasia. S. Levy (Ed.), Elsevier/North-Holland, New York, pp. 235-248,1982.

Herberman, R.B.: Immunoregulation and natural killer cells. In Molecular Immunology, in press.

Herberman, R.B.: Lymphoid cells in immune surveillance against malignant transformation. In The Role of Lymphoid Cells in Host Defense Mechanisms. Advances in Host Defense Mechanisms, Vol. 2. A.S. Fauci and J.I. Gallin (Eds.), Raven Press, New York, in press.

Liotta, L.A., Goldfarb, R.H., Brundage, R., Siegal, G.P., Terranova, V., and Garbisa, S.: Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane. Cancer Res., 41:4629-4636, 1981.

Sulica, A., Gherman, M., Galatiuc, C., Manciulea, M., and Herberman, R.B.: Inhibition of human natural killer cell activity by cytophilic immunoglobulin $G^1$. J. Immunol., 128:1031-1036, 1982.

Herberman, R.B.: NK cells and natural defenses against cancer and microbial diseases. In Proceedings of the First Latin American Symposium on Clinical Immunology. N. Bianco and G. Torrigiani (Eds.), in press.

Herberman, R.B.: Immunodiagnosis of human cancer. In Proceedings of the First Latin American Symposium on Clinical Immunology. N. Bianco and G. Torrigiani (Eds.), in press.

Herberman, R.B.: Current status and prospects for immunotherapy of cancer. In Proceedings of the First Latin American Symposium on Clinical Immunology. N. Bianco and G. Torrigiani (Eds.), in press.

Kedar, E., Ikejiri, B.L., Gorelik, E., and Herberman, R.B.: Natural cell-mediated cytotoxicity in vitro and inhibition of tumor growth in vivo by murine lymphoid cells cultured with T cell growth factor (TCGF). Clin. Immunol. Immunother., in press.

Herberman, R.B.: Uses and limitations of tumor markers. In Oncodevelopmental Markers: Biological, Diagnostic and Monitoring Aspects. W.H. Fishman (Ed.), Academic Press, New York, in press.

Riccardi, C., Vose, B.M., and Herberman, R.B.: Regulation by interferon and T cells of IL-2 dependent growth of NK progenitor cells: A limiting dilution analysis. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Santoni, A., Riccardi, C., Barlozzari, T., and Herberman, R.B.: Natural suppressor cells for murine NK activity. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Santoni, A., Riccardi, C., Barlozzari, T., and Herberman, R.B.: C. Parvum-induced suppresor cells for mouse NK activity. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Riccardi, C., Allavena, P., Ortaldo, J.R., and Herberman, R.B.: Cloned lines of mouse natural killer cells. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Sulica, A., Gherman, M., Manciulea, M., Galatiuc, C., and Herberman, R.B.: Negative Regulation of human NK activity by monomeric IgG. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman, (Ed.), Academic Press, New York, in press.

Djeu, J.Y., Timonen, T., and Herberman, R.B.: Production on interferon by human natural killer cells in response to mitogens, viruses and bacteria. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman, (Ed.), Academic Press, New York, in press.

Riccardi, C., Barlozzari, T., Santoni, A., Cesarini, C., and Herberman, R.B.: Regulation of in vivo reactivity of natural killer (NK) cells. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman, (Ed.), Academic Press, New York, in press.

Gorelik, E., and Herberman, R.B.: Role of natural-cell-mediated immunity in urethan-induced lung carcinogenesis. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman, (Ed.), Academic Press, New York, in press.

Gorelik, E. and Herberman, R.B.: Depression of natural antitumor resistance of C57BL/6 mice by leukemogenic doses of irradiation and its restoration by transfer of bone marros or spleen cells from normal, but not beige, syngeneic mice. J. Natl. Cancer Inst., in press.

Serrate, S.A. and Herberman, R.B.: Natural cell-mediated sytotoxicity against spontaneous mouse mammary tumors. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Gorelik, E., Rosen, B., and Herberman, R.B.: Depression of NK reactivity in mice by leukemogenic doses of irradiation. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Kedar, E. and Herberman, R.B.: Induction of NK-like anti-tumor reactivity in vitro and in vivo by IL-2. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Grossman, Z. and Herberman, R.B.: Hypothesis on the development of natural killer cells and their relationship to T cells. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Goldfarb, R.H. and Herberman, R.B.: Augmentation of natural killer activity by retinoic acid. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Goldfarb, R.H., Herberman, R.B. and Sugimura, T.: Inhibition of natural killer cell cytotoxic reactivity by tumor promoters and cholera toxin. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Goldfarb, R.H., Timonen, T.T. and Herberman, R.B.: The role of neutral serine proteases in the mechanism of tumor cell lysis by human natural killer cells. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Kedar, E., Ikejiri, B.L., Srendi, B., Bonavida, B. and Herberman, R.B.: Propagation of mouse cytotoxic clones with characteristics of natural killer (NK) cells. Cell. Immunol., in press.

Herberman, R.B.: Natural killer cells. Hospital Practice, 17(4):93-103, 1982.

Fruehauf, J.P, Bonnard, G.D. and Herberman, R.B.: The effect of lentinan on production of interleukin-1 by human monocytes. Immunopharmacology, in press.

Herberman, R.B.: Natural killer (NK) cells and their possible relevance to transplantation biology. Transplantation, in press.

Herberman, R.B., Ortaldo, J.R., Riccardi, C., Timonen, T., Schmidt, A., Maluish, A. and Djeu, J.: Interferon and NK cells. In "UCLA Symposium on Molecular and Cellular Biology, Vol. XXV", in press.

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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Natural Cell-Mediated Immunity in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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|---|---|---|---|---|
| PI: | J.R. Ortaldo | Biologist | BRTB | NCI |
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| | D. Sabolovic | Guest Worker | BRTB | NCI |

COOPERATING UNITS (if any) NCI-FCRF; Naval Medical Ctr.; Uniformed Services Univ. Health Sci.; IMI, NIDR; MET, NCI; LCI, NIAID; I, NCI; Bureau of Biologics; Roche Inst. of Molecular Biology, Nutley, NJ.

LAB/BRANCH
Biological Research and Therapy Branch

SECTION
Natural Immunity Section

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SUMMARY OF WORK (200 words or less - underline keywords)

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 large variety of NK-susceptible target cells. Interferon caused augmentation of NK and K cell activities of LGL and only LGL demonstrated either spontaneous or interferon-activated NK activity. The mechanism of augmentation by IFN appeared to be multiple: 1) increase in lytic efficiency, 2) increase in lytic binders, 3) increase in number of binders, and 4) increased recycling of effector cells. Natural, recombinant and hybrid recombinant alpha interferon molecules have all been shown to augment NK activity but vary widely in their potency relative to antiviral activity. Activation of one of the interferon metabolic pathways with macromolecular substrates (2'-5')pppApApA resulted in increased NK activity. Cultures of highly purified LGL in the presence of T cell growth factor demonstrated typical morphology and cytotoxic patterns of fresh NK cells. Subpopulations, cultures and clones of LGL (NK cells) are being studied against a variety of NK targets.

PROJECT DESCRIPTION

The objectives of this project are:

1. Studies of natural cell-mediated immunity to tumors in man and analysis of the phenotypic, biochemical and functional characteristics of the effector cells;

2. Studies of the factors regulating the development and activation of these cells;

3. Analysis of the role of natural immunity in the immune response and the interaction of natural effector cells with other components of the immune system;

4. Evaluation of patients with cancer for the correlation between immune parameters and clinical course of disease;

5. Investigations on the effects of biological response modifiers on natural cell-mediated immunity; and

6. Studies on the role of natural immunity in resistance against tumor growth and in other diseases.

## 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), that possess a distinct morphology with Giemsa staining, containing azurophilic granules, with pale, blue-staining cytoplasm. A considerable portion of these LGL form lytic conjugates with NK-susceptible targets. These cells have been isolated from peripheral blood lymphocytes using discontinuous Percoll density gradients and subsequent further purification by rosette formation with sheep erythrocytes at 29°C, to minimize contamination with small, typical T lymphocytes. Using these procedures, we have been able to reproducibly obtain fractions containing >90% LGL by morphological analysis and containing <1% classical T cells as determined by morphology and monoantibody analysis. Virtually all of the LGL have Fc receptors for IgG and they possess most, if not all, of the NK cell activity against NK-susceptible targets. We have extended our previous results by examining in detail a wide variety of NK-susceptible targets, including both lymphoid and anchorage-dependent lines. A high degree of correlation existed between the presence of LGL and NK cytolysis of this large panel of targets. Fractions which were devoid of LGL were also devoid of NK activity. Also, fractions which were devoid of LGL could not be induced to become cytotoxic by the addition of activating agents such as interferon or interferon-inducers. Detailed analysis of treatment of LGL with interferon, which causes augmentation of NK activity, has indicated that interferon has multiple effects on NK cells, depending on the target cell tested. The effects include 1) the increase in the rate of the reaction, 2) an increase in the recycling of NK cells, 3) an increase in the number of lytic binders present in the population, 4) an increase in the number

of binders against certain NK targets (especially seen with anchorage-dependent target cells). By using a single cell agarose cytotoxicity assay, virtually all of the above mechanisms have been shown to be operative. Target cell selection seems to dictate which mechanism predominates. However, a small proportion of the LGL, at least 10-15%, did not appear to be cytolytic cells and could not be induced with interferon to become cytolytic. It remains to be elucidated whether these cells possess other immunological functions or if they are either pre-NK cells or post-NK cells. We have also demonstrated LGL in human spleen cells and in most mammalian species including man, nonhuman primates, dogs, cats, sheep, rodents, cattle and sheep. The functional association of LGL morphology with NK cell function has been difficult in several species due to the lack of proper targets. However, this association has been seen with human, nonhuman primates, rat and mouse effector cells.

Because of the ability to obtain highly purified LGL fractions and their strong association with human NK cell activity, we were able to perform detailed phenotypic studies with these effector cells and compare them to the phenotype of typical small, mature T lymphocytes. This has been performed using a series of monoclonal antibodies which have been recently become available. This analysis was performed using fluorescence activated cell sorter which gives both the frequency of positive cells as well as the relative density of the antigen on the surface of the cells. A considerable proportion of LGL reacted with antibodies OKM1 (60-80%) and anti-Ia (15-30%). 25% reacted with OKT8, which is similar to its expression on T cells. In contrast, however, very few LGL reacted with OKT3 or OKT4 antibodies, which react with a high proportion of classical T lymphocytes. OKT11A and 9.6 antibodies, which react with the sheep erythrocyte receptor, also reacted with approximately 50% of the LGL. OKT10 (which as been found to react mainly on pro- or early thymocytes) reacted with a high proportion of LGL, whereas none of the other peripheral blood lymphoid cell populations were positive. By depletion of the cells on antibody monolayers against the mouse monoclonal antibodies, we were able to determine that most, if not all, of the active NK cells were OKT10 positive and the majority were also OKM1 positive. A low proportion of the functional NK cells possessed the OKT8 or Ia antigens. Studies in progress are involved with the examination of LGL (NK cell) phenotypic subpopulations with regard to 1) their proliferative frequency in vitro culture in the presence of IL-2 (T cell growth factor); 2) the frequency of precursors of cells with cytotoxic reactivity against a panel of NK-susceptible targets (lymphoid, adherent carcinomas and primary allogeneic tumor cells; and 3) their clonality.

The ability to isolate and identify effector cells responsible for NK activity has led us to examine the cytotoxic mechanisms involved in cytolysis. T cell cytotoxicity has been demonstrated to involve three major steps: 1)target cell binding, 2) programming for lysis and 3) cytolysis. Because of the ability to demonstrate and examine LGL target cell binding, we were able to examine a variety of agents and determine whether they affect either binding or cytolysis. We first examined a variety of enzymes which were shown previously to have some effects on NK activity. Both papain and trypsin strongly diminished both cytotoxicity and the conjugate-forming ability of LGL. Conversely, lipase, which was previously demonstrated not to diminish the NK activity, showed no significant decrease in the ability of the cells to form

conjugates or kill NK-susceptible targets. The analysis of a variety of metabolic agents, which included cholera toxin, prostaglandins, phorbol myristate acetate (PMA) and Staph protein A, demonstrated that while cholera toxin and prostaglandins diminished the cytotoxicity, these had no major effect on the binding of the effector-target cell. Conversely, PMA significantly depressed both the binding and the cytotoxicity of the effector-target interaction. Further analysis of cyclic nucleotides, in addition to a chelating agent (EDTA), demonstrated that cyclic AMP sharply diminished the cytotoxic reaction, but did not effect the binding, whereas the EDTA greatly diminished both the binding and the cytotoxicity. These analyses have enabled us to conclude that a protein receptor is involved in the effector-target interaction as is determined by the papain and trypsin sensitivity of the binding. Monosaccharides, which have been previously reported to be involved in the recognition by mouse NK cells, were examined for their ability to inhibit effector-target cell interactions. Using various monosaccharides and their 6-phosphate derrivities (mannose, galactose, and fructose), inhibition of NK activity was demonstrated; however this inhibition was at a post-binding stage. In addition, most agents which affected cytotoxicity appear to effect a post-binding stage of cytotoxicity, with only EDTA and PMA affecting the binding step. EDTA has been shown in the T cell system to abrogate binding due to its ability to bind magnesium which is required for binding. The mechanism of the effects of PMA requires further elucidation.

## II. The Specificity of Human NK Cells

The nature of the recognition by NK cells of a wide range of tumor cells and some normal cells remains to be elucidated. We previously demonstrated, by cold target inhibition and by depletion on selective tumor cell monolayers, the ability to remove reactivity against certain targets with complete maintenance of reactivity against other target cells. This has suggested that NK cells have a variety of recognition structures and that their specificity is clonally distributed. We have begun a detailed biochemical analysis of initial binding steps of purified LGL to NK-susceptible targets. We have found that solubilized material from the membranes of K562 target cells, inserted into lipid vesicles, can efficiently inhibit effector-target cell interactions, but does not inhibit the subsequent cytolytic reaction. This model system should now enable us to biochemically characterize the nature of the antigen(s) on the target cells and to determine the possible diversity of structures on different target cells. Target cell structures, isolated from K562 (a myeloid leukemia cell line) has been demonstrated to be a glycoprotein (sensitive to 65°C, sensitive to trypsin, adherent to Con-A columns) of 100-150,000 MW. The specific activity of the material (based on 50% inhibition of binding) can be increased 25 to 100 fold by lectin column purification and elution with alpha-methylmannoside. In addition, these NK target structures have been shown to be specific, not inhibiting ADCC or non-human NK effectors (rat LGL). However, inhibition of binding against several NK-sensitive targets has been seen.

III. Regulation of Human NK Activity

Interferon has been shown to have a variety of effects on immune reactivity, including the ability to rapidly augment cell-mediated cytotoxic responses, such as the reactivity of NK cells, and macrophages, or monocytes. Some of these effector mechanisms may have in vivo importance in the resistance against tumor growth or against infections by various microbial agents. To obtain better insight into the nature of the diversity of such effects by interferon, various preparations of natural human leukocyte, recombinant leukocyte, and recombinant hybrid alpha interferons were tested for their ability to augment the reactivity of NK cells and monocytes. These interferon species were tested at several antiviral titers, to determine possible quantitative differences in their ability to modulate immunological functions. At higher doses of interferon (i.e. >500 units) all of the interferon species demonstrated significant augmentation of both NK activity and of monocyte-mediated cytolysis and cytostasis. However, if low levels of interferon were employed (10-50 units), appreciable differences among the various leukocyte interferons were seen. Work is in progress with restriction enzyme derived recombinant hybrid interferon molecules. Pure recombinant A and D hybrids have been shown to vary in their antiviral activity and their effects on NK cells. In our previous experiments, the species specificity of recombinant interferon was maintained with the leukocyte species and recombinant A interferon. However, recombinant D, being reactive with murine cells, has been demonstrated to augment the activity of murine NK cells. The examination of A/D hybrids may enable the determination of the location of the active sites of the antiviral and immune modulatory functions on the interferon molecule. Collectively these results demonstrated substantial quantitative differences in the ability of the various species of human leukocyte interferons to significantly augment levels of cell-mediated functions. Such results should have a significant impact in choosing the interferon species for appropriate clinical trials. In addition, this demonstration that homogenous natural and recombinant interferons have potent effects on effector mechanisms provides a basis for optimal design of new clinical trials for the therapy of cancer.

The interferon induced increase in NK activity is paralleled by an increase of (2'-5')oligo A synthetase, a major pathway involved in the antiviral action of interferon. By introducing the product of the enzyme, (2'-5')pppApApA, into the NK cells by the calcium phosphate percipitation method, the effect of interferon was mimicked. The mechanism by which the (2'-5')pppApApA increased the NK activity is presently not clear. It was shown that when this molecule is introduced into non-lymphoid cells it activates a (2'-5')oligo A-dependent endonuclear, which results with the degradation of RNA in the cells. It is also possible that the introduction of the (2'-5')pppApApA into NK cells results in a limited inhibition of synthesis of certain regulatory proteins.

In addition to agents which augment NK activity, we have studied the suppressive effects of adenosine triphosphate (ATP). The inhibition of NK activity by ATP was specific, not seen by other phosphorylated-nucleotides (UTP, GTP, CTP). The inhibition was shown dependent on the terminal high energy phosphate, in that, ATP or ATNP (a nitrated ATP not capable of releasing the terminal phosphate) were not inhibitory. This potent regulation of NK activity is presently not thoroughly understood, but appears to be at the membrane level.

In addition to the ability of LGL to grow in response to purified IL-2 (see below) this molecule appears to regulate NK activity and be produced, in part by LGL. In lieu of this, we are studying LGL and small T lymphocytes for 1) their mechanism of augmentation by IL-2, whether it be via the production of IFNs or other lymphokines, and 2) the presence of IL-2 receptors and their regulation on LGL and T cells. The latter receptor analysis is being performed using intrinsically labeled ($S^{35}$) IL-2 and a binding assay.

## IV. Cytotoxicity by Cultured Cells

A major limitation for the detailed analysis and characterization of NK cells is that they represent a small proportion of the lymphoid cells in the peripheral organs. A potential solution to this problem was suggested by the observation that peripheral blood lymphocytes can be cultured in the presence of partially purified T cell growth factor (interleukin 2 or IL-2) and these continuously growing cell lines have two distinct cytotoxic capabilities: 1) an NK-like activity and 2) a polyclonally activated T Cell-like activity. The highly enriched LGL cultures, as well as the T cell cultures, maintained rapid growth in the presence of IL-2. The T cells maintained relative stability in culture, demonstrating an increase in the ability to form rosettes at 29°C with sheep erythrocytes, and showing a small increase in the expression of the OKT3 antigen. However, they did not express new antigens as determined by a battery of monoclonal reagents, with the exception of the expression of Ia, which has been previously reported on activated T lymphocytes. In contrast, the LGL cultures demonstrated some very significant changes in phenotype. OKT10, OKM1, and Fc receptors are present on a high proportion of fresh LGL, whereas cultured LGL demonstrated a dramatic loss of these phenotypic characteristics by 10 days. Conversely, the appearance of Ia antigens and OKT3 antigens as well as the ability of a subpopulation of cultured LGL to form rosettes with sheep erythrocytes at 29°C became quite apparent at about the same time in culture (approximately 10 days). Analysis of the cytotxic capacity of these cultured LGL and T cells was performed. The cultured LGL demonstrated killing against NK-susceptible targets and primary tumors, which was exhibited by both the fresh and the cultured LGL to a similar degree and their activity was boosted by interferon. These cultures are of practical importance in the facilitation of the biochemical analysis of NK cells and T cells and in the determination of the controversy of several issues concerning these effector cells. It seems particularly likely that clones of NK cells and T cells will be very useful in their detailed characterization. However, the lack of an NK-specific marker which is maintained in culture, makes the analysis of NK cells and T cells from a mixed population difficult and therefore requires the subpopulation analysis for proper identification of the cytotoxic effector mechanisms studied.

## SIGNIFICANCE OF BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

Natural cell-mediated immunity may play an important role in immune surveillance against tumors. Understanding and determining the in vitro role of natural immunity in human tumor systems should be very useful in the understanding of

the significance of human natural cell-mediated immunity in vivo. The recent findings of the morphologic counterpart of NK cells greatly facilitates the studies of the mechanisms and relevance of NK cells in vivo and in vitro. The further characterization of the NK cell phenotype offers the ability to both monitor NK cells in clinical situations.

In addition, the finding that interferon, both natural and recombinant leukocyte interferons, is a potent modulator of NK cell activity provides an important possible mechanism for therapeutic effects of interferon in clinical protocols. In addition, the finding that NK cells may be cultured in the presence of growth factors in vitro offers the possibility for therapeutic trials with highly purified populations of NK cells.

PROPOSED COURSE OF PROJECT

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 phenotyping of human LGL, especially attempts to find monoclonal antibodies specific for LGL. Particular focus will be on reagents which provide insight into the lineage of these cells, especially their possible relationship to either T cells or monocytes; 2) further characterization of the function of LGL and comparison with non-LGL which possess Fc receptors. 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) studies of the possible differentiation of LGL both in vivo and in vitro and whether they can develop into a typical T cell or other lymphoid cell population; 4) the in vitro cloning of NK cells from cultures of highly purified populations of LGL analyzing cytotoxic specificity, and phenotypic expression; 5) a detailed examination of the biochemical mechanism involved in augmentation of NK and ADCC by interferon. Some of the biochemical mechanisms of interferon induction of antiviral resistance have been determined. We will continue to apply the same approaches to study the biochemical mechanisms involved in interferon-induced augmentation of NK and other cell-mediated cytotoxic activities; 6) further studies in human to examine the in vivo behavior of NK cells. Because of the ability to isolate LGL and typical T cells on Percoll gradients, in vivo studies are planned on a limited basis to examine recirculation of lymphocyte subpopulations as well as the possible in vivo differentiation of isotopically or fluorescein-labeled cells; and 7) the cytolytic activity of NK cells against autologous primary tumor cells and the reactivity of expanded and/or interferon activated NK cells on autologous and allogeneic primary tumor cells.

Another area of continued interest in our studies of NK cells is the characterization of the specificity of their interaction with target cells. The reactivity patterns of clones of cultured NK cells should be very helpful in this regard. In addition, experiments will be continued to fractionate cell membranes of NK-susceptible target cells and characterize the nature of the structures involved in the conjugate formation between highly purified NK cells and target cells. Further separation and biochemical identification of soluble materials will be performed and reagents prepared (monoclonal antibodies) to purify, identify, and enumerate target cell structures.

PUBLICATIONS

Herberman, R.B., Ortaldo, J.R., Rubinstein, M., and Pestka, S.: Augmentation of natural and antibody-dependent cell-mediated cytotoxicity by pure human leukocyte interferon. J. Clin. Immunol., 1:149-153, 1981.

Ortaldo, J.R., and Herberman, R.B.: Specificity of natural killer cells. In NK Cells: Fundamental Aspects and Role in Cancer. Human Cancer Immunology, Vol. 6. B. Serrou (Ed.), Elsevier/North-Holland, Amsterdam, p. 17-35.

Ortaldo, J.T., Timonen, T., Mantovani, A., and Pestka, S.: The effect of interferon on natural immunity. In The Biology of the Interferon System. E. de Maeyer, G. Glasso, and H. Schellekens (Eds.), Elsevier/North-Holland Biomedical Press, Amsterdam, p. 241-244.

Herberman, R.B., and Ortaldo, J.R.: Natural killer cells: Their role in defenses against disease. Science, 214:24-30, 1981.

Ortaldo, J.R., Sharrow, S.O., Timonen, T., and Herberman, R.B.: Determination of surface antigens on highly purified human NK cells by flow cytometry with monoclonal antibodies. J. Immunol., 127:2401-2409, 1981.

Ortaldo, J.R., and Timonen, T.T.: Modification of antigen expression and surface receptors on human NK cells grown in vitro. In Proceedings of the 14th International Leucocyte Culture Conference, 1981, p. 286-289.

Herberman, R.B., Ortaldo, J.R., Timonen, T., Reynolds, C.W., Djeu, J.Y., Pestka, S., and Stanton, J.: Interferon and natural killer (NK) cells. In The Interferon System: A Review to 1982. Texas Reports on Biology and Medicine, Volume 41, 1981-82. S. Baron, F. Dianzani, and J. Stanton (Eds.), University of Texas Medical Branch, Galveston, in press.

Ortaldo, J.R., Timonen, T.T., Vose, B.M., and Alvarez, J.A.: Human natural killer cells as well as T cells maintained in continuous cultures with IL-2. In The Potential Role of T Cell Subpopulations in Cancer Therapy. A. Fefer (Ed.), Raven Press, New York, in press.

de Landazuri, M.O., Lopez-Botet, M., Timonen, T., Ortaldo, J.R., and Herberman, R.B.: Human large granular lymphocytes: Spontaneous and interferon-boosted NK activity against adherent and nonadherent tumor cell lines. J. Immunol., 127:1380-1383, 1981.

Herberman, R.B., Ortaldo, J.R. Mantovani, A., Hobbs, D.S., Kung, H-f, and Pestka, S.: Effect of human recombinant interferon on cytotoxic activity of natural killer (NK) cells and monocytes. Cell Immunol., 67:160-167, 1982.

Goldfarb, R.H., Timonen, T., and Herberman, R.B.: Mechanisms of tumor cell lysis by natural killer cells. In Advances in Experimental Biology and Medicine. W. Clark and P. Golstein (Eds.), in press.

Ortaldo, J.R., and Timonen, T.T.: Analysis of fresh human NK cells and their IL-2 maintained cultures: Comparison with other lymphoid populations. In Human Cancer Immunology. B. Serrou (Ed.), Elsevier/North-Holland Biomedical Press, Amsterdam, in press.

Ortaldo, J.R.: Natural killer cells: A separate lineage? In NK Cells and Other Natural Effector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Ortaldo, J.R., Herberman, R.B., and Pestka, S.: Augmentation of human natural killer cells with human leukocyte and human recombinant leukocyte interferon. In NK Cells and Other Natural Effector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Timonen, T., Ortaldo, J.R., and Herberman, R.B.: Analysis of natural killer activity of human large granular lymphocytes at a single cell level. In NK Cells and Other Natural Effector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Timonen, T., Ortaldo, J.R., and Herberman, R.B.: Cultures of purified human natural killer cells. In NK Cells and Other Natural Effector Cells. R.B. Herberman (Ed.), Academic Press, New York, in press.

Serrate, S.A., Vose, B.M., Timonen, T., Ortaldo, J.R., and Herberman, R.B.: Association of human natural killer cell activity against human primary tumors with large granular lymphocytes. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Maluish, A.E., Ortaldo, J.R., and Herberman, R.B.: Modulation of NK activity by recombinant leukocyte interferno in advanced cancer patients. In Natural Cell-Mediated Immunity, Volume 2. R.B. Herberman (Ed.), Academic Press, New York, in press.

Timonen, T., Reynolds, C.W., Ortaldo, J.R., and Herberman, R.B.: Isolation of human and rat natural killer cells. J. Immunol. Methods, in press.

Timonen, T., Ortaldo, J.R., and Herberman, R.B.: Analysis by a single cell cytotoxicity assay of natural killer (NK) cell frequencies among human large granular lymphocytes and of the effects of interferon on their activity. J. Immunol., in press.

## MONOCYTE/MACROPHAGE SECTION (PROPOSED)

This report summarizes the activities of the proposed Monocyte/Macrophage Section for its first year since its initiation in January of 1982. The proposed Monocyte/Macrophage Section of the Biological Research and Therapy Branch is a direct extension of the previously existing Basic Mechanisms Section of the Biological Development Branch of the Biological Response Modifiers Program. As part of the reorganization of the Intramural Research Program of the Biological Response Modifiers Program, the former Basic Mechanisms Section was divided in January of 1982 along the lines of the major scientific projects in the section: monocytes/macrophages and natural killer cells. This division occurred following the merger of the LID and BRMP; the programatic interest in the areas of monocyte and NK cell research coupled with the large numbers of researchers in this area prompted the formation of the proposed Monocyte/Macrophage Section and the Natural Immunity Section. Dr. Herberman was made Head of the Natural Immunity Section and Dr. Henry C. Stevenson has served as Acting Head of the proposed Monocyte/Macrophage Section. Researchers with primary interest in natural killer cells were assigned to work in the newly created Natural Immunity Section. This included an investigator in the former Basic Mechanisms Section, Dr. Craig Reynolds. Two other senior investigators in the Basic Mechanisms Section were reassigned to different sections in the newly organized Biological Response Modifiers Program on the basis of their immunologic expertise: Dr. Stephen Sherwin was reassigned to the proposed Lymphokines/Cytokines Section due to his interest in transforming factors, and Dr. Jack Pearson and Dr. James Knost were reassigned to the Monoclonal Antibody/Hybridoma Section. Two investigators in the Basic Mechanisms Section were assigned to the proposed Monocyte/Macrophage Section, Dr. Henry C. Stevenson and Dr. Eugenie Kleinerman. In addition, three other investigators were added to this Section: Dr. Luigi Varesio was reassigned from the Lymphokines Section to the proposed Monocyte/Macrophage Section because of his interest in RNA synthetic events that accompany macrophage cytotoxicity. Dr. Thomas Hoffman was a Senior Investigator in the former Laboratory of Immunodiagnosis (LID) who was assigned to the proposed Monocyte/Macrophage Section because of his interest in the phospholipid metabolism of monocytes. Finally, Dr. Howard Holden (also a member of the former LID) was assigned to the proposed Monocyte/Macrophage Section because of his long-standing interest in developing *in vivo* animal models of macrophage function.

The major area of research for the proposed Monocyte/Macrophage Section is the study of monocytes and their tissue counterparts, macrophages, and the role of these cells in the defense against cancer. The investigators in the proposed Monocyte/Macrophage Section performs a wide variety of research investigations that fall into six general categories: 1) investigations on the phenotypic, biochemical and functional characteristics of monocytes and macrophages; 2) in-depth *in vitro* investigations into the ability of monocytes and macrophages to destroy tumor targets; 3) analysis of the factors regulating the development and activation of macrophages and monocytes; 4) analysis of the role of monocytes and macrophages in the immune response and the interaction of these cells with other components of the immune response; 5) investigations the production of biologic response modifiers (BRMs) by monocytes and macrophages and the effects of BRMs on the functional activities of these and other component cells of the immune response; 6) development of *in vivo* models to test the relevance of of monocytes and macrophages to host defense against malignancy.

Monocytes have been found to have several potential roles in host defense against cancer. They can have direct cytotoxic effects against tumor cells, they can act as accessory cells for the generation of the immune response, they can produce a wide range of potent immunomodulatory agents known as monokines, and under the appropriate circumstances, they can act as suppressor cells of various immunologic functions. We have continued detailed studies regarding each of these functions of monocytes and macrophages and their underlying mechanisms. We have developed in vivo animal models to test the relevance of our in vitro observations and we have developed strategies whereby laboratory data generated in animals can be applied to clinical situations.

Animal models have been developed which allow us to compare the contribution of macrophage cytotoxicity against tumor cells vis-a-vis the cytotoxicity exerted by natural killer cells. Animals that were treated in a fashion that reduced their natural killer cell function (anti-asialo GM1 treatment) showed a much greater degree of metastatic disease following i.v. inoculation with B16 tumor cells. When these animals were treated with adoptive transfer of activated tumoricidal macrophages, the numbers of metastases in these partially NK-cell deficient animals was reduced. Of extreme interest is the finding that the adoptive transfer of unactivated macrophages into animals resulted in a greater number of metastases found in these animals. Additional in vivo animal experimentation indicates that activated macrophages when given in adoptive transfer systems have different trafficking patterns from unactivated macrophages. Two monoclonal antibodies directed against murine macrophage cell surface antigens have been recently produced, 36 and 52. These monoclonal antibodies react strongly with bone marrow derived macrophages and thioglycollate-elicited peritoneal macrophages, but react weakly with resident peritoneal cells. These antibodies also have some reactivity against many of the macrophage-like cell lines that currently exist. These monoclonal antibodies are being characterized in an attempt to provide reagents that can identify subpopulations of murine macrophages based on their membrane antigenic characteristics. An assay for plasminogen activator activity has been developed and is used as an indicator for murine macrophage activation when these cells are treated with macrophage activating factor (MAF). Although there is a correlation between the cytotoxic function of macrophages treated with MAF and their plasminogen activator production, there is no positive correlation between macrophages activated by endotoxin and their plasminogen activator activity. Two murine T cell hybrids have been produced that produce MAF.

The ability of human monocytes to destroy tumor targets is an area of continued interest by the investigators of this section. Human monocytes have been shown to be capable of killing a wide range of human and mouse tumor targets. It has been documented that monocytes have a baseline spontaneous cytotoxicity against many types of tumor cells, and that this baseline spontaneous level of monocyte cytotoxicity can be augmented by a variety of agents including alpha interferon and MAF-containing human lymphokine preparations. Studies into the lymphocyte-monocyte interactions have shown that there are helper lymphocytes that are capable of secreting soluble factors that augment monocyte cytotoxicity and similarly there are suppressor lymphocytes and macrophages which under the appropriate circumstances can down-regulate monocyte cytotoxic function. Studies have been performed that document the role of liposomes in human monocyte activation. It has been shown that various activating factors, including MAF-

containing lymphokines, can be packaged into multilamellar liposomes and that these packaged activating substances will be preferentially phagocytized by human monocytes. Following ingestion of MAF-containing liposomes (but not empty liposomes) macrophages have enhanced tumoricidal properties.

Detailed analysis of membrane events that are observed on monocytes and macrophages following activation signals have been performed. These studies show that changes occur at the cell membrane level which alter the ability of the cell to undergo transmethylation reactions of phospholipid pathways. These changes in transmethylation activity are correlated with the activation status of the monocyte. It has been demonstrated that some highly purified interferon preparations are capable of inhibiting transmethylation reactions at the level of the monocyte membrane. Similarly, tumor promotors such as phorbol myristate acetate were shown to decrease phospholipid methylation in a variety of peripheral blood mononuclear cells but this effect was most prominent in monocytes. In addition, the transmethylation observed was shown to be of the competitive type based on extensive kinetic studies. Of interest was the finding that although phorbol myristic acetate (PMA) is capable of promoting the differentiation of myeloid-like cell lines based on the acquisition of highly differentiated functional phenotypes, PMA was shown to have minimal effects on the phospholipid transmethylation of these already transformed cell lines.

In parallel with the development of animal models which test the relevance of monocytes in vivo, the investigators of the proposed Section have made a strong commitment to applying research information obtained from animal systems to the human situation. We have established a group of highly immunologically characterized normal human donors which are carefully scrutinized vis-a-vis a number of their immune parameters, particularly focusing on their monocyte and NK cell function. We are able to obtain very large numbers of human monocytes from these individuals and they are purified by a sterile suspension technique known as elutriation. Hundreds of millions of these cells obtained from a single normal human can also be cryopreserved with complete preservation of all functions tested so far. Techniques have been developed that allow us to separate monocytes into different size fractions using the elutriator apparatus and also using a fluorescence activated cell sorter. Cells separated by this technique and by techniques that discriminate monocytes on the basis of their density are being tested for differential cytotoxic function or differential ability to release monokines. Monoclonal antibodies against these distinct size subpopulations of monocytes are currently being raised. We have also utilized activated normal human monocytes to establish a gene library of these cells in bacteria. Similarly, successful fusions between malignant human monocytes and normal human monocytes (monocyte hybridomas) have been completed; we are currently analyzing the function of these genetically manipulated cells for distinct human monocyte functions that are of interest to the program.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09225-02 BRTB |
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PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Human Monocyte-Mediated Cytotoxicity In Vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
| PI: | Eugenie Kleinerman | C.O. | BRTB | NCI |
| OTHERS: | C.W. Reynolds | Staff Fellow | BRTB | NCI |
| | R.H. Wiltrout | Staff Fellow | BRTB | NCI |
| | James Braatz | Expert | BRTB | NCI |

COOPERATING UNITS (if any)

Cancer Metastasis and Treatment Laboratory, NCI-FCRF

LAB/BRANCH

Biological Research and Therapy Branch

SECTION

Monocyte-Macrophage (proposed)

INSTITUTE AND LOCATION

NCI-NIH, Frederick, Maryland 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms involved in monocyte activation and interactions that take place between monocytes and lymphocytes have been examined. Peripheral blood monocytes become cytotoxic after cocultivation in vitro with autologous unstimulated "helper" lymphocytes or with a soluble factor secreted by these helper cells. These monocytes are also controlled by suppressor lymphocytes. The relationship between loss of suppressor activity and the generation of helper activity is being investigated. The exact biochemical signals that trigger monocyte activation are not known at this time, however, many activating substances have been described, e.g., muramyl dipeptide, macrophage activating factor, that stimulate cytotoxic activity of monocytes. These activating factors can be packaged into multilamellar liposomes and these liposomes are phagocytized by monocytes. The factor(s) are then released intracellularly and render the monocyte cytotoxic. Therefore an internal signal rather than a surface binding stimulus is probably crucial in mediating activation. In addition, encapsulated MAF is more efficient than the free unencapsulated form. The use of liposome-encapsulated substances can be a powerful tool to deliver different agents into the monocyte in an attempt to study the biochemical signals that are important in activation.

## PROJECT DESCRIPTION

### OBJECTIVE

The objectives of this project are to: 1) better understand the mechanism involved in the development of a cytotoxic monocyte from non-cytotoxic peripheral blood mononuclear leukocytes (MNL); 2) define interactions taking place between lymphocytes and monocytes in the generation of these cytotoxic cells; a) the role of helper lymphocytes, b) the role of soluble factors produced by helper lymphocytes, and c) role of suppressor lymphocytes; 3) examine the specificity of the cytotoxic function of these activated monocytes by testing their ability to kill normal as well as tumor targets.

In addition, the purpose of the projects performed this year were: 1) to determine the effect of various cancer chemotherapeutic agents and X-irradiation on monocyte-mediated killing; 2) to standardize a monocyte-mediated tumoricidal assay to be used to measure the activation potential of various lymphokine preparations, soluble helper factors (described above) and other biological response modifiers; 3) produce a human lymphokine supernatant from peripheral blood MNL that activates the tumoricidal function of human monocytes; 4) determine if this lymphokine supernatant can be encapsulated within the aqueous interior of multi-lamellar liposomes; and 5) determine whether human blood monocytes can be rendered tumoricidal following in vitro incubation with this liposome-encapsulated lymphokine.

### METHODS EMPLOYED

To address the question of mechanisms involved in the development of a cytotoxic monocyte from a non-cytotoxic peripheral blood MNL and also to better understand the interaction between monocytes and lymphocytes, a well established assay of red cell target killing was employed that uses no exogenous stimulus to activate the cells to become cytotoxic.

Human peripheral blood mononuclear leukocytes (MNL) become cytotoxic toward a variety of red blood cell targets after culturing in vitro for three to seven days. (Routinely $^{51}Cr$-labeled chicken red blood cells are used as targets in an 18-hour assay.) Since the development of this cytotoxic activity occurs in the absence of added antibody or lectin to the culture, and the killer cell has been ascertained to be a monocyte, we have named this phenomenon Culture-Induced Monocyte-Mediated Cytotoxicity (CIMMC). MNLs are not cytotoxic when assayed immediately following isolation and only develop cytotoxicity after being cultured. The cytotoxic phenomenon usually peaks on day 5, then begins to slowly decrease and is normally absent by day 10. Because no exogenous stimulant is employed to activate the killer cell, this assay system serves as a good model for examining both the mechanism by which human monocytes develop into cytotoxic effector cells and the interaction that occurs between monocytes and lymphocytes.

In addition to the above red cell target assay, we have developed an assay measuring monocyte-mediated cytotoxicity against a human melanoma tumor target, A375.

Monocytes are isolated by combining the isolation techniques of continuous Percoll gradients and plastic adherence to FCS-coated plates, plus extensive washing, to obtain monocyte populations with greater than 99% purity.

Briefly MNL from peripheral blood are first isolated on Ficoll-Hypaque gradients. 40-50 x $10^6$ MNL are then layered onto performed continuous Percoll gradients and spun at 1000g x 20 min. This yields 4 bands of cells. Monocytes are contained in the 2nd band, ~ 5mm above the middle of the gradient and are 70-80% pure. The monocytes are then adjusted to contain 1 x $10^6$ monos/ml in RPMI 1640 with 5% human AB serum. 1 x $10^5$ monos are plated onto microtiter plates that have previously been coated with FCS. After a 1 hour incubation, the non-adherent cells are aspirated and the plates washed x 3. The combination of these two techniques yields a greater than 99% pure monocyte population. The activating or control medium is added for 18-24 hours, then aspirated off. The adherent tumor targets labeled with [$^{125}$I] IUdR are then added to the adherence-purified monocytes, so no removal of effector cells is necessary. 72 hours later the assay is harvested and the residual $^{125}$I counts remaining in the live adherent tumor cells are determined.

## MAJOR FINDINGS

### I. Effect of X-Radiation on the Development of CIMMC

Radiation was used to better define and understand the interaction between human killer monocytes and their suppressor lymphocytes in the development of CIMMC. As stated previously, human mononuclear leukocytes become spontaneously cytotoxic to a variety of red blood cell targets after 7 days of *in vitro* culture. This phenomenon was independent of exogenous stimulus by antigen, antibody, or mitogen. Monocytes have been shown to be the cytotoxic cell, with their activation controlled by lymphocyte suppressor cells. X-irradiation enhanced the magnitude of monocyte-mediated cytotoxicity generated and stimulated an earlier appearance of this cytotoxicity when compared with that of unirradiated mononuclear leukocytes. This effect of X-irradiation (150 to 600R) on separated populations of mononuclear leukocytes, enriched for either lymphocytes or monocytes, was examined; this enhanced cytotoxicity appeared to be secondary to inactivation of the lymphocyte suppressor cells.

### II. Effect of Various Chemotherapeutic Agents on CIMMC

We have previously shown that *cis*-Diamminedichloroplatinum (II) (*cis*-DDP), an active antineoplastic agent, and X-irradiation enhanced human CIMMC. To ascertain whether this is a unique property of *cis*-DDP or common to other effective antineoplastic agents, we studied the effect of L-phenylalanine mustard (L-PAM), adriamycin (ADR), actinomycin D (Act. D) and 4'-(9-Acridinylamino) Methanesulfon-m-Anisidide (m-AMSA) on spontaneous monocyte-mediated cytotoxicity. *Cis*-DDP, ADR, L-PAM and m-AMSA under appropriate *in vitro* conditions all increased spontaneous monocyte-mediated cytotoxicity. Activation involved either 1) direct monocyte activation (*cis*-DDP and ADR); 2) inactivation of suppressor lymphocytes (X-irradiation); 3) a combination of the two (L-PAM); or 4) mechanisms not yet

elucidated (m-AMSA). In contrast, incubating mononuclear leukocytes with 0.4 µg/ml Act. D depressed monocyte-mediated killing. Thus, while stimulation of "non-specific" killing is not a unique property of cis-DDP, it is not a universal effect of all chemotherapeutic agents. The stimulation and enhancement of already existing host defense mechanisms may be an important additional way in which chemotherapeutic agents exert their anti-tumor effect.

III. Evidence for the Role of a Helper Lymphocyte in the Generation of CIMMC

Until recently, we believed that these cytotoxic monocytes were only controlled by suppressor lymphocytes and that these suppressor cells were somehow inactivated during our in vitro culture process. The following experimental evidence suggesting this interpretation:

1) When fresh autologous lymphocytes were added to cultured MNL, expression of cytotoxicity was abrogated.

2) The suppressor cells required 24 hr to suppress optimally.

3) The lymphocytes lost their ability to suppress cytotoxicity after being in culture for 5-7 days.

4) Suppressor activity could be regenerated with the addition of pokeweed mitogen to the culture for 24 hrs.

However, we now have evidence that monocytes also require a helper cell to develop their cytotoxic function. This hypothesis stemmed from other observations that we were making in the laboratory:

1) Purified monocyte preparations developed cytotoxic function much later (day 10) or not at all when compared to MNL preparations.

2) In our experiments with actinomycin D, we found that when purified lymphocyte populations were treated with actinomycin D for 15 minutes, washed and recombined with purified autologous monocytes, CIMMC failed to develop. However, if untreated lymphocytes were also added to this combination, ($M + L^{ActD} + L$), CIMMC developed the same as in the control cultures.

Together these data suggested to us that lymphocytes may play an important helper role in the generation of CIMMC, and that this function is eliminated by treatment with actinomycin D.

To directly address this hypothesis, purified lymphocytes (99% esterase negative) were cultured for 3 days, harvested, and then added to fresh Percoll-enriched autologous monocytes (80% esterase positive) for 48 hours. CIMMC was then assayed. Monocytes incubated with "pre-cultured" lymphocytes for 48 hours were cytotoxic while monocytes incubated alone or with fresh lymphocytes were not. This further supports our new hypothesis, that a helper lymphocyte is necessary in the development of CIMMC and that part of the time required for development of activity is related to a period of activation of this helper activity.

IV. Development of a Assay that Measures the Tumoricidal Activity of Purified Human Monocytes

Our understanding of the mechanisms involved in macrophage recognition and destruction of tumor target cells has evolved from in vitro studies. However, most of these in vitro studies of activation and subsequent cytotoxicity of macrophages have been done either in rodent systems or by using human monocytes which have been isolated by adherence and then exposure to EDTA. It is my contention that chelation of both $Ca^{++}$ and $Mg^{++}$ may alter the membrane structure of the monocyte and so too alter the stage of activation of the cell. It is thus important to develop an assay where monocytes can be obtained without exposure to chelating agents. In our laboratory we have developed an assay (described above) measuring monocyte-mediated cytotoxicity against a human melanoma tumor target A375. We find that this assay is reproducible in our hands and gives us low levels of spontaneous tumor cell killing by unstimulated monocytes as opposed to the findings of Mantovani et al. (Int. J. Cancer 23:28-31, 1979).

V. Production of Human Lymphokine-Containing Supernatant that Induces Monocyte Tumoricidal Activity

We have been able to produce a human lymphokine supernatant from peripheral blood mononuclear cells (MNL) using Sepharose-bound Con A. This allows us to spin out the mitogen so it is not present during the assay. This cell-free supernatant activates both fresh and cultured peripheral blood monocytes to become tumoricidal against the human melanoma target A375. We have already shown that supernatants from MNL alone or Con-A and media alone do not activate tumoricidal activity. Furthermore, soluble Con A itself does not directly induce monocytes to become tumoricidal. Finally, all lymphokine supernatant preparations have been shown to be free of endotoxin and interferon including $\gamma$-interferon. We therefore feel that the supernatant that we are producing contains Macrophage Activating Factor (MAF). This lymphokine supernatant can easily be encapsulated within the aqueous interior of liposomes.

VI. Activation of Human Monocyte-Mediated Tumoricidal Function by Liposomes Containing Human Lymphokines

The purpose of these studies was to determine whether human blood monocytes can be rendered tumoricidal following in vitro incubation with a lymphokine (derived from peripheral blood mononuclear cells incubated with Con-A) that had been encapsulated within the aqueous interior of liposomes. We undertook these investigations because of the data demonstrating that appropriately activated macrophages may be important in host defense against neoplasia. Previous studies in rodent systems have shown that liposomes containing heterologous lymphokines can be engulfed by macrophages and render them tumoricidal. Furthermore, the multiple I.V. injections of liposomes containing lymphokines have been associated with regression of metastases in murine systems. We report here that human peripheral blood monocytes can be rendered tumoricidal in vitro following their interaction with a human lymphokine in either free form or encapsulated

within multilamellar liposomes. Human monocytes incubated for 24 hr. with human lymphokine contained within 100 nmole phospholipid resulted in the reproducible generation of cytotoxicity (50-70%) against A375 human melanoma cells at a 1:10 T/E ratio. Activation by liposome-entrapped lymphokine was very efficient and required less than 1/800th the concentration required for free, unencapsulated lymphokine. We conclude that human monocytes can be activated by liposome-entrapped lymphokine in vitro. Since the administration of free lymphokine in vivo may lead to the development of undesirable-entrapped lymphokine, the use of liposomes may circumvent such consequences and thus have therapeutic potential.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

Monocytes/macrophages are effector cells which function as part of the host's defense mechanism against invading pathogens. Monocytes, once activated, may phagocytize and/or destroy antigenic material (a range of self-cells and non-self invading cells) and need no previous exposure to the invading organism or antigen to function efficiently. It is believed that monocytes may be one of the first forms of naturally occurring host defense mechanisms that have been preserved phylogenetically. Indeed invertebrate species such as starfish (Echinoderm), blood worms (Annelid) and keyhole limpets (Mollusc) possess large mononuclear cells in their "blood stream" much like human monocytes but have no capabiilties of forming antibodies. Yet, these creatures survive in an environment that is loaded with micro-organisms.

Little is known about the biochemical mechanisms involved in turning monocytes into activated, cytotoxic effector cells. Because these activated effector cells may be crucial to host defense against both invading pathogens and in tumor immunity, understanding how the cell becomes activated is as important as understanding how the cell lyses its target once it is activated.

To study the mechanisms involved in monocyte activation and interactions that take place between monocytes and lymphocytes, an assay system is needed in which no exogenous stimulus is added to activate the monocytes and lymphocytes so that manipulations can be done to inhibit certain metabolic pathways (such as phospholipid methylation) to ascertain their role in the process. We believe that the red cell killing assay described meets these requirements and it is our goal in the next one year to try to sort out the interactions that are taking place between monocytes and lymphocytes in this assay and to determine whether red cell killing can provide a model for tumor cell killing. Once biochemical pathways can be described that are responsible for differentiating the monocyte to its tumoricidal state, it may be easier to synthesize compounds specifically designed for this function, which would hopefully be useful in adjuvant chemotherapy or in conjunction with other biological response modifiers.

Even though at the present time we don't understand the biochemical signals involved, because of the important role that macrophages may play in host defense against infection and neoplasms, a search for agents that can empirically stimulate or augment macrophages function in vitro and in vivo has be generated. Macrophages/monocytes obtained from healthy donors are usually not cytotoxic

to tumor cells in vitro but can be rendered cytotoxic following interaction with a variety of agents such as soluble lymphokines from mitogen-stimulated lymphocytes, double-stranded RNA and bacterial products, e.g., LPS and muramyl dipeptide (MDP). However, the administration in vivo of free lymphokine(s) or other free soluble activating factors has proven difficult due to the development of undesirable side effects such as anaphylaxis and granuloma development. The concept of devising a carrier vehicle to transport the activating compound directly into the effector cell would seem to circumvent the problem of undesirable side effects and has therefore received increasing attention. Particular success has been obtained using phospholipid vesicles (or liposomes) as these carrier vesicles. Biologically active materials such as MDP can be encapsulated inside liposomes, and macrophages have been shown to incorporate these liposomes intracellularly. After cellular uptake of the liposome via phagocytosis and/or fusion, the encapsulated material is released intracellularly in an active form.

Studies in rodents have shown that liposomes containing MDP or lymphokines can be engulfed by macrophages. Furthermore, these macrophages subsequently become tumoricidal. Multiple i.v. injections of liposomes containing lymphokines have been associated with regression of metastases in murine systems. To date, however, no experiments involving human monocytes and liposome-encapsulated lymphokines have been reported. The above experiments were the first step in attempting to determine if this idea was feasible in human subjects, and hopefully will lay the ground work for in vitro studies using patients monocytes and eventually for clinical trials.

## PROPOSED COURSE OF THE PROJECT

During the next year we would like to characterize the helper cell and better understand its function in the development of CIMMC. This will be done using monoclonal antibodies to deplete or reconstitute our lymphocyte pool and compare the development of CIMMC. In addition, we would also like to ascertain whether there is a suppressor cell as originally hypothesized. Again monoclonal antibodies will be employed to deplete subpopulations of lymphocytes to see the effect on the development of CIMMC. We believe that these helper lymphocytes functon by manufacturing a "helper factor". We already have preliminary evidence that unstimulated purified cell-free lymphocytes produce such a soluble factor after 3 days in culture and this factor in turn activates the monocyte to become cytotoxic by itself. It is our hope to also begin some purification procedures in the next year, in an attempt to isolate and characterize this factor. If we can purify this factor, future work will be directed at ascertaining whether there is a specific receptor on the monocyte membrane. Binding of the specific receptor may trigger the biochemical processes crucial to the cell's activation process.

Work will also continue using multilamellar vesicles or liposomes containing lymphokine to activate human monocytes. These activated monocytes will be studied for the specificity of their cytotoxic functions by testing their ability to lyse other tumor targets and various normal target cells.

In addition, we will attempt to determine if our human lymphokine supernatant is species-specific by testing its ability to activate macrophages from other species (i.e. mice and rats). The lymphokine supernatant will be tested both within and free of liposomes. Theoretically there may be at least 2 parts to the MAF molecule. One part may be responsible for binding onto the monocyte membrane while another part may independently trigger activation. Binding may be species-specific while activation is not. This could potentially be sorted out using the lymphokine both within and outside liposomes, since the encapsulated form does not bind to the membrane. Thus, liposome-encapsulated lymphokine may be shown to transcend species barriers.

PUBLICATIONS

Kleinerman, E.S., Louis, J.S., Wahl, L.M., and Muchmore, A.V.: Pharmacology of human spontaneous monocyte-mediated cytotoxicity. I. Enhancement by Salicylates and Steroids. Arthritis Rheum., 24: 131-137, 1981.

Kleinerman, E.S., Decker, J.M., and Muchmore, A.V.: In vitro cellular regulation of monocyte function: Evidence for a radiosensitive suppressor. J. Reticuloendothelial Society 30: 373-380, 1981.

Kleinerman, E.S., and Zwelling, L.A.: The effect of cis-diamminedichloroplatinum (II) on immune function in vitro and in vivo: A review. Cancer Immunol. and Immunother., 12: March, 1982.

Kleinerman, E.S., Zwelling, L.A., Schwartz, R., and Muchmore, A.V.: Effect of four commonly used chemotherapeutic agents on naturally occurring human spontaneous monocyte-mediated cytotoxicity. Cancer Res., 42: 1692-1695, 1982.

Muchmore, A.V., and Kleinerman, E.S.: Activation of spontaneous monocyte-mediated cytotoxicity in humans by X-irradiation and cis-Diamminedichloroplatinum (II). In Chirigos, M.A., Mitchell, M., Mastranglo, M.J., and Kim, M. (Eds.): Progress in Cancer by Immune Modifiers. New York, Raven Press, 1981, vol. 19, pp. 101-106.

Muchmore, A.V., and Kleinerman, E.S.: Spontaneous monocyte mediated cytotoxicity in man: Evidence for T helper activity. In Herberman, R.B. (Ed.): NK Cells and Other Natural Effector Cells. New York, Academic Press, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09248-01 BRTB |
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PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Human Monocytes in the Immune Response to Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|---|---|---|---|---|
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COOPERATING UNITS (if any)

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Biological Research and Therapy Branch

SECTION

Monocyte/Macrophage Section (proposed)

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, MD 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 5.5 | 4.5 | 2.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human monocytes are normal cells with numerous functions which have potential roles for preventing new tumors or eradicating established malignancies. Studies have been performed which have allowed us to establish a group of highly immunologically-characterized normal human donors which are part of a longitudinal analysis of normal human monocyte function and other components of the immune response. Investigations have particularly focused on the potential antitumor functions of normal human monocytes. We have been able to isolate large numbers of sterile human monocytes by elutriation and subsequently cryopreserve them in suspension. We have studied the spontaneous cytotoxicity of monocytes to a wide range of tumors and how this function is up-regulated by macrophage activating factors. We developed methodology for separating human monocytes into subsets by size and density and we are testing these subsets for distinct differences in tumor cytotoxicity and BRM release. We have isolated a gene library from activated human monocytes and we have transfected this library into bacteria in preparation for analyzing the function of cells that have been genetically engineered with human monocyte genes.

PROJECT DESCRIPTION

## OBJECTIVES

The general objectives of this project are to a) establish a pool of normal human donors to be used for in depth research studies of their immune response; b) to establish a leukapheresis facility in which peripheral blood, leukapheresis specimens, and bone marrow specimens can be obtained for subsequent in-depth research analysis; c) to establish a laboratory facility in which large amounts of sterile human leukocytes can be separated into their component subtypes (platelets, granulocytes, red cells, T cells, B cells, NK cells and monocytes) for subsequent in-depth study; d) establishment of a cryopreservation bank of the aforementioned cells; e) establishment of a computerized monitoring facility whereby a longitudinal analysis of the immune response of normal human donors can be performed; f) the preservation and proliferation of human monocytes in vitro; g) the characterization of functional subsets of human mononuclear phagocytes; h) the development of monoclonal antibodies with reactivity for human monocyte subsets; i) attempts to develop an in-depth understanding of the nature of human monocyte cytotoxicity against tumor targets; j) development of a reproducible assay for screening human macrophage activating factors (MAF) preparations for their activity on human monocytes; k) isolation of the gene segments from activated human monocytes that code for the tumoricidal function of monocytes and/or the ability of monocytes to release high priority biologic response modifiers; l) the development of fusion products between monocyte tumors and normal monocytes (monocyte hybridomas) that secrete large amounts of high priority biologic response modifiers.

## METHODS EMPLOYED

Human volunteers undergo extensive testing to ensure their normality, which includes detailed immunologic studies and histocompatibility typing. Leukocytes are removed from normal donors by phlebotomy, manual leukapheresis, machine leukapheresis, and bone marrow aspiration. Normal donors have immunologic monitoring performed on a monthly basis, consisting of lymphocyte blastogenesis to mitogens, natural killer cell activity, total T cells and subpopulations, B cell enumeration, and macrophage cytostatic activity. Human leukocytes are subfractionated into mononuclear leukocytes, granulocytes and red cells by Ficoll-Hypaque gradients; granulocytes and red cells are separated from one another by dextran sedimentation; platelets are purified by elutriation; lymphocytes and monocytes are separated from one another by elutriation; lymphocytes are fractionated into T cells by E-rosette methodology; NK cells are isolated by removing adherent cells followed by Percoll gradients; monocytes are subfractionated by albumin gradients, the fluorescence cell sorter, and reactivity with monoclonal antibodies. Functional assays of human monocytes include release of monokines such as interferon, colony stimulating factor and interleukin-1; the ability of monocytes to spontaneous kill tumor targets is measured in vitro as is the enhancement of this function by macrophage activating factors; the ability of monocytes to migrate randomly, in vitro is measured as is the inhibition of this function by monocyte migratory inhibitory factors; the in vitro phagocytic capabilities of monocytes for latex beads and IgG-coated particles is measured; the ability of monocytes to perform accessory cell

functions in mitogen-and antigen-stimulated T cells and mitogen-and antigen-stimulated B lymphocytes is determined; purity and character of human monocyte preparations is assessed by staining with esterase, peroxidase, acid phosphatase, lysozyme and Wright's stains. Monocytes are sized by a computer-monitored automated cell sizer. Specific antigenic determinants on monocyte membranes are detected by assessing the amount of $D_R$ antigens on their surfaces, by analyzing the amount of monocyte-specific antigens on their surfaces, and by examining the ability of monocyte preparations to bind highly monocyte-specific lectins (Pokeweed mitogen, concanavalin A). Several assays to determine the ability of monocytes to kill tumor targets are employed, including the ability of monocytes to lyse radiolabeled tumor targets, and direct visualization of the interaction between monocytes and tumor targets under a video microscope. Monocytes are fused to HAT-sensitive human monocyte tumors to produce monocyte hybridomas using standard hybridoma technology. Gene segments that code for products of activated monocytes are obtained by isolating the messenger RNA produced by activated human monocytes, producing cDNA copies of these messages, incorporating this cDNA into highly specialized plasmid vectors, and transfecting these plasmids into bacterial cultures and then (following the appropriate amount of expansion) transfecting these messages into eukaryotic cells. The eukaryotic cells thus transfected will be analyzed for their ability to perform well-known monocyte functions such as biologic response modifier release and the possession of monocyte associated surface membrane antigens. Cryopreservation technology has been applied to the long-term storage of large numbers of purified human lymphocytes and monocytes. These cells are frozen in a sterile fashion with autologous serum for subsequent use in laboratory assays. All research laboratory data are coordinated into a central computer bank under the name of the normal donor from whom the cells were obtained.

## I. Longitudinal Analysis of the Function of Normal Human Monocytes and Other Cellular Components of the Immune Response

Previous studies using normal human cells for immunologic research have demonstrated a great deal of variability in the responses measured in normal humans. This variability has in the past been attributed to the outbred nature of the human population, the fact that many "normal people" have some type of disease process ongoing that they are unaware of, and the fact that many Americans consume drugs, such as aspirin, which can modulate the immune response. To generate reliable data with regard to the monocyte function of normal humans, we have established a large collection of volunteers that have undergone careful medical scrutiny to ensure their normalcy. In addition these normals have had a wide battery of immunologic assays performed, both initially and then on a regular basis to assess the constancy of the observed immune responses of these individuals. In addition these donors have been histocompatibility typed. We are currently assessing the reproducibility of laboratory information obtained from these individuals vis a vis the immunologic data accrued from unselected normal volunteers. We are performing phlebotomy, machine leukapheresis, manual leukapheresis and bone marrow aspirations on these donors as required for subsequent laboratory experimentation (detailed below). The various components of human blood are separated into red cells, platelets, granulocytes, T cells, B cells, natural killer cells and monocytes as required by ongoing experimentation. The purity of these cell preparations is excellent. These sterile purified

leukocyte subpopulations are then cryopreserved for subsequent laboratory examinations. The results of all experimental laboratory experiments (such as macrophage cytotoxicity, natural killer cell function, B cell function, interferon production, MIF responses, chemotactic responses, T cell subpopulations and others) are placed on computer file indexed by the name of the donor. We are in the process of making correlations between the laboratory examinations performed by widely disparate laboratories using a single donor's white cells at a single point in time. Moreover we are observing the "immunologic pedigree" of each normal donor in a longitudinal fashion.

## II. Long-Term Culture of Human Monocytes and Expansion of Human Monocyte Numbers In Vitro

Previous work in our laboratory has established that human monocytes can be maintained in culture for a prolonged period of time. We have demonstrated a number of distinct changes in the biology of monocytes that occur during in vitro culture. These include increases in the size of human monocytes, increases in the phagocytic capability of human monocytes and changes in the enzyme markers of these cells, such as an increase in 5'nucleotidase and a loss of peroxidase activity during the first week of culture. Studies are now ongoing to look at the differences in other functional capabilities of cultured human monocytes, such as cytotoxic function, ability to release biologic response modifiers (monokines), and ability to perform other accessory functions of lymphocyte activation. We have developed a system in which monocytes can be maintained in culture and can mature into macrophages while in suspension. We are currently exploring this system in great detail to see if it can be utilized in such a fashion that will allow us to expand the numbers of human monocytes in vitro. To date no reliable system for allowing monocytes to proliferate in culture has been developed and our program would like to see this capability established, since it is at least theoretically possible that such technology would allow us to perform immunoreconstitution experiments on cancer patients that were defective in monocyte function. Current experimentation in this area has focused on two specific techniques: a) the use of low density suspension cultures that allow monocytes to generate their own growth factors, and b) the addition of purified exogenous human colony stimulating factors (monocyte growth factors) that constitute a growth signal for the monocytes that we have maintained in suspension culture.

## III. Characterization of Human Monocyte-Mediated Tumor Cytotoxicity

Probably no one of the multifaceted functions of human monocytes is of more immediate interest to the Program than is their ability to kill tumor cells in vitro. We are engaged in experimentation which is designed to test which tumor targets monocytes kill best, which preparation of monocytes are optimal killers, the isolation of soluble factors (MAF) which can enhance the tumoricidal function of monocytes, and understanding the mechanisms of the actual tumor cell killing event. To date we have been successful in isolating several hundred million purified human monocytes from a normal individual by leukapheresis followed by elutriation, and utilizing these purified cells as a resource for performing large experiments relating to the tumoricidal function of these cells. We

have tested the ability of these cells to kill a wide range of tumor targets including human colon carcinoma, human melanoma, human breast, human B cell leukemia and human erythroleukemia. We have documented that our monocyte preparations have a spontaneous cytotoxic capability against these targets, and the characteristics of tumor cell killing by monocytes appears to be distinct from that observed using purified natural killer cells. Moreover, we have shown that human monocytes are capable of killing targets that are relatively NK cell insusceptible. We have identified a number of compounds which are capable of augmenting the cytotoxic function of human monocytes including alpha interferon and MAF-containing human lymphokine preparations. We are currently designing a reproducible assay for measuring MAF activity in various lymphokine preparations; the ultimate goal of this assay is to purify MAF for subsequent human clinical trials.

IV. Characterization of Functional Subsets of Human Mononuclear Phagocytes

Our work and the work of others have shown that human monocytes have a number of functional capabilities which may be very important vis-a-vis the ability of the cancer patient's immune system to control the growth of malignant cells. Examples of these critical functions include the ability of human monocytes to destroy tumor targets in vitro, and the ability of human monocytes to release a variety of biologic response modifiers (monokines) which may have profound effects in the up-regulation of the immune response. Animal models are being devised to assess the relevance of these phenomena to the function of the immune response in vivo. We are currently investigating whether all human monocytes have the same functional properties or whether certain monocytes have distinct functional capabilities vis-a-vis the others. We are particularly interested in isolating (if it exists) a monocyte subpopulation with markedly enhanced tumor cytotoxicity and/or a subset of human monocytes that has markedly enhanced abilities to produce monokines of interest to our program. To date, we have employed three general strategies to explore the possibility that human monocyte subsets exist: a) the separation of human monocytes on the basis of size; b) the separation of monocytes on the basis of their density; c) the separation of monocytes on the basis of distinct membrane antigenic determinants that they might possess. We have been successful in isolating cells on the basis of size utilizing two techniques, elutriation and fluorescence cell sorter analysis. Cells obtained by these two techniques are currently being assessed for their differential ability to function as cytotoxic cells, accessory cells and/or cells that can release monokines such as interferon and Interleukin-1. Similarly, we have been able to separate monocytes on the basis of their density utilizing albumin gradients and the fluorescence cell sorter. Finally, we have a number of monoclonal antibodies in our possession which recognize distinct membrane determinants present on human monocytes. We have selected monocytes that have these antigens in high vs. low density using complement-mediated lysis and the fluorescence cell sorter. As described previously, all of the various sub-populations of monocytes separated by these techniques are being tested for their differential ability to function as cytotoxic cells, accessory cells, or monokine releasers. We have also taken monocytes isolated on the basis of size and injected them into mice in preparation for generating monoclonal antibodies against small, medium, and large human monocytes. We have performed the fusion of the spleens of these mice and we are now preparing to screen the

monoclonal antibodies produced by these hybridomas. If distinct functional subsets of human monocytes are found, we will attempt to further isolate and purify these subsets and attempt to maintain them in culture. The ultimate objective of these studies is to identify functional subsets of human monocytes if they exist, to assess cancer patients for any defects in monocyte subset function that they might have, and to develop technologies whereby any defective monocyte subsets cancer patients could be reconstituted.

V. Genetic Engineering of Human Monocytes: Establishment of an Activated Monocyte cDNA Library and Characterization of Monocyte Hybridomas

As we longitudinally examine the function of monocytes in the immune response of normal humans, and as we document the various in vitro capabilities of human monocytes, we are constantly attempting to extrapolate information that we obtain about normal human monocyte function to possible defects that may exist at these levels in the cancer patient. Similarly, as we study the functional capabilities of human monocytes, we are constantly striving to move from a biologic understanding of these phenomena to a chemical understanding of these phenomena. For these reasons, we have been engaging in research to understand the biochemical basis for human monocyte function. The major thrust of these efforts has been to understand the functions of monocytes on the basis of the distinctive genes that are operative in these cells. Moreover, we are investigating the mechanisms of macromolecular synthesis which lead to altered phenotypic behavior in human monocytes following the activation event. We have demonstrated that macromolecular synthesis is distinctly different in activated human monocytes as opposed to unactivated human monocytes, and we are focusing particularly on the RNA messages which are produced in activated monocytes. We have activated a very large number of monocytes from a single normal donor and we have isolated the messenger RNA from these activated cells, converted this message into cDNA form, incorporated this cDNA into plasmids, and have established a gene library in bacteria. Following amplification of these messages in bacteria, we will transfect this cDNA into eukaryotic cell systems and screen these eukaryotic cells for distinct monocyte functions that they may possess by virtue of the genetic engineering manipulations that have been performed on them. We are particularly interested in the acquisition of the ability to secrete monokines, and the acquisition of cytotoxic function.

We are also applying hybridoma technology to the problem of isolating gene segments from human monocytes. We have successfully converted a human monocyte cell line (U937) into a HAT-sensitive cell line. We have now performed several fusions of this cell line with normal human monocytes in an attempt to create a proliferating, immortal cell line with distinct functional capabilities of normal human monocytes. Such fusion cell products (monocyte hybridomas) are currently being screened for their ability to perform unique monocyte functions (monokine secretion and tumor cytotoxicity) that are not possessed by the parent U937 line. We are particularly focusing on the ability of these immortal cells to secrete monokines of potential programmatic interest in oncologic clinical trials.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

One of the major research thrusts of the Biological Research and Therapy Branch is to develop new insights and understandings into the role of the immune response as an immunosurveillance weapon to prevent clinical malignancy or as a mechanism whereby established malignancies can be reduced or eliminated. The acquisition of theoretical concepts and practical knowledge in this area has been extremely slow, particularly in the applications of basic research to clinical human situations. We have a major commitment to understanding more about the total spectrum of signals that can augment the human immune response to neoplasia (biologic response modifiers = BRMs), and we have a major commitment to understanding the mechanisms whereby these BRMs are operative in the normal human and in the patient with malignancy. As we understand more about each leukocyte subtype that participates in the immune response and we acquire a deeper understanding of the mechanisms whereby leukocyte subtypes cooperate with one another as they participate in the immune response, we are constantly striving to place these new mechanistic understandings into the clinical setting, whereby we may be able to up-regulate the inefficient immune system of the cancer patient.

The monocyte and its tissue counterpart, the macrophage, have been documented to play pivotal roles in the immune system, particularly vis-a-vis host defense. It is known that these cells are critical for the phenomenon of antigen processing, that they are required as accessory cells for a variety of T lymphocyte and B lymphocyte functions, that they secrete a variety of biologic response modifiers (monokines) that have dramatic immunoregulatory functions, that these cells are capable of producing a wide range of complement components, that these cells are major participants in the phenomenon of antibody-dependent cellular cytotoxicity (ADCC), and it has been demonstrated that monocytes have the capability of destroying tumor targets in vitro. Applications of current understandings about this multifaceted cell to the setting of the patient with malignancy are still forthcoming. A great deal of work still remains to be performed in order to translate current in vitro systems into workable understandings of the in vivo functions of monocytes in humans. Moreover, a great deal of work remains to be performed in order to translate valuable information obtained in animal models to the clinical situation. Our laboratory is completely committed to furthering our understanding of the role of human monocytes in the immune response to neoplasia in the human setting.

We are committed to understanding the mechanisms of monocyte-mediated cytotoxicity against tumors in humans. Moreover, we have developed an assay whereby activators of human macrophage cytotoxicity can be detected. Understanding of the mechanisms of tumor killing by macrophages and the factors which control this function should allow us to up-regulate this function in the cancer patient in the future. Similarly, monocytes are the predominent secretors of a wide variety of biologic response modifiers that are of particular interest to this program (such as interferon, interleukin-1, and colony stimulating factor). We are committed to understanding the mechanisms whereby these soluble substances exert their powerful immunomodulatory effects. In addition, we are devising strategies (such as genetic engineering and monocyte hybridoma technology) whereby we can obtain maximal amounts of purified monokines for subsequent clinical trials.

PROPOSED COURSE OF THE PROJECT

During the next year, we will continue to examine the role of human monocytes in the normal immune response. We will particularly focus on understanding the mechanism whereby monocytes kill tumor targets and we will begin experiments whereby this process is visualized directly on a single cell basis. Moreover, we will continue to explore those signals which modulate the ability of monocytes to kill tumor targets, particularly focusing on macrophage activating factors (MAF). We will embark on a series of experiments whereby large amounts of MAF can be isolated and purified, potentially to the degree of purity required for clinical trials.

Similarly, we will continue with laboratory investigations analyzing the factors which control the differentiation and proliferation of monocytes in vitro. As we gain more experience in this area we hope to be able to expand the numbers of monocytes obtained from a normal human or a cancer patient ten-fold within a period of about three weeks. When this technological advance is achieved, we will prepare for clinical trials of this valuable immunoreconstituting resource.

Genetic engineering of human monocytes appears to be a very promising area of laboratory investigation. A gene library of activated human monocytes has been established and over the course of the next year detailed experimentation will focus on the exact messages that are contained in that library. We will attempt to establish a cell line which possesses functional monocyte genes monokines that are of programmatic interest. Similarly, we will screen the function of monocyte hybridomas that are being established for their ability to secrete monokines that could potentially be used in clinical trials. Similarly, we will continue to perform in-depth investigations to determine whether functional subsets of human monocytes can be recovered.

PUBLICATIONS

1. Stevenson, H.C., and Fauci, A.S.: Purification of human monocytes by counter-current centrifugation elutriation. In Herscowitz, et al. (Eds.): Manual of Macrophage Methodology. New York, Marcel Dekker, 1981.

2. Stevenson, H.C., and Fauci, A.S.: Effects of corticosteroids on the functoin and distribution of human lymphocytes. In Franklin, E., et al. (Eds.): Clinical Immunology Update. New York, Elsevier/North-Holland, 1981.

3. Stevenson, H.C., and Fauci, A.S.: The effect of glucocorticoids and other hormones on the inflammatory and immune response. In Rosenstreich, et al., (Eds.): The Cell Biology of Immunity and Inflammation. New York, Elsevier/ North-Holland, 1981.

4. Fauci, A.S., Stevenson, H.C., Whalen, G., and Andrysiak, P.: Monocyte-mediated immunoregulation of human B cell reactivity. In Revillard, J.P. (Ed.): Symposium Human B Lymphocyte Immunobiology and Cancer Immunotherapy. New York: Raven Press, 1981.

5. Stevenson, H.C., and Fauci, A.S.: Immunodeficiency secondary to pharmacologic agents. In Chandra, R.K. (Ed.): Immunodeficiency Disorders. London: Churchill Livingstone, 1981.

6. Stevenson, H.C., Katz, P., Wright, D.G., Contreras, T.L., Jemiomek, J.K., Hartwig, V.M., LaFlor, W., and Fauci, A.S. Characterization of the negatively-selected human monocyte and their suspension cell culture derivatives. Scand. J. Immunol. 14:243-256, 1981.

7. Fauci, A.S., Stevenson, H.C., Whalen, G., Andrysiak, P.: Monocyte-mediated immunoregulation of human B cell function. In Fauci, A.S., and Bailieux, R.E. (Eds.): International B Cell Workshop--1980: Human B Cell Function, Activation, and Immunoregulation. New York: Raven Press, 1981.

8. Stevenson, H.C.: Macrophages as accessory cells in lymphoproliferative responses. In Bellanti, J.A., and Herscowitz, H.B. (Eds.): The Reticuloendothelial System: A Comprehensive Treatise. New York: Plenum Press, 1982 (in press).

9. De Shazo, R.D., and Stevenson, H.C. Generalized dermatitis to aminophyline. Annals of Allergy 46: 152-154, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09249-03 BRTB formerly Z01 CB 08501-02 LID |
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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Lipid Metabolism in the Function of Human Mononuclear Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH

LAB/BRANCH
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SECTION
Monocyte/Macrophage Section (Proposed)

INSTITUTE AND LOCATION
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| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 4.0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Aspects of lipid metabolism were studied, using highly-developed techniques with purified mononuclear cell subpopulations, including natural killer (NK) cells and monocytes. Phospholipid and neutral lipid methylation or arachidonate metabolism were shown to be altered during NK activity, interferon treatment, and chemotaxis, or tumor promotion. Chemotaxis and superoxide generation by monocytes, evaluated by automated techniques, were correlated with changes in phospholipid metabolism. Spontaneous cytotoxicity by adherent human peripheral blood leukocytes against tumor cell lines was shown to be mediated by NK-like cells, in addition to, or instead of classical monocytes. The characteristics of the effector cells were extensively studied using well-defined monoclonal antibodies against cell surface components. Differentiation of myeloid or monocyte-like cell lines by phorbol myristate acetate (PMA) interferon (or 2'-5' oligioadenylate) and dexamethasone (or lipomodulin) was studied based on changes in morphology, antigenic expression and function in antibody-dependent cytotoxicity and ability to produce superoxide.

## PROJECT DESCRIPTION

### OBJECTIVES

To study the role of lipid metabolism, particularly in the plasma membrane in regulation of immune effector functions of human mononuclear cells (lymphocytes and monocytes), control of differentiation processes, and response to biologically active agents. To examine the role of various subpopulations of lymphoid cells and these metabolic pathways in spontaneous cytotoxicity.

### MAJOR FINDINGS

I. Lipid Metabolism

A. Natural Killer (NK) Activity

Preliminary studies implicating a role for phospholipid methylation in NK-target cell interaction were confirmed using both peripheral blood lymphocytes and purified (Percoll density gradient fractionated) large granular cells. Initial observations on a role of phospholipase $A_2$ in NK lysis were extended by using purified lipomodulin, a naturally occurring inhibitor of the enzyme. Similar to other pharmacologic phospholipase $A_2$ inhibitors, treatment of NK cells with lipomodulin strongly inhibited NK capacity. This occurred with either pretreatment of the effector cells or direct addition to the assay. Since lipomodulin had no detectable effects on conjugate formation (binding) with target cells, it appeared that this enzyme is involved in some post-binding step leading to lysis.

Large granular cells were found to demonstrate a particular distribution of arachidonate in their lipids. In addition to incorporation into phospholipids, arachidonate was found to comprise a considerable portion of di- and tri-glycerides In this respect LGL were more similar to monocytes.

B. Interferon

Our initial observation on inhibition of phospholipid methylation by interferon was confirmed using α-(recombinant) and β-interferon. The mechanism responsible for this inhibition was extensively investigated. Turnover studies and pulse-chase experiments showed no increased metabolism of preformed phospholipids. A direct effect on the methyltransferase was ruled out using cell-free extracts as a source of enzymatic activity. Studies of the methionine pool proved that this effect was not a result of impairment of methionine entry into the cell.

Inhibition of phosphatidylcholine synthesis via the transmethylation pathway was observed within 5 minutes of IFN treatment. Each transmethylated product was shown to be inhibited to the same extent. Tests of other interferons, including two purified preparations of α-recombinant IFN showed that not all interferons shared the property of inhibiting transmethylation. A α-interferon preparation showed a small, but significant degree of inhibition.

C. Tumor Promotors

Phorbol esters with tumor promoting activity (e.g. phorbol myristate acetate (PMA), mezerein) were shown to decrease phospholipid methylation in peripheral blood mononuclear cells. This effect was most prominent in monocytes. Inhibition of transmethylation was shown to be of the competitive type, based on extensive kinetic studies. This effect was seen at doses of PMA which were not toxic, and depended on the methionine concentration. PMA was also shown to increase incorporation of choline into phosphatidylcholine via the CDP-choline pathway in these cells.

PMA was shown to have minimal effects on already transformed cell lines. These included myeloid and monocyte-like cell lines which are capable of responding to PMA by differentiation towards mature monocytes (see below).

D. General Aspects of Phospholipid Metabolism in Peripheral Blood Mononuclear Cells

Techniques of extraction and purification of various species of lipids were refined and applied to quantification in the fentemolar range of phospholipid synthesis by small numbers of highly purified subpopulations of peripheral blood mononuclear cells. The technique of measuring phospholipid methylation by incorporation of methionine into phospholipids was evaluated using a variety of extraction procedures and a number of different chromatographic systems. A technique which accomplished preservation of lyso-phospholipids in the extract, in the absence of non-specific hydrolysis, which also yielded a high degree of purity and (>90% of radioactivity accounted for in phospholipids) was developed. Other techniques for evaluating synthesis of phosphatidylcholine by the CDP-choline or CDP-ethanolamine pathways were similarly established.

Techniques for measuring labeling, purification, and quantification of the amino-phospholipids on the external aspect of the plasma membrane were adopted for use with the type and number of effector cells available to us after purification procedures. This method, involving trinitrobenzene sulfonate (TNBS), can be combined with radioactive labeling to determine the quantity of phosphatidyl-ethanolamine facing the outside of the cell thereby obtaining a direct measure of membrane effects which contribute to fluidity.

E. Monocyte Function

Metabolism of lipids unique to mnocytes were found to have a high rate lipid metabolism phospholipid methylation. These pathways were affected by interferon and phorbol esters as described above, and apparently accounted for the major changes observed in peripheral blood. In addition, monocytes were found to contain large amounts of neutral lipids, as had been observed by others. Significantly, certain as yet unidentified species of neutral lipids have been shown to be methylated. Preliminary evidence shows that the degree of this methylation is altered by interferon. Techniques for preparative scale separation using reverse-phase thin-layer chromatography and high-performance liquid chromatography yielded sufficient quantities of pure methylated product to allow analysis by gas chromatography and mass spectroscopy.

Neutral lipids of monocytes were also identified as a source of unsaturated fatty acids released in response to certain stimuli, such as interferon, PMA, or formylated peptides (see below).

F. Changes in Phospholipids Methylation and Chemotaxis

Published studies have implicated phospholipid methylation (PLM) as a mechanism of transducing signals derived from interaction of cell membrane-receptor with chemoattractants. These studies including our own on NK cells during their interaction with susceptible targets, are based on pharmacologic inhibition studies and observations on increased arachidonic acid release from cells capable of responding to chemoattractants. In our laboratory, using purified monocytes obtained by elutriation, we confirmed inhibition of phospholipid methylation by high doses of chemoattractants such as f-met-leu-phe, but failed to find such with C5a. Also, conditions of varying methionine concentration known to favor (low) or prevent (high) regulation of IIM by chemoattractant had no effect on chemotaxis. In our assay system (see below), under conditions documented to decrease PIM (i.e. treatment with PMA), the predominent effects appeared to be on random motility, rather than on chemotaxis. This seems to refute the contention in the literature that chemotaxis is accompanied by changes in PIM.

II. Monocyte Function

A. Spontaneous Cytotoxicity

Studies begun last year on cytotoxicity against adherent breast, kidney (mouse), colon, or brain tumor cell lines were continued. Suitable conditions for labeling targets and their incubator with effector cells were determined. Extensive comparison between cytotoxicity by unfractionated mononuclear cells and populations of non-adherent, adherent, small lymphocyte-enriched, and monocyte-enriched (by elutriation) cells was performed.

Non-adherent as well as adherent cells had cytotoxic capacity, as did lymphocytes as well as monocytes. Interferon or lymphokine boosted cytotoxicity in both lymphocyte-enriched and monocyte-enriched fractions. Culture of mononuclear cells in fetal bovine serum for up to 6 days resulted in enhanced cytotoxic capacity in unfractionated and non-adherent fractions. Close inspection of cell morphology in adherent or monocyte-enriched fractions revealed the presence of significant numbers of non-phagocytic cells (up to 15-20% in adherent and 10% in elutriated fractions). For most of the target cells tested, cytotoxicity was greatest in elutriated fractions enriched for intermediate-sized cells, i.e. those between typical T cells and large (phagocytic) monocytes.

This observation implicating NK-like cells in cytotoxicity of adherent tumor cell lines was characterized in greater detail using monoclonal antibodies. Peripheral blood mononuclear cells, adherent, or elutriated populations were depleted of monoclonal antibody-reactive cells by complement-dependent lysis or separated into monoclonal antibody positive or negative subsets by an indirecting rosetting technique and Ficoll-Hypaque density gradient separation. The experimental data indicated that in either peripheral blood mononuclear

cells (PBMNC) or monocyte-enriched populations, effector cells which are cytolytic for adherent human or mouse tumor target cells were B43.4.1 (or OKM1)-positive lymphocytes with E-rosette receptors. These effector cells were BRL.1-, BRL.2-, B52.1.1-, B44.1.1-, B13.4.1- and DR antigen-negative cells, unlike classical monocytes. Some cells which are cytotoxic for mouse cell line TU-5, an adherent SV-40 transformed kidney tumor-line, may bear B52.1.1 or other monocyte-associated antigens.

B. Monocyte Chemotaxis.

An assay system for monocyte chemotaxis through a Millipore filter in a Boyden chamber was set up using unfractionated mononuclear cells or monocytes purified by elutriation. Dependence on the amount of protein and the cell concentration was observed. Chemotaxis was assayed in response to formylated peptide as well as $C_5a$ (zymosan-activated serum). An automated system for analyzing and storing such data was developed.

Monocyte chemotaxis was found to correlate poorly with inhibition of phospholipid methylation. Doses of formylated peptide which caused greatest inhibition of phospholipid methylation as assessed by methionine incorporation were found to inhibit random motility as well as chemotaxis. The dose of f-met-leu-phe which caused maximal chemotaxis ($10^{-8}$M) resulted in little inhibition of PLM. Other agents which caused decreased PLM (PMA, interferon) failed to cause chemotaxis; rather, they inhibited random motility. Furthermore, at concentrations of methionine where effects of PLM are not apparent, chemotaxis was observed to take place unimpaired. Thus, alterations in PLM do not appear to be required for chemotaxis of human monocytes.

C. Superoxide Production

A rapid microassay system which employes "through-the-well" spectrophotoscopic measurement was used to measure superoxide ion ($O_2$) generation in peripheral blood mononuclear cells or tumor cells induced to differentiation by a variety of agents. Monocytes, but not lymphocytes, were found to produce superoxide in response to PMA or a variety of other agents, including lymphokines with macrophage activating factor activity (which did, however, contain residual mitogen). Interferon ($\beta$, or $\alpha$) failed to show consistent effects on either spontaneous or PMA-induced superoxide production

When PBMNC or purified monocytes were allowed to differentiate in vitro into macrophages, the ability to produce superoxide was lost. This effect was dependent on the type of serum used for culture, taking place optimally in human serum and poorly in fetal bovine serum.

The acquisition of the ability to produce superoxide production was also used as an index of differentiation by certain tumor cell lines. DMSO, hypoxanthine, or actinomycin D were able to induce differentiation of a number myeloid cell lines including HL-60, KG-1, K562, and RM-1 towards more mature granulocyte-like cells. PMA was able to induce H2-60 and U937 (a monocyte-like cell line) towards monocytes (see below).

D. Differentiation of Myelomonocytic Cell Lines

Preliminary observations on the superoxide production by differentiated cell lines stimulated more detailed studies on differentiation. Of particular interest proved to be the initial observation that U-937 cells, when cultured with interferon for 6 days, manifested both superoxide production and morphologic changes typical of monocyte-macrophages. Detailed studies of human fibroblast (β) or leukocyte (α) interferon on differentiation of a human histiocytic lymphoma-derived cell line (U937) or promyelocytic leukemia-derived cell line (HL-60) were performed. When cultured with β-IFN (40-1,000 U/ml), U937 cells showed gross morphologic and microscopic changes consisting of clumping, increased prominence of cytoplasmic granules and membrane ruffling. After culture with β-IFN, the number of U937 cells reactive with B43.4.1 monoclonal antibody, which is specific for human monocytes, natural killer cells and neutrophils, increased from fewer than 10% of untreated U937 cells to 47%. β-IFN treatment also enhanced antibody dependent cellular cytotoxicity (ADCC) against chicken red blood cells and phorbol myristate acetate (PMA)-induced superoxide production by U937 cells. The same morphologic, phenotypic and functional changes were also observed when U937 were treated with purified α-IFN, which was produced by recombinant DNA technology. In contrast, HL-60, which differentiates towards cells of the monocyte lineage in response to PMA (based on the above criteria), and towards granulocytes in response to dimethylsulfoxide (DMSO), did not differentiate when cultured with IFN. No consistent relationship between induction of differentiation and changes in phospholipid methylation were observed.

In collaboration with A. Schmidt, the effect of (2'-5')oligoadenylate on differentiation of U937 cells was also examined. This oligonucleotide is the product of (2'-5')oligo(A) synthetase, which is induced in interferon-treated cells. Treatment of U937 cells with (2'-5')oligoadenylate was able to mimic the effects of interferon, inducing differentiation as evidenced by all the criteria cited above. HL-60 cells, however, were not induced to differentiate by (2'-5')oligo-isoadenylate. Thus, for U937 cells differentiation by IFN may be mediated through its induction of (2'-5')oligoisoA.

Differentiation of U937 cells towards monocytes was also studied using corticosteroids (dexamethasone) and lipomodulin (see above). Both were found to induce differentiation of U937 cells which could be inhibited by monoclonal anti-lipomodulin antibody. These data suggested that dexamethasone-induced differentiation of U937 is mediated via lipomodulin. Possibly lipomodulin exerts the activity needed to arrest cell growth and differentiate these cells by interfering with TIA activity usually associated with transformation or stimulation of growth (blastogenesis).

III. Monoclonal Antibodies to Cell Surface Components

Using an enzyme-linked immunosorbent assay, clones were derived which reacted with human monocyte cell surface components. However, more detailed analysis of their reactivity with other cell types in other assays such as fluorescent activated cell sorting or indirect rosette-formation failed to identify any one as uniquely reactive with monocytes or a subpopulation thereof.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

The place of lipid metabolism in immune regulatory and effector function and the role of monocytes in human defense against malignancy are both relatively unexplored areas of immunologic investigation. We have combined our interests in both these areas.

Phospholipids are components of the cell membrane which in large part determine its functional state. Changes in lipid metabolism result in profound effects on the interaction of the cell with its environment. We are attempting to gain an understanding of how agents which affect the immune system may mediate their effects by effecting changes in phospholipid metabolism. Monocytes have been suggested to play a significant role in controlling tumor cell proliferation in experimental animals and humans. Furthermore, a number of immunoadjuvants have been shown to exert their action via effects on the monocyte. Cytotoxicity and cytostasis represent in vitro correlates for in vivo functions of monocytes which are active against neoplastic cells.

PROPOSED COURSE OF THE PROJECT

I. Lipid Metabolism

A. Functional Studies

Initial studies implicating PIM in NK-target interaction are still open to interpretation. NK assays will be carried out under conditions known to induce a specific change in lipid methylation. To avoid the lack of specificity of general metabolic inhibitors on non-methylation pathways, we will use different concentrations of methionine, since we have shown that regulation of PL methylation depends on the level of methionine. Interferon boosting of NK cytotoxicity will be examined under the conditions where the effect on cell phospholipid methylation is known.

B. Analytical Studies of Lipid Metabolism in Natural Killer Cells

1. The studies described above have focused on changes in one pathway of lipid metabolism. The relationship between NK activity and lipid metabolism will be examined by evaluation of changes occuring during NK-target cell interaction. Preliminary work on NK cell activity has been hampered by the global nature of interference with methylation reactions accomplished by pharmacologic inhibitors, i.e., deozadinosine.

Comparison will be made between NK cells or target cells alone or together. Evaluation of the effector-target interaction will be made under conditions where binding can take place and under conditions known to prevent it. For example, we plan to compare lipid metabolism under calcium and magnesium free conditions.

Specific pathways to be examined will be synthesis of phospholipid by the CDP-choline, CDP-ethanolamine and transmethylation pathways, methylation of neutral

lipids, fatty acid distribution into species of phospholipids. Plasma membrane redistribution of endogenous phospholipids will also be examined.

2. Boosting of NK by interferon, and lipid metabolism. The effect of interferon on each of the above pathways will be carried out as described in a separate section of this protocol. The studies outlined will be performed using effectors and/or target cells pretreated with interferon.

BIOCHEMICAL ALTERATIONS ASSOCIATED WITH INTERFERON TREATMENT

1. Fatty acid composition and plasmalogen content of the external plasma membrane phospholipids. Purified populations of mononuclear cells (monocytes, LGL) or tumor cells will be treated with different interferons ($\alpha$, $\beta$, $\gamma$). TNBS will be used to label amino groups on the external side of the cell. Phospholipids will be extracted into organic solvents, and TNBS-Phosphatidylethanolamine purified by preparative thin layer chromatography. Fatty acid composition of TNBS-PE will be analyzed by reverse phase HPLC and gas chromatography after hydrolysis and methyl esterification of the fatty acids. Plasmalogen content will be determined after introducing $^{14}$C-ethanolamine into TNBS-PE and performing hydrolysis under alkaline and acidic controlled conditions. Identification of products of hydrolysis will be accomplished by analytical thin layer chromatography.

2. Phospholipid and neutral lipid methylation will be analyzed as already described in purified populations of cells treated with interferons. Phospholipid methyl-transferase activity of interferon-pretreated cell extracts will be assayed by measuring the enzymatic transfer of the methyl group from (Methyl-$^{3}$H)-S-adenosylmethionine (SAM) into exogenous phospholipid brought into aqueous solution by sonication and detergent solubilization. Phospholipid methylation will be quantified by measuring the radioactivity incorporated into the phospholipids extracted by organic solvents, and identified by thin layer chromatography.

3. Cholesterol incorporation into cell membranes will be measured by labeling interferon treated purified cells with radioactive cholesterol, and extracting the cells with organic solvents. For the above labeling procedures, different periods of incubation with the isotope or interferon will be evaluated.

4. Protein methylation will be examined in vitro using whole cells or extracts. Cells will be treated under different conditions with interferons. The TCA precipitate will be cleared of labeled nucleic acid by hot TCA extraction, and of lipids by extraction with organic solvents. Protein carboxymethylation will be quantified by measuring the amount of radioactive methanol formed during alkaline hydrolysis of the treated TCA precipitate. Protein N methylation will be estimated by incorporation of (1-$^{14}$C) methionine into the TCA precipitate prepared under the same conditions and comparison with the incorporation of (Methyl-$^{3}$H)-Methionine.

Cell free assays will be performed by using homogenates of treated cells as crude enzyme preparations, or after further purification by 105,000g centrifugation, (Methyl-$^{3}$H)-SAM as methyl donor, and exogenous proteins as methyl acceptor substrates.

II. Monocyte Function

A. Spontaneous Cytotoxicity

Further studies within this group are not planned given the conclusion that this activity is mediated in large part by NK-like cells. Interest in adherent NK cells will be continued in collaboration with the Natural Immunity Section of our Branch to determine their role (if any in immune surveillance in vivo).

B. Chemotaxis

We plan to further evaluate the effects of phospholipid transmethylation in purified monocytes exposed to chemotactic peptide. We will examine the effects of inhibitors of the s-adenosyl-methionine (SAM)-dependent transmethylation reactions on the monocyte chemotaxis, chemokinesis and random migration including an evaluation of the "true chemotaxis" (in order to differentiat the effect on the intrinsic ability of the cells to migrate from that to the ability to recognize a chemotactic gradient). We will also investigate whether or not drugs known to prevent or reverse deactivation in neutrophils exert a similar action on monocytes and whether they exert effects on SAM-dependent transmethylation reactions.

If similar mechanisms hold true for monocytes as for neutrophils, we consider evaluations of sera or effusion fluids from patients with malignancy on chemotaxis, activation-deactivation, and methylation. If lipid methylation proves not to be integral to monocyte chemotaxis, we plan to probe the role of other transmethylations reactions, principally protein carboxymethylation, in chemotaxis be human cells.

PUBLICATIONS

Hoffman, T., Hirata, F., Bougnoux, P., Fraser, B.A., Goldfarb, R.H., Herberman, R.B., and Axelrod, J.: Phospholipid methylation and phospholipase $A_2$ activation in cytotoxicity by human natural killer cells. Proc. Natl. Acad. Sci. 78: 3839-3843, 1981.

Douillard, J.Y., and Hoffman, T.: Basic facts about hybridomas. In Schwartz, L. (Ed.): Compendium of Immunology. New York, Van Nostrand, Rheinhold Co. Ltd., Vol. II, in press.

Jett, J.R., Hoffman, T.M., Connor, R.J., and Herberman, R.B.: Effect of various immunoregulatory substances on the growth inhibitory activity (GIA) of human monocytes against lymphoblastoid cell lines. Immunopharmacology 4: 105-113, 1982.

Douillard, J.Y., and Hoffman, T.: Enzyme-linked immunosorbent assay for screening monoclonal antibody production using enzyme-labeled second antibody. In van Vunakis, H., and Langone, J.J. (Eds.): Methods in Enzymology. Immunochemical Techniques, Part. E. New York, Academic Press, in press.

Hoffman, T., Bougnoux, P., Hattori, T., Chang-Z-l., and Herberman, R.B.: Phospholipid metabolism during NK cell activity: Possible role for transmethylation and phospholipase $A_2$ activation in recognition and lysis. In Herberman, R.B. (Ed.): Natural Cell-Mediated Immunity. New York, Academic Press, Volume 2, in press.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 09250-07 BRTB formerly Z01 CB 08517-06 LID |
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PERIOD COVERED October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Macrophage Immunobiology in Experimental Tumor Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
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COOPERATING UNITS (if any)

Litton Bionetics, Inc.; CorBel Laboratories; Laboratory of Microbial Immunity, NIAID, NIH; Surgery Branch, NCI, NIH

LAB/BRANCH Biological Research and Therapy Branch

SECTION Monocyte/Macrophage Section (proposed)

INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701

| TOTAL MANYEARS: 5.4 | PROFESSIONAL: 4.4 | OTHER: 1 |
|---|---|---|

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were performed to investigate the role of macrophages against tumor cells in vivo and to compare the contribution of these cells to that of natural killer (NK) cells. Animals inoculated with Brewer's thioglycollate-elicited macrophages 2 days before to 15 minutes after iv inoculated of B16 tumor cells, had reduced clearance of the tumor cells and developed a greater number of metastases. Animals treated with antibody to asialo $GM_1$ had reduced NK activity and a greater number of metastases. Adoptive transfer of activated macrophages reduced the number of metastases in anti-asialo $GM_1$-treated mice. Studies of the homing of adoptively transferred macrophages indicated that the traffic pattern of these cells is in part dictated by the type of agent used to elicit the peritoneal macrophages. Two new monoclonal antibodies have been developed that are specific for macrophages. Both recognize antigens that are found on thioglycollate-elicited cells and on cultured macrophages from bone marrow but are weakly expressed on resident and peptone-elicited macrophages. Plasminogen activator production has been used as an indicator for activation with macrophages treated with macrophage activation factor (MAF) but there is not a positive correlation with macrophages activated by endotoxin. T cell hybrids producing MAF have been developed from Ly 1+ 2+ and Ly 1+ 2- enriched populations of T cells.

## PROJECT DESCRIPTION

### OBJECTIVES

The general objectives of this project are: 1) to gain insight into the changes that occur during the process of macrophage activation; 2) to determine the relative roles of macrophages and natural killer (NK) cells in control of metastasis and tumor formation; 3) to examine the interactions between tumor cells and macrophages; 4) to identify and characterize macrophage subpopulations and macrophages at different stages of differentiation and/or activation, and 5) to develop T cell hybrids that produce macrophage activation factor (MAF).

### METHODS EMPLOYED

Lymphoid cells were obtained from the spleen, lymph nodes, peritoneal cavity, peripheral blood, thymus and tumor of tumor bearing and normal mice. Normal and tumor cell lines were maintained in tissue culture. A variety of tumors induced by viral, chemical or unknown agents were maintained by transplantation in syngeneic animals. Immune and normal lymphocytes and tumor cells were stored in a viable frozen state. Inbred strains of mice were employed for several different allogeneic and tumor model systems. Immunological competence and/or cellular immune reactivity were measured in vitro by the following tests: lymphocyte stimulation, lymphocyte- and macrophage-mediated cytotoxicity in $^{51}Cr$ and $^{111}InOx$ release assays, production of lymphokines, measurement of plasminogen activator production, suppression of mitogen-induced immune functions and inhibition of growth of lymphoma cells. Normal spleen cells from immune animals were fused with plasmacytoma tumor cells and the resulting hybrids cloned to select lines that produce monoclonal antibodies to macrophage cell surface antigens. Normal spleen cells stimulated with mitogen were also fused to T cell lymphoma cells and the hybrids selected for the production of MAF. Monoclonal antibodies were tested in a radioimmune assay, a cytotoxicity assay or by immunofluorescence with flow microfluorometry.

### I. Migration of Adoptively Transferred Macrophages (MØ)

Previous studies from our laboratory have shown that MØ elicited with Brewer's thioglycollate (thio) medium, which contains agar, accumulate rapidly in the lung and remain there in significant numbers for up to 72 hours. Very few of these cells migrate to the spleen. In contrast, when MØ are elicited with proteose peptone or thioglycollate broth (which contains no agar), a different migration pattern is observed. MØ elicited with peptone or thio broth appear to be monocyte-like in morphology while MØ elicited by Brewer's thio appear to be fully differentiated MØ (large and vacuolated with a ruffled membrane). MØ elicited by thio broth and peptone rapidly traverse the lung, migrate to the liver or migrate to the spleen (up to 20-25%), a striking contrast to the homing pattern observed for MØ elicited with Brewer's thio. These observations correspond with additional data which indicate that fully activated MØ lose their spleen homing capability. We have therefore studied ways to get activated

MØ to home in a similar way as normal macrophages. This has been accomplished by activating PEC in vivo with MVE-2 and poly I:C/L:C. It is known that these cells require contact with these agents for only a short period of time for them to differentiate to activated, cytotoxic MØ. Consequently, we "activate" the macrophages for 3 hours in vivo, remove them for labeling in vitro while they remain morphologically unactivated, then inject them i.v. into recipients. These cells home with a pattern similar to that of cells that have not been activated and differentiate to activated cells in situ.

## II. Enhancement of Metastasis Formation In Vivo by Macrophages

It has been previously shown in our laboratory that certain macrophage (MØ) populations are capable of dramatically suppressing natural killer (NK) cell activity in vitro. Since one postulated role for NK cell activity in vivo is regulation of tumor cell dissemination (metastasis), experiments were designed to determine whether administration of macrophage populations which suppressed NK activity in vitro also augmented metastasis formation in vivo. Thioglycollate-elicited macrophages were injected intravenously from 2 days before to 15 minutes after an intravenous injection of B16 melanoma cells. These studies revealed that macrophage treatment significantly decreased the rate of tumor cell clearance from the lungs (which is considered to be an in vivo assay for natural immunity) and concomitantly increased the incidence of lung metastases. Numerous other cell types failed to mediate these effects including lymphocytes, unrelated tumor cells and macrophage-like cell lines. Thioglycollate MØ lysates obtained by repeated cycles of freezing and thawing were as effective at augmenting metastasis as whole cells. Studies are currently underway to more definitively elucidate the mechansism(s) involved in macrophage-mediated metastasis augmentation by focusing on the regulation of natural immunity in vivo by adoptively transferred MØ.

## III. Modulation of In Vivo Natural Immunity and Tumor Metastasis by Treatment with Anti-Asialo GM1 (as GM1) Serum

Treatment of mice cells in vivo with anti-as GM1 or of spleen cells in vitro with anti-as $GM_1$ serum + C has been shown to abrogate NK cell activity, while having minimal effects on other cells of the immune system. Based on these observations, experiments have been performed to evaluate the role of NK activity in vivo by depletion of that function through intravenous injections of anti-as $GM_1$. In vivo injection of anti-as $GM_1$ results in complete abrogation of splenic NK activity and corresponding significantly reduces the rate of lung clearance of intravenously injected tumor cells. This abrogation of NK activity and decrease in lung clearance correlates closely with an increased incidence of lung and liver metastases in the B16 melanoma "artificial" metastasis model. These results lead us to postulate that NK activity in vivo may contribute significantly to the control of metastases. This hypothesis has been confirmed by the reconstitution of the antimetastatic defenses by transfer of normal spleen cells to anti-as $GM_1$ treated recipients.

IV. Use of Anti-as GM1 to Compare the Relative Efficiency of Macrophages and NK Cells Against Metastases.

It has been reported that NK cells or macrophages can function as anti-metastatic effectors in vivo. However, the data in most cases fail to take into account effects on the host's immune system other than those attributed to the specific cell type under study. Specifically, it is not clear what effects activation or transfer may have on endogenous NK activity of the host. A series of experiments has been designed to investigate the ability of activated MØ to mediate anti-metastatic function in animals, whose NK activity has been suppressed by in vivo administration of anti-as $GM_1$ serum.

Preliminary studies have focused on the specificity of anti-as $GM_1$ serum for depleting NK activity in vivo and its parallel effects on macrophage function. Treatment of mice with anti-as $GM_1$ did not appreciably reduce the susceptibility of peptone-elicited macrophages to be activated for cytotoxicity in vitro. More notable effects were observed when activated macrophages were treated with anti-as $GM_1$ in vitro or in vivo. Treatment of MØ activated by MVE-2 with anti-as $GM_1$ plus complement in vitro significantly reduced, but did not abrogate, macrophage-mediated cytotoxicity. A similar reduction in MØ-mediated cytotoxic activity was observed when anti-as $GM_1$ treated mice were used as the source of MØ activated in vivo with MVE-2. Further studies are underway to determine the expression of anti-as $GM_1$ on various subpopulations of monocyte/MØ.

Since NK activity is totally abrogated by anti-as $GM_1$ treatment and cytotoxic MØ function is not, effects of MØ transfer into anti-as $GM_1$ treated recipients was used as a model for MØ-mediated modulation of metastasis. These studies demonstrated that transfer of activated MØ i.v. could mediate a dramatic reduction in hepatic metastases in anti-as $GM_1$ treated recipients. These studies indicate that activated MØ may directly act as anti-metastatic effectors and that both NK cells and activated MØ participate in the defense against metastasis.

V. Suitability of Various Isotopes as Cellular Labels for In Vivo Tumor Cell Clearance Assays

The standard assay for assessing lung clearance of i.v. injected tumor cells and its correlation with NK status of the host has employed [$^{125}$I]dUrd as the tumor cell label. While [$^{125}$]dUrd is suitable for these purposes in many situations, it has several disadvantages. Chief among these are toxicity for certain cells and a relatively long labelling period which requires cell division, raising the question about the label distribution within a population of cells.

Several other isotopes which label by cytoplasmic incorporation, and therefore do not require cell division for uptake have been widely used for cell migration studies. Perhaps the two most widely used isotopes are $^{111}$InOx and $^{51}$Cr, both of which have the added virtue of requiring a relatively short labeling period. $^{111}$InOx is particularly advantageous in this regard. Studies which compared rates of lung clearance of [$^{125}$]d Urd, $^{111}$InOx, and $^{51}$Cr reveal the three isotopes to be similar in clearance kinetics. Cells labelled with any of these isotopes were cleared in a similar manner. Based on these findings, it is concluded that all three labels are suitable for the assessment of in

vitro NK activity by lung clearance assay. However, when liver clearance data was analyzed, it became evident that the two cytoplasmic labels ($^{111}$InOx and $^{51}$Cr) were retained in the liver, while [$^{125}$I]dUrd was eliminated. This finding led to the conclusion that both $^{111}$InOx and $^{51}$Cr are significantly taken up by the liver after they are released from tumor cells and therefore may not be suitable for clearance or migration studies where analysis of liver uptake is required.

VI. Monoclonal Antibodies to Macrophage Cell Surface Antigens

We are interested in examining the activation and differentiation of macrophages. It appears that there are different subpopulations of macrophages, which vary in their stage of activation or differentiation. These subpopulations have been separated on the basis of their size, density or surface characteristics. Reports from several laboratories indicated that there also are antigenic differences among macrophages obtained from different anatomical sites and among macrophages at different stages of activation and/or differentiaion. However, the antisera utilized to identify these differences were very difficult to prepare because very large numbers of adsorptions were required to make the antisera specific. Monoclonal antibodies would be more desirable because these adsorptions would not be necessary and the reagents would be against monospecific determinants. We initiated these experiments to develop monoclonal antibodies against cell surface antigens on macrophages in an attempt to provide reagents that could identify subpopulations of macrophages based on their antigenic characteristics.

Hybridomas were produced by the fusion of the mouse myeloma, NSI, with spleen cells from rats immunized with a macrophage cell line, P388D$_1$, or with bone marrow cultured macrophages that had been activated with poly I:C. After fusion, the hybrids were selected in HAT medium and then cloned and tested for the production of cytotoxic antibodies to various targets. Clones were selected that appeared to be producing antibodies specific for macrophages and further characterization was performed.

One clone, 32.50 has been previously obtained and characterized. 32.50 reacts with resident, elicited and activated macrophages. It does not bind to normal, unstimulated lymphocytes. However, it does react with stimulated lymphocytes, bone marrow cells and granulocytes. Two other monoclonal antibodies have been recently produced, 36 and 52. These antibodies appear to be specific for macrophages since they do not react with bone marrow cells, granulocytes, thymocytes, lymph node cells or spleen cells. They both react strongly with bone marrow-derived macrophages and Brewer's thioglycollate-elicited peritoneal macrophages. The antigens to which these antibodies react are expressed weakly on peptone elicited and resident peritoneal cells. The pattern of reactivity appears to be different between these antibodies when tested against macrophage-like cell lines, although both bind to and lyse the majority of lines tested.

VII. Use of Plasminogen Activator (PA) Production as an Indicator of Macrophage Activation

Plasminogen-dependent fibrinolysis is an excellent assay for measuring the products of activated lymphocytes that activate macrophages. We compared secretion of PA from several macrophage-like cell lines with that of thioglycollate-

elicited macrophages and found that the cell lines produced PA constitutively and therefore did not respond to stimulation with MAF. Several of these lines produced large amounts of PA and would be good candidates for a source of PA for purification. During the study, we also found that a significant part of the PA produced by thioglycollate-elicited macrophages is cell-associated and would not be detected if supernatants alone were tested. Non-plasminogen dependent proteolysis was small when compared to PA secretion.

We attempted to correlate PA production with the induction of macrophage-mediated cytotoxicity by endotoxin (LPS). We found that LPS does not induce PA production even though it does induce cytotoxicity. In addition, if the macrophages have been previously activated to produce PA, the addition of LPS markedly reduced the levels of PA production. This observation helps to explain the diverse reports on the effect of LPS on PA production since we found that macrophages must be activated before they are sensitive to the inhibitory effects of LPS.

VIII. Production of T-T Hybrids Utilizing Lyt Subpopulations

Lyt subpopulations were isolated by the use of monoclonal antibodies and a panning technique. Both Lyt 1+2- and Lyt 1+2+ cells were capable of producing MAF, as well as small quantities of interferon, when stimulated with mitogens. Studies on the role of macrophages in the production of MAF by T cell subpopulations indicates that during the first 24 hours peritoneal macrophages act as helper cells while at 48-56 hours there was no longer help and even some suppression was observed.

Experiments in which T cell subpopulations were utilized for fusion with a T cell lymphoma to produce hybrids indicated that there was an increased frequency of hybrids when the subpopulations were employed as compared to unfractionated spleen cells. One such hybrid which produces MAF (as detected by PA production and activation for cytotoxicity) has been selected for further study of the surface phenotype and the production of MAF.

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The immune response to neoplasia is very complex and involves not only the interaction between host effector cells and tumor cells, but also interactions among the various subpopulations of leukocytes. Some cells and/or factors may serve to amplify the immune response, while others may suppress it. We have been studying some of these interactions on several levels to learn more about the host effector mechanisms that are antagonistic to the growth of the tumor and about the factors or cells that regulate the development of these responses.

Macrophages are capable of modulating the host's response to the tumor, both by their ability to act as accessory cells in the development of an immune response and by their capacity to suppress development of immune functions. We have found that macrophages exert their suppressor activity very early during the development of the immune response and may act by inhibiting protein synthesis. A delicate balance may exist between the accessory function and suppressor function of macrophages and the ability of a host to deal with a tumor

may be critically affected by the net state of balance between these regulatory functions. Understanding all these interactions, what influences them and how they can be modulate to the benefit of the host, will give us information that we can use to assist the host in dealing with its tumor.

Macrophages can also have direct antitumor activity. Studies on the process by which macrophages become activated have indicated that there are multiple stimuli which are required for macrophages to become cytolytic. In addition, there appears to be several different stages of differentiation which may be represented by distinct subpopulations of macrophages. Identifying the mechanism of macrophage activation and the subpopulations involved in this process should provide information that can be used to establish immunotherapy regimens.

Natural killer cells may have an important role in the protection of the host from tumor induction (immune surveillance) or metastasis, or in the antitumor response after different types of immunotherapy. Studies on the mechanisms regulating their functional activity should not only provide insight into the control of the cytotoxic activity of these cells but also provide the means to modulate the levels of activity in vivo, thereby enabeling more detailed experiments about their in vivo relevance to be performed.

## PROPOSED COURSE OF THE PROJECT

During the next year we will continue to examine the role of macrophages in host resistance to tumor. We will also compare the relative contribution of NK cells and macrophages against metastatic and primary tumor growth. In addition, we will continue to identify macrophages at different stages of differentiation and/or activation by the use of monoclonal antibodies.

The role of macrophages in the host response to tumor growth will be evaluated by utilizing animals whose NK activity has been specifically depressed by treatment with anti-asialo $GM_1$. Macrophage and T cell functions will be assessed to determine the effects on their functional activity as a result of treatment with the antisera. Subsequently, growth of metastatic tumor nodules will be measured in animals that have been treated to activate their macrophages or that have received activated macrophages by adoptive transfer.

Additional monoclonal antibodies against cell-surface antigens of macrophages will be developed. Attempts will be made to obtain reagents which react with macrophages at different stages of activation and/or differentiation or with different subpopulations of macrophages. These monoclonal antibodies will be characterized and then utilized to study activation and/or differentiation of macrophages and to assess the role of macrophages in a variety of immune responses and in response to tumor growth.

Finally, suppressor macrophages will be induced in vitro and characterized for their surface antigens using monoclonal antibodies to try to identify the phenotype of these cells. Several assays will be performed to examine the functional activities that the suppressor macrophages can express. Once suppressor macrophages have been characterized in vitro, similar studies will be performed to examine the phenotype and functional activities of suppressor macrophages in tumor-bearing mice.

PUBLICATIONS

Brunda, M.J., Taramelli, D., Holden, H.T., and Varesio, L.: Suppression of Murine Natural Killer Cell Activity by Normal Peritoneal Macrophages. In Herberman, R.B. (Ed.): NK Cells and Other Natural Effector Cells. New York, Academic Press, volume 2, in press.

Brunda, M.J., Varesio, L., Herberman, R.B., and Holden, H.T.: Interferon-independent, lectin-induced augmentation of murine natural killer cell activity Int. J. Cancer, in press.

Gorelik, E., Wiltrout, R.H., Brunda, M.J., Holden, H.T., and Herberman, R.B.: Augmentation of metastatis formation by thioglycollate-elicited macrophages. Int. J. Cancer, in press.

Jones, C.M., Varesio, L., and Herberman, R.B.: Interferon Activates Macrophages to Produce Plasminogen Activator. In Dumond, D. (Ed.): Proceedings of the 3rd International Conference on Human Lymphokines. New York, Academic Press, in press.

Mattes, M.J., and Holden, H.T.: The distribution of Helix pomatia lectin receptors on mouse lymphoid cells and other tissues. Eur. J. Immunol. 11: 358-365, 1981.

Wiltrout, R.H., Taramelli, T., and Holden, H.T.: Measurement of macrophage-mediated cytotoxicity against adherent and nonadherent target cell by release of $^{111}$Indium-oxine. J. Immunol. Methods 43: 319-331, 1981.

## IMMUNOPHARMACOLOGY SECTION

On September 4, 1981, the Director, NCI, approved the transfer of the Virus and Disease Modification Section from the Developmental Therapeutics Program, Laboratory of Chemical Pharmacology, to the Biological Response Modifiers Prgram (BRMP), Biological Development Branch. The title of the section was changed from Virus and Disease Modification Section (HNC6728) to Immunopharmacology Section (HNC6822).

During the period of November 1980 to December 1981, the section occupied rented laboratory space at Litton Bionetics located in Kensington, Md. The section was assigned space in Building 560 at the Frederick Cancer Research Facility in December of 1981.

Personnel in this section include:

Michael A Chirigos, Ph.D., Section Head
Elizabeth Read, Permanent/Part-time Technician
Anna Bartocci, Ph.D., Visiting Fellow
Erich Schlick, M.D., Guest Worker
Mario Piccoli, M.D., Guest Worker
Klaus Hartung, M.D., Guest Worker

The Immunopharmacology Section (ImPhS) conducts studies to define: 1) the host's humoral and cellular immune response to tumor growth; 2) the specific changes in the host's immune response that occur as a result of tumor cytoreductive therapy; 3) the mechanisms involved in enhancing specific cellular components of the immune system; and, 4) the role of specific agents capable of reconstituting and/or augmenting the immune response when used in concert with tumor cytoreductive therapy.

During this year considerable progress has been made. Poly ICLC was examined in-depth for its immunoregulatory capacity. This agent was found to induce a high titer of interferon which correlated with enhanced macrophage and Natural Killer cell tumor lytic activity. A combination of Poly ICLC with cytoreductive treatment with Cytoxan resulted in a synergistic antitumor effect. Azimexon was found capable of reconstituting the number of bone marrow cells accompanied by a similar increase in granulocyte-monocyte colony-forming units in mice which were exposed to various doses of irradiation (100 to 400 rads). The results indicate a direct stimulatory effect of azimexon on the proliferation of nucleated bone marrow cells which is due to stimulation of hemopoietic precursor cells, mediated by a colony stimulating factor(s) for granulocytes and macrophages. Six BRMs were examined for their capacity to stimulate the delayed type hypersensitivity response (DTHR), which is a T cell driven response. Levan, Lentinan, Mannozym, $MVE_2$, Poly ICLC and IFN enhanced the DTHR and were found to correlate with their capacity to regulate macrophage and NK cell tumor lytic activity. Significant therapeutic response was achieved against an alveolar lung carcinoma when $MVE_2$ was combined with cytoreductive chemotherapy. The synergistic antitumor

effect was considered to be due to the capacity of $MVE_2$ to enhance alveolar macrophage tumor lytic activity. Results of pharmacokinetic studies of $MVE_2$ indicate that the scheduling of treatment is critical in regulating effector cell responses. Single or multiple treatment with $MVE_2$ increased macrophage tumoricidal activity, equally, however NK cell augmentation was best following single dose treatment. $MVE_2$ and Poly ICLC were found to be effective adjuvants in combination with drug chemotherapy (Cytoxan, Adriamycin, 5-fluorouracil) in treating a mammary adenocarcinoma.

The Immunopharmacology Section is currently engaged in the following projects: 1) establishing the correlations of stimulated macrophage and/or NK cell tumoricidal activity with tumor retardation by cytoreductive therapy and biological response modifiers (BRMs); 2) determine, for a selected number of BRMs, the optimal dose, route, and treatment regimen resulting in maximum activity and duration of effects on effector cell systems; 3) study the effects of BRMs on the production of colony stimulating factor or on the number of bone marrow myeloid precursor cells; and 4) investigate whether augmentation of effector cells by BRMs will also alter cell regulating factors such as prostaglandins, cyclic AMP and cyclic GMP, IFN, and colony stimulating factor(s).

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06146-05 BRTB |
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PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Immune Regulation by Immune Modifiers and Cytoreductive Therapy in the Tumored Host

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | M.A. Chirigos | Head, Immunopharmacology | BRTB, NCI |
| OTHERS: | A. Bartocci | Visiting Fellow | BRTB, NCI |
| | E. Schlick | Guest Worker | BRTB, NCI |
| | M. Picolli | Guest Worker | BRTB, NCI |
| | K. Hartung | Guest Worker | BRTB, NCI |
| | H.B. Levy | Lab of Viral Diseases | NIAID |

COOPERATING UNITS (if any)

Armed Forces Radibiological Research Inst, DOD; Laboratory of Viral Diseases, NIAID

LAB/BRANCH: Biological Research and Therapy Branch

SECTION: Immunopharmacology Section

INSTITUTE AND LOCATION: NCI-FCRF, Frederick, Maryland 21701

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

High titers of interferon (IFN) induced by Poly ICLC correlate with enhanced macrophage (Mϕ) and Natural Killer Cell (NKC) tumor lytic activity (TLA). Poly ICLC combined with Cytoxan cytoreductive therapy results in a synergistic antitumor effect. Bone marrow cell (BMC) depression resulting from irradiaton or cytoreductive treatment is reversed by Azimexon treatment through stimulated proliferation of nucleated BMC and colony stimulating factor (CSF). Lipopolysaccharide, Poly ICLC and IFN stimulate cellular secretion of CSF. Suppression of the delayed type hypersensitivity response (DTHR) by human chorionic gonadotropin was abrogated by indomethacin and aspirin indicating suppression is mediated through prostaglandin. Enhanced DTHR achieved with 6 biological response modifiers (BRMs): Levan, Lentinan, Mannozym, $MVE_2$, Poly ICLC, IFN, was correlated with their capacity to regulate Mϕ and NKC TLA. Significant therapeutic response was achieved against an alveolar lung Ca with combined cytoreductive chemotherapy and MVE. Pharmacokinetic studies of BRMs indicated that treatment scheduling is critical in regulating Mϕ and NKC TLA.

PROJECT DESCRIPTION

OBJECTIVES

The focus of this project is to examine the immunological and pharmacological mechanisms by which biological response modifiers (BRMs) regulate the various cellular components of the immune system (e.g. T cells, B cells, macrophages, natural killer cells, granulocytes and bone marrow myeloid precursor cells). BRMs are examined to assess whether they exert a direct effect on the various cellular elements of the immune system, or if their effect is mediated through the induction of soluble cellular components (e.g. interferon, colony stimulating factor, prostaglandin) and through some nonimmunologic effects on the host. BRMs which are found to enhance cellular immune response(s) are combined with antitumor cytoreductive therapy to establish a more effective therapeutic treatment modality. Diagnostic tests are developed for monitoring the effect of immunoadjuvant therapy which influences tumor regression.

METHODS EMPLOYED

A. Established transplantable tumors (leukemia, melanoma, mammary adenocarcinoma, alveolar carcinoma) or newly developed ones (carcinogen-induced or virus-induced) are employed for in vitro and in vivo studies.

B. Immune responses, both humoral and cellular, are measured by standard procedures or by new methods developed by section staff. These assays include T and B cell mitogens, IgM and IgG antibodies to sheep red blood cells, T and B cell immunofluorescent antibodies, macrophage and lymphocyte cytotoxicity assays, natural killer cell activity, delayed type hypersensitivity, granulocyte-macrophage stem cell assay, protaglandin and cyclic adenosine monophosphate assays.

MAJOR FINDINGS

Poly-ICLC, a polyinosinic:polycytidylic acid stabilized with poly-L-lysine in carboxymethylcellulose, was tested in mice for its immunoregulatory activity. Poly-ICLC was found to enhance T cell responsiveness but not B cells. It augmented the delayed type hypersensitivity response significantly. The results indicate Poly-ICLC has T cell stimulatory effects. Macrophage tumoricidal activity also was markedly enhanced both in vitro and in vivo after exposure to Poly-ICLC, and natural killer cell cytotoxicity was significantly augmented in vivo. Both macrophage and natural killer cell activity was maintained for over 3 days after only one treatment. The extended period of tumor cell cytotoxicity exhibited by macrophages and natural killer cells may correlate with induction by Poly-ICLC of early and high levels of interferon, which are maintained in the serum for a longer period of time. Poly ICLC, when combined with Cytoxan for treating MBL-2 systemic leukemia, was found to provide synergistic therapeutic effects. Survival time of leukemic mice receiving combined treatment was significantly longer than that achieved on treatment by either agent alone.

From our previous studies we have found that treatment with azimexon (a 2-cyanoaziridine derivative): 1) protected against lethal doses of x-irradiation or cytoreductive drug (Cytoxan); 2) increased mouse granulocyte-macrophage and monocyte-macrophage progenitor cells; 3) acted synergistically with Cytoxan treatment in increasing survival time of L1210 tumor bearing mice; and 4) increased delayed-type hypersensitivity (DTH) responses.

To evaluate the cellular mechanism(s) through which azimexon was expressing its effects, we examined the number of granulocyte-macrophage committed (CFU-C) stem cells/femur in mice treated with either azimexon, whole body irradiation, or Cytoxan. In vitro and in vivo experiments with serum from mice treated with either irradiation or Cytoxan followed by azimexon, showed the presence of a serum factor(s), which stimulates the proliferation of nucleated bone marrow cells. The results indicate a direct stimulatory effect of azimexon on the proliferation of nucleated bone marrow cells, which is due to stimulation of the hematopoietic precursor cells, probably mediated by colony stimulating factor (CSF). Several cell lines were incubated in vitro in the presence of BRMs (Lipopolysaccharide [LPS], Poly ICLC, αIFN, MVE-2 and azimexon to assess production and secretion of CSF. Of the 5 BRMs, only LPS, Poly ICLC, and αIFN were found to stimulate the production of significant levels of CSF by normal peritoneal exudate macrophages, the WEHI-3 cell line and the L1210 tumor cell.

The fetus during normal pregnancy is regarded as an intrauterine transplant but instead of the expected rejection, a parasitic graft-host relationship is established. This relation is similar to that of an autologous tumor which can circumvent host immune factors and grow progressively. Since the human chorionic gonadotropin (HCG) is considered an absolute requirement for successful continuation of pregnancy and since experimental evidence indicates that HCG is immunosuppressive, studies were conducted to determine what effect crude and purified human chorionic gonadotropin had on murine delayed type hypersensitivity to sheep red blood cell antigens. A significant inhibition of delayed type hypersensitivity (DTH) reaction was observed in mice treated with different doses of crude or highly purified preparations of human chorionic gonadotropin (HCG). The α and β subunits of HCG and desialylated HCG were without immunosuppressive effect. The inhibition of the DTH reaction was completely reversed by the simultaneous injection of indomethacin or aspirin, two known inhibitors of prostaglandin synthesis. These findings indicate that HCG itself can suppress the T cell-driven DTH response and that the mechanism involves the release of prostaglandins.

Six BRMs were tested for their effects on delayed-type hypersensitivity reactions (DTH) to sheep erythrocytes in normal $CD_2F_1$ mice. The agents tested Levan, Lentinan, Mannozym, MVE2, Poly ICLC, and highly purified αIFN gave significant increases in the DTH response above the non-drug treated controls. The schedule of doses for each agent was taken from previous experiments from this section, to maximally effect Natural Killer cell activity, macrophage activation and IFN induction. αIFN was capable of eliciting a significant DTH response when given four hours after the initial injection with sheep erythrocytes. In addition, λ carrageenan, a macrophage cytotoxic agent which can render macrophages inactive, was found to suppress the DTH response to levels slightly above saline controls.

Carrageenan-induced suppression of the DTH response could be abrogated by co-administration with BRMs to levels attained by the BRM agents alone. The enhanced DTH response obtained with the six BRMs correlated well with their capacity to regulate macrophage and NK cell activity. This DTH assay could serve as an additional cell mediated assay for evaluating potential BRMs.

A series of maleic anhydride divinyl ether (MVE) polyanions, synthesized with molecular weight ranging from 12500 to 52600, were found capable of enhancing macrophage tumoricidal activity against MBL-2 leukemia cells. These agents also augmented natual killer cell activity against the YAC lymphoma and M109 adenocarcinoma cell lines. This response appeared to be dependent upon MVEs ability of activating macrophage tumoricidal activity. When these agents were combined with Cytoxan cytoreductive therapy an enhanced antitumor (curative) response was achieved. The striking combined effect may be attributable to the effective tumor cytoreductive response to Cytoxan, followed by an augmented immunological response by MVE, through the action of activated macrophage and NK cell tumoricidal effect on residual tumor cells.

The M109 alveolar carcinoma, a tumor which is refractory to several chemotherapeutic agents, was employed to assess the effect of combined chemo-immunotherapy. A marked reduction of lung tumor lesions was achieved, resulting in a longer remission period and a significant number of long term survivors, with combined BCNU chemotherapy and MVE2 immunotherapy. Ancillary studies suggest that this treatment was successful because the primary chemotherapy reduced the tumor burden sufficiently so that tumoricidal macrophages, activated by the secondary MVE2 treatment, further reduced residual tumor cells in the lung.

Results of a recent preliminary study, where MVE2 was administered as a single or multiple treatment (1 x per week for 2, 3, or 4 weeks), show that macrophage tumoricidal activity is increased to 80% or greater in all treatment regimens compared to placebo-treated controls, and that a single treatment was as effective as multiple treatments. Macrophage activity was maintained for 11 days post treatment. In contrast, whereas single treatment with MVE2 increased NK cell activity, with a peak occurring at 3 days and decreasing to normal levels by the 11th day, multiple treatment (2, 3, or 4 treatments) did not augment NK cell activity. This study is being pursued to determine the mechanism by which NK cells become hyporesponsive to MVE2. Two possibilities will be examined: (1) among the MVE2-activated macrophages, a population of macrophages are acting as suppressor cells on NK cells; or, (2) activated macrophages are synthesizing prostaglandin E which is known to inhibit NK cell activity and act through a feedback inhibition mechanism to depress macrophage tumoricidal activity.

Two BRMs, MVE2 and Poly ICLC were further studied as adjuvants to surgery and/or chemotherapy in treating the 16/C mammary adenocarcinoma. Several significant responses were obtained: (1) a marked reduction of established

tumors was achieved with the combination of Cytoxan, Adriamycin, 5-Fluorouracil (CAF), as well as with the two drug combination of Cytoxan and Adriamycin (CA). Three cycles of chemotherapy with either the CA or CAF were highly effective. (2) One cycle of chemotherapy (CAF) prior to surgical removal of the primary tumor, followed by an additional 2 cycles of CAF, was consistently more effective than primary surgery followed by three cycles of CAF. (3) Surgery of primary tumor, followed by MVE2 or Poly ICLC treatment, resulted in a longer extension in survival time to a decrease in the number of metastatic lung lesions. (4) MVE2 or Poly ICLC injected by various routes in tumor-bearing mice, in order of response, were found to be most effective when these agents were administered by the intratumoral > intraperitoneal > intramuscular route. (5) Treatment of mice, bearing established tumors, with CAF and MVE2 or Poly ICLC resulted in a greater extension of survival time than that attained with CAF alone or MVE2 or Poly ICLC alone. Two BRMs were evaluated for their capacity to stimulate antibody responses (IgM or IgG) in vivo or in vitro. Muramyl dipeptide when tested at various concentrations did not stimulate IgM or IgG antibody responses in vivo, but significantly enhanced antibody response in vitro. MVE2 at various doses was effective in stimulating IgM and IgG in vivo and enhanced antibody response in vitro. MVE2 was blastogenic when incubated in vitro with splenic lymphocytes, and was shown to enhance the blastogenic response to minimum concentrations of lipopolysaccharide.

## SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

The Immunopharmacology Section investigates and develops potential therapeutic agents which may alter biological responses important in the resistance to cancer growth and metastasis. The section investigates agents of chemical or biological origin which include immunoaugmenting, immunomodulating and immunorestorative agents, interferon and interferon inducers, lymphokines and thymic factors. The multidisciplinary approach represented by this project is entirely directed towards: increasing the host's antitumor response through augmentation and/or restoration of cellular effector mechanisms; increasing the host's cellular and humoral immune responses by the administration of natural or synthetic effectors or mediators; and, increasing the ability of the host to tolerate damage of normal cellular components resulting from cytotoxic modalities of cancer treatment. Experimental studies conducted with chemical (levamisole, azimexon, poly ICLC, cimetidine, etc.) and biological (interferon, BCG, thymosin fractions, levan, lentinan, glucan, etc.) agents in respect to their: immunoadjuvant effects; most efficacious treatment regimens; and, usefulness in combined treatment modalities, has provided information leading to the inclusion of some of these agents for the treatment of human breast, colon and rectal carcinomas, head and neck tumors, and leukemias. The ability of several immune modulators to specifically 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 areas described under objectives will be pursued.

The major treatment modality for various cancers is chemotherapy. In many cases, this cytotoxic treatment results in host 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. Myelopoiesis is controlled by specific regulatory macromolecules. These colony stimulating factors (CSF) are found to be increased in situations where there is an increased granulopoiesis and monocyte-macrophage production. Studies will be continued to assess the capacity of selected BRMs to stimulate colony stimulating activity in mice under various host conditions: 1) normal; 2) tumor-bearing; and 3) tumor-bearing undergoing cytoreductive therapy.

The uniqueness of BRMs is that they affect cellular components of the immune system by either reconstituting or augmenting the activity of, or increasing the proliferation of, T-, B-, monocytes-macrophages, and/or bone marrow cells. More effective chemotherapy has been developed based on combining treatment with drugs that affect specific stages in cellular replication. A similar approach will be applied in studies with BRMs.

Little is known about the pharmacokinetics of the effects of BRMs on various immunologic effector mechanisms as related to such variables as dosage, route of administration, regimen of treatment (particularly when used in combination with cytoreductive therapy), and the duration of the effects on various effector cells.

Many published reports deal with a single exposure of BRM and its effect on an effector cell response (e.g., macrophage, NK cell, cytotoxic T cells, ADCC). Relatively few studies have been performed to establish the effectiveness of single vs. multiple doses on effector cell responses and the duration of the effects, or the possible development of hyporesponsiveness of effector cells to BRM. Very little information is available concerning the effector cell responses to combined chemotherapy and BRM treatment. Since treatment with various chemotherapeutic agents may lead to a depression of hematopoeitic 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.

Prostaglandin E (PGE) production by murine tumors has been shown to increase with tumor size and to be decreased after treatment with the PG synthetase inhibitor indomethacin. T-cell effector functions and mitogenesis are major targets of the immunosuppressive effects of PGE of tumor origin. It is possible that this immunosuppression appears at the tumor site first, enabling the tumor to escape host defense mechanisms.

Since macrophage activation may lead to the secretion of two cell regulating factors (CSF and PGE) which exert mutually opposing actions on tumoricidal effector cells, it is important to examine macrophage activating BRMs for their capacity to regulate secretion of PGE and/or CSF. A concomitant increase of intracellular cAMP, which is believed to mediate the inhibition of proliferation resulting from exposure of effector cells to PGE, will also be monitored.

Since prostaglandins regulate effector cell responses, it will be of value to assess the prostaglandin-inducing capacity of a selected number of BRMs. Different effector cell populations (peritoneal exudate macrophages; splenic adherent cells; non-adherent cells; and total spleen cell population) will be incubated in vitro with different concentrations of BRMs known to stimulate macrophage tumoricidal activity (IFN, LPS, MVE2, Poly ICLC, Tuftsin) for 1 to 4 days. The cell culture supernatants will be examined for the presence of prostaglandin. That effector cell population found to produce high concentrations of prostaglandin will also be examined for its cAMP content. Indomethacin, an inhibitor of prostaglandin synthesis will be used as a probe to assess whether it reverses some of the inhibitory effects that have been attributed to PGE.

PUBLICATIONS

1. Chirigos, M.A.: Measurement of antitumor and adjuvant effects of interferon with transplantable and other tumors in mice. In Pestka, S. (Ed.): Interferons: Methods in Enzymology. New York, Academic Press, 1981, Vol. 79, pp. 414-419.

2. Chirigos, M.A., and Jacques, P.J.: Polysaccharides and related substances. In Hadden, J., Chedid, L., Mullen, P., and Spreafico, F. (Eds): Advances in Immunopharmacology, Pergamon Press, 1981, pp. 485-490.

3. Chirigos, M.A., Papademetriou, V., Bartocci, A., and Read, E.: Immunological and tumor responses to various immunotherapeutic agents. In Hadden, J., Chedid, L., Mullen, P., and Spreafico, F., (Eds.): Advances in Immunopharmacology, Pergamon Press, 1981, pp. 217-225.

4. Chirigos, M.A., Papademetriou, V., Bartocci, A., Read, E., and Levy, H.B.: Immune response modifying activity in mice of polyinosinic: polycytidylic acid stabilized with poly-L-lysine, in carboxymethylcellulose (Poly-ICLC). Int. J. Immunopharmacol, 3:329-337, 1981.

5. Papademetriou, V., Bartocci, A., Steel, L.K., Read, E., and Chirigos, M.A.: Immunoregulatroy effect of human chorionic gonadotrupin in murine peritoneal macrophages. In Chirigos, M.A., Mastrangelo, M.J., Mitchell, M., and Krim, M. (Eds.): Progress in Cancer Research and Therapy, 1981, Vol. 19, Raven Press, pp. 117-129.

6. Chirigos, M.A., Bartocci, A., Papademetriou, V., and Read E.: Enhancement of macrophage tumoricidal activities in vitro and in vivo by immune modifiers. In Chirigos, M.A., Mastrangelo, M.J., Mitchell, M., and Krim, M. (Eds.): Progress in Cancer Research and Therapy, Raven Press, 1981, Vol. 19, pp. 77-87.

7. Lo Buglio, A.F., Robinson, P., Chirigos, M.A., and Solvay, M.: Human monocyte direct cytotoxicity to tumor cells. In Chirigos, M.A., Mastrangelo, M.J., Mitchell, M., and Krim, M. (Eds.): Progress in Cancer Research and Therapy, Raven Press, 1981, Vol. 19, pp. 107-115.

8. Jeng, J.C., McCarthy, K.F., Chirigos, M.A., and Weiss, J.C.: Effect of azimexon (BM 12-531) on mouse granulocyte-macrophage progenitor cells. Experentia 38:132-133, 1982.

9. Chirigos, M.A., Mitchell, M., Mastrangelo, M.J., and Krim, M. (Eds.): Mediation of Cellular Immunity in Cancer by Immune Modifiers by Immune Modifiers. Progress in Cancer Research and Therapy. Raven Press, 1981, Vol. 19, 275 pp.

10. Goldstein, A.L., and Chirigos, M.A. (Eds.): Lymphokines and thymic hormones. Their potential utilization in cancer therapeutics. Progress in Cancer Research and Therapy. Raven Press, 1981, Vol. 20, 324 pp.

BALTIMORE CANCER RESEARCH PROGRAM
SUMMARY
Laboratory of Clinical Biochemistry
October 1, 1981 to September 30, 1982

This was a year of radical change and pertubations in the Laboratory of Clinical Biochemistry as a result of the conversion of the Baltimore Cancer Research Program to the University of Maryland Cancer Center. While it was important to maintain the continuity and high grade of collaborative research that was ongoing, the Federal Laboratory of Clinical Biochemistry initiated a process of separating the Federal administrative process from the newly developing University of Maryland Cancer Center. These two tasks were accomplished successfully during the year; and we have continued our collaborative research projects.

Research in the biochemical pharmacology of antineoplastic agents has continued to delineate the role of free radical intermediates of several drugs. We have been investigating the anthracycline antibiotics, the actinomycin series, and the mitomycins in detail. In this year we have emphasized the mitomycins and shown that the alkylating activity of mitomycin is triggered through a free radical intermediate rather than a hydroquinone as has previously been postulated. We have developed new methodology and technology for the isolation of mitomycin derivatives. Our evidence also postulates that a new active center of the mitomycin molecule is possible.

We are continuing our study of the anthracycine antibiotics. This year our efforts in the study of clinical pharmacology have yielded interesting data on interactions of the clinical pharmacology of anthracyclines with such drug response modifiers as hyperthermia and drug-drug interactions with drugs such as cyclophosphamide and tetrahydrocannabinol.

Among our studies of the anthracycline antibiotics, we have identified a drug binding cytoplasmic protein from mouse liver. This protein binds anthracyclines in a quantitative and reproducible fashion. This determination has been made through an affinity analog of daunorubicin which we have synthesized in our laboratory and are also using to examine other drug receptor sites.

A new research effort has been devoted to the examination of cell surface membrane proteins and glycoproteins through a new qualitative and quantitative technique of two-dimensional electrophoresis. After several years of developmental efforts, this technique has now been applied to the study of cell membranes of specific cell cultured types, namely the HL-60 and K562. Experiments were designed to examine the cell membrane protein content to see if the composition of cell surface membranes correlates with specific type of cells. We find that types of leukocytes have specific cell membrane protein "signatures". Also changes occur in the protein and glycoprotein composition during cellular differentiation growth and development. It is also apparent that changes occur in cell membrane in the process of drug resistance. Specific drug resistant lines of cells are being developed and examined for changes of specific cell membrane protein formation.

The identity of cell lines has always been a thorny issue amongst cell biologists. In an examination of several lines of K562 cells, which originated from a single clone, we have found that there appears to be clonal evolution in the K562 since there are significant differences amongst cell lines of this well used and established cell type.

In a coordinated study of the new anticancer agent diaziquone or 2,5-diaziridinyl 3,6-bis

carboxyethylamino 1,4-benzoquinone (AZQ), we have concluded the study of the distribution and metabolism of this agent in mice. We have evidence of a very wide tissue distribution, of very rapid metabolism, tissue binding and free radical formation of AZQ. We have shown time dependent and temperature dependent binding of the agent to cellular macromolecules. The free radical species of AZQ differs considerably from that seen with anthracyclines, since we are able to generate a different mode of radical with whole cells. In a collaborative clinical pharmacology study of AZQ, we have administered this agent to patients in a Phase I pharmacokinetic study to patients with implanted Ommaya reservoirs. This study demonstrates the effectiveness of AZQ delivery to CSF and verified the utility of drug development of potential drugs for brain tumor treatment.

The Laboratory of Clinical Biochemistry will cease to exist July 1, 1982; and the Federal employees of the Laboratory will be transfered to the National Cancer Institute in Bethesda. We will maintain selected aspects of our research program and initiate new projects, especially along the lines of drug resistance in the cell membrane studies.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06012-12 LCB |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Pharmacology of Antineoplastic and Other Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| | R.D. Friedman | Chemist | LCB, NCI |
| | S. Tipping | Clinical Associate | LCB, NCI |
| | S. Pan | Associate Professor | UMCC, U of Md |
| | P.L. Gutierrez | Assistant Professor | UMCC, U of Md |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.8 | 1.3 | 0.5 |

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The conversion of anthracycline antibiotics as examplified by daunorubicin to 7-deoxyaglycone metabolites occurs via a protonated free radical anion intermediate. Intact daunosamine is the leaving group. Mitomycin C a quinone anticancer antibiotic in gastrointestinal tumors, is metabolized to a free radical by NADPH cytochrome P-450 reductase and xanthine oxidase. Nine metabolites were resolved by reversed phase high performance liquid chromatography. Identification by mass spectrometry showed cis and trans 2,7 diamino-1-hydroxymitosene were formed. Besides the reactive center at $C_1$ following bioreduction, a second reactive center at $C_7$ is implicated by these results.

Project Description:

An electrochemical flow cell (Miner and Kissinger, Biochem. Pharmacol., 28, 3285, 1979) was used to generate the semiquinone and hydroquinone forms of the anthracycline antibiotic daunorubicin (DNR). Flow cell reduction of DNR in dimethylformamide/tetraethylammonium perchlorate (0.1 M) at −0.700 V vs. Ag/AgCl produced the semiquinone free radical. Electron paramagnetic resonance (EPR) spectroscopy of this species gave a 30 line spectrum (g=2.0040, spectrum width=10.3 Gauss) that decayed very slowly (days) when anaerobic. The absorbance spectrum of the radical showed maxima at 383 nm and 509 nm. Thin layer chromatographic (TLC) analysis of this species yielded only DNR. Anaerobic aqueous reduction of DNR in 0.05 M KCl at −0.600 V gave the hydroquinone (absorbance maximum 430 nm) which was stable. Exposure of the hydroquinone solution to air, however, caused immediate return of the red color, precipitation of 7-deoxyaglycone and near quantitative yield of daunosamine as assayed by TLC and gas chromatography mass spectrometry. EPR analysis of air-exposed hydroquinone solutions exhibited a 38 line spectrum (g=2.0037, spectrum width=16.5 Gauss) that decayed to an asymmetric spectrum typical of 7-deoxyaglycone. These results provide the first evidence that intact daunosamine is the leaving group in the reductive glycosidic cleavage of DNR, and that the cleavage is homolytic. The results also imply that cleavage proceeds only through a protonated radical anion and not directly through the hydroquinone.

In the presence of electron donors and anaerobic conditions, NADPH cytochrome P-450 reductase (EC 1.6.2.4) and xanthine oxidase (EC 1.2.3.2) readily and similarly metabolize mitomycin C (MC). Reverse-phase high pressure liquid chromatography was used to separate and to detect metabolites. Mitomycin C (MC) and 9 derivatives were resolved by reversed-phase high performance liquid chromatography. The column used was a 10 cm x 8.0 mm $C_{18}$ (10 μ) radial compression cartridge fitted with a 7 cm x 2.1 mm guard column packed with CO:PELL ODS. Compounds were eluted with a 13 minute linear gradient from 100% potassium phosphate (0.01M, pH 7.0) to 50% phosphate, 50% methanol at a flow rate of 3.0 ml/min. Peaks were detected at 365 nm and 313 nm. Cis-and trans-2,7-diamino-1-hydroxymitosene (k' 14.4 and 11.7) were generated by acidic hydrolysis of MC (k' 12.7). These isomers were purified on a 50 cm x 9.4 mm M9 ODS-3 semi-preparative column and treated with acetic anhydride/pyridine. Electron ionization mass spectroscopy confirmed their identifies (m/z 404, $M^+$; 361, -($COCH_3$); 343,-($HOCONH_2$)). Catalytic hydrogenation of MC generated 2,7-diaminomitosene (k' 16.6). Purification, acetylation and mass spectral analysis confirmed its identity (m/z 303, -($COCH_3$); 285,-($HOCONH_2$)). Alkylation reactions of MC at pH 3.5 produced 4 peaks (k' 3.1, 4.1, 6.5, 7.1) dependent on phosphate concentration. All seven of the above compounds had absorbance spectra typical of 7-aminomitosene. Two additional minor peaks (k' 13.4, 17.7) were detected in all preparations that had absorbance spectra similar to 2-amino-1,7-dihydroxymitosene. NADPH cytochrome P450 reductase and xanthine oxidase were found to readily metabolize MC anaerobically in the presence of electron donors. Electron spin resonance studies demonstrated a free radical formation during the anaerobic activation of MC by both enzymes (g=2.0046). Nine metabolites were detected that co-chromatographed with the chemically generated species. Cis and trans-2,7-diamino-1-hydroxymitosene were conclusively verified in these incubations by mass spectroscopy following purification and acetylation. Besides the reactive center expressed at $C_1$ following bioreduction, a second reactive center expressed at $C_7$ is implicated by these results.

Publications:

Ahmed, N.K., Felsted, R.L., and Bachur, N.R.: Daunorubicin reduction mediated by aldehyde and ketone reductases. Xenobiotica 11: 131-136, 1981.

Nakazawa, H., Chou, F.E., Andrews, P.A., and Bachur, N.R.: Chemical reduction of actinomycin D and phenoxazone analogues to free radicals. J. Organic Chem. 46: 1493-1496, 1981.

Pan, S., Pedersen, L., and Bachur, N.R.: Comparative flavoprotein catalysis of anthracycline antibiotic reductive cleavage and oxygen consumption. Molec. Pharmacol. 19: 184-186, 1981.

Nakazawa, H., Andrews, P.A., and Bachur, N.R.: Isolation of daunorubicin derivatives by counter-current chromatography with the horizontal flow-through coil planet centrifuge. J. Chromat. 205: 482, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06014-12 LCB |
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Control Mechanisms Affecting Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Biochemistry

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INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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| 3.0 | 3.0 | 0 |

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new technique of two dimensional electrophoresis to examine, distinguish and quantify cell surface membrane proteins and glycoproteins was developed and applied to HL-60 and K562 cell differentiation. The technique may be useful in the study of cellular drug resistance. K562 cells were induced to differentiate by hemin and studied for comparative morphology, cytochemistry, and cytogenetics. The data indicate the existance of clonal evolution in the K562 line. The synergetic interaction of cytosine arabinoside and deoxyguanosine were studied in HL60 and K562 cells.

Project Description:

The human promyelocytic leukemic cell line, HL-60, was induced to differentiate in vitro by treatment with dimethylsulfoxide and retinoic acid. Cell surface membrane proteins and glycoproteins were labeled with I-125 by the methods of lactoperoxidase/$H_2O_2$ or Iodo-gen and analyzed by two-dimensional polyacrylamide gel electrophoresis and autoradiography. A minimum of twelve cell surface proteins were unchanged, three proteins (MW 95,000; 87,000; and 77,000) were lost and seven new components (MW 280,000; 250,000; 162,000; 143,000; 58,000; 56,000; and 50,000) appeared during cell differentiation. Both chemical inducers gave similar results. The major labeled cell surface protein (MW 95,000) lost during cell differentiation correlated with the loss of cell transferrin binding and was identified as the transferrin receptor by affinity absorption of extracts of I-125 surface membrane-labeled cells with transferrin-Sepharose beads. This affinity purified component had molecular weights of 200,000 and 95,000 daltons under non-reducing and reducing conditions, respectively, confirming its dimer structure. Two-dimensional electrophoresis of cell surface membrane labeled normal granulocytes confirmed the absence of the transferrin receptor and the presence of several of the major new cell surface proteins identified during HL-60 maturation. This technology is being applied to the study of drug resistance in specific cell lines.

We studied K562 cells from three laboratories which we designated A, B, C, including passage 251 from B. Lozzio, deignated C, for cellular and molecular properties including soft agar cloning efficiency, hemoglobin induction by sodium butyrate or hemin, cell surface protein markers and cytogenetics. Cell properties which were similar when maintained in RPMI medium 1640 + 10% fetal calf serum are morphology, histologic staining properties, growth kinetics (doubling times 20-28 hrs), cloning efficiencies in 0.3% agar (30-45%) and absence of hemoglobin production in the presence of sodium butyrate as determined by benzidine and indirect immunofluoresence assay for hemoglobin F. In contrast, incubation with hemin (5-50 μM) produced intracellular hemoglobin-positive granules. K562A proved most sensitive with 100% of cells responding at 20 μM compared to 11.3% of K562B ($p<0.05$). Results with K562C were intermediate. Cytogenetically, a near-triploid mode (67-73 chromosomes) was observed in each of the cell suspensions; furthermore, cells from all three sources contained the same long acrocentric marker chromosome, indicating a common origin. However, whereas 95-100% of the cells were $Ph^1$-positive in K562A and C, 10% of the cells contained a $PH^1$ in K562B. Cell membranes were labelled with I-125 by the lactoperoxidase/$H_2O_2$ method and protein markers identified by 2-D gel electrophoresis. K562A contained a 93,000 and 85,000 mw membrane protein with isoelectric points at pH 5.3-5.7 and 5.5-6.05, respectively. These proteins did not appear in K-562B or C. Our results demonstrate that the K562 cell line is heterogenous.

Manipulation of culture and environmental conditions of K562 cells can induce the differentiation of these leukemic blasts into progenitors of erythroid, myeloid, and monocytoid cells. We have studied the ultrastructure and cytochemistry of cells obtained from three sources, all capable of differentiating in the presence of hemin along the erythroid pathway. By comparing these cells with uninduced cells and with bone marrow from a patient with erythroleukemia we followed the sequence of cellular changes as hemoglobin positive erythrocyte-like particles accumulated in the growth medium. Differences in morphology of our uninduced cells compared to those originally described by Lozzio et al suggests clonal evolution of leukemic cell lines. Differentiating cells from induced cultures (5-50 μM hemin added to medium) were easily recognized by the coalescence of electron dense granules, many of them peroxidase positive, with dilated

Golgi cisternae in the centrosome region of the cell. Large masses of annulate lamellae often indicative of rapidly proliferating and differentiating cells, are closely associated with the active Golgi cisternae. As differentiation proceeds many of the cells show an intense positive reaction for hemoglobin demonstrated at the ultrastructural level by fixing cells in a tannic acid-aldehyde mixture. The synthesis of hemoglobin is accompanied by the breakdown of organelles and the expulsion from the degenerating cells of large spherical membrane bound particles 4-9 μM in diameter. These erythrocyte-like particles possess lipid inclusions and internal membranous configurations. The percentage of cells capable of generating these particles is directly related to the concentration of hemin. Occasionally some cells from induced cultures with multiple nuclei, heavily condensed chromatin, and a reduced number of organelles were observed. These cells bear a striking resemblance to leukemic erythroblasts. We propose the existence of clonal evolution which may influence the experimental results obtained by investigators using this cell line.

Deoxyguanosine (dGuo) and ara-C synergistically inhibit cloning of the human leukemia cell lines HL-60 and K562 (Akman et al., Proc. AACR, p. 282, 1980). We have investigated the mechanism of synergy, comparing the effect of dGuo to that of thymidine (dThd), which increases ara-C triphosphate (ara-CTP) pool size and augments incorporation of ara-C into nucleic acid in other cell lines. We incubated log phase HL-60 cells with 0.1 mM dGuo or 1 mM dThd, concentrations equitoxic for HL-60 cloning, for 24 hrs and analyzed changes in cell cycle and deoxycytidine triphosphate (dCTP) pool size. Both nucleosides increased cellular vulnerability to ara-C and caused partial synchrony in early S phase as shown by flow cytometry. dGuo (0.1 mM) reduced dCTP pool size by 29%, as measured with the E. coli DNA polymerase assay, versus a 61% reduction by dThd (1 mM). In subsequent experiments, we analyzed ara-C metabolism by co-incubating HL-60 cells with 10 nM $^{3}$H-ara-C, a concentration which does not inhibit cell cloning in absence of deoxynucleosides, and with dGuo or dThd in concentrations from 0.01 to 5.0 mM. dThd increased incorporation of $^{3}$H-ara-C into DNA, with a maximal increase of 370% at 1.0 mM. As assayed by HPLC, dThd increased ara-CTP pool size up to a maximum of 145% of control at 0.6 mM. dGuo also increased ara-CTP pool size to a maximum of 193% of control at 0.01 mM, the lowest concentration tested. However, dGuo increased incorporation of ara-C into DNA only marginally (129% of control) at 0.01 mM, and reduced incorporation at higher concentrations. These data suggest that dGuo and dThd exhibit synergy with ara-C by different mechanisms, since dThd augments its incorporation into DNA, while dGuo does not.

Publications:

Felsted, R.L., Leavitt, R.D., Chen, C., Bachur, N.R., and Dale, R.M.K.: Phytohemagglutinin isolectin subunit composition. Biochim. Biophys. Acta 668: 132-140, 1981.

Felsted, R.L., Li, J., Pokrywka, G., Egorin, M.J., Spiegel, J., and Dale, R.M.A.: Comparison of Phaseolus vulgaris cultivars on the basis of isolectin differences. Int. J. Biochem. 13: 544-557, 1981.

Ross, D.D., Akman, S.A., Schrecker, A.W., and Bachur, N.R.: Effects of deoxynucleosides on cultured human leukemia cell growth and deoxynucleotide pools. Cancer Res. 41: 2141-2146, 1981.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06016-12 LCB |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pharmacodynamics of Cancer Chemotherapeutic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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| | B.M. Fox | Research Chemist | UMCC, U of Md |
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COOPERATING UNITS (if any)

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Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Preclinical studies of 2,5 diaziridinyl-3,6-bis (carboxyethylamino) 1,4-benzoquinone (AZQ) were done in mice with $^{14}C$ labeled AZQ and with various murine and human leukemic cells. These studies show evidence of wide tissue distribution, rapid metabolism, tissue binding, and free radical formation. Other drug studies show interaction between hyperthermia and doxorubicin. A cytoplasmic protein in mouse liver cells has been identified by gel electrophoresis to be an anthracycline binding protein.

Project Description:

We investigated the metabolism and disposition in mice of AZQ, an antitumor agent currently undergoing clinical testing. $^{14}$C-AZQ, dissolved in 5% dimethylacetamide/95% phosphate buffer (0.01 M, pH 6.5), was administered IV to mice at a dosage of 6 mg/kg. At specified times after injection, mice were killed and plasma and organs were obtained and analyzed for total radioactivity and for chloroform-extractable $^{14}$C. Plasma total radioactivity declined in triexponential fashion. Chloroform-extractable plasma $^{14}$C declined very rapidly in a similar fashion, and by 3 hrs, was about 0.2% of its initial concentration. Within 30 min after injection, more radioactivity remained in the aqueous phase than was extracted into chloroform. When analyzed by TLC, all plasma chloroform-extractable radioactivity cochromatographed with parent AZQ. Tissue distribution of $^{14}$C was extensive. $^{14}$C concentrations (DPM/gm tissue) were initially greatest in heart and lung, but after 2 min post-injection, were greatest in kidney, and after 10 min, post injection total $^{14}$C in all tissues exceeded total $^{14}$C in plasma. The apparent volume of distribution of AZQ was approximately 1 l/kg, in contrast to the volume of 0.05-0.25 l/kg reported for humans. Also, the total body clearance of AZQ was 0.5 ml/min or 75 ml/min/sq m which is less than the value of 120-370 ml/min/sq m reported for humans. Extraction of tissues with chloroform showed conversion of AZQ into nonchloroform-extractable metabolites. This conversion was reproduced in vitro by liver homogenate at 37°C but not at 0°C.

We investigated AZQ's in vitro interaction with and antitumor effect on various murine and human leukemic cells. L1210 cells were able to accumulate AZQ from RPMI 1630 medium with or without newborn calf serum, a process which was temperature dependent and which was not affected by sodium azide. AZQ produced a dose-dependent inhibition of L1210 $^{3}$H-thymidine incorporation, but this inhibition was slow to develop requiring 6 hours to become apparent. The minimal inhibitory concentration of AZQ for this process was 0.05-0.25 nmole/ml. AZQ was a much less effective inhibitor of L1210 cell $^{3}$H-uridine and $^{14}$C-valine incorporation. AZQ produced dose-dependent inhibition of the growth of suspension cultures of L1210 and HL60 cells with minimal inhibitory concentration of 0.1-0.3 nmoles/ml. AZQ produced a dose-dependent increase in oxygen consumption when added to L1210, HL60 and K562 cells and was converted to an AZQ anion free radical by these cells. Both of these processes were duplicated by L1210 cell lysate, however, NADPH was required in addition to AZQ. Anaerobic conditions were not required for AZQ anion radical production by intact cells or by cell lysate. When AZQ's aziridine rings were opened by acid treatment, the resulting molecule was accumulated by L1210 cells, but did not provoke $O_2$ consumption, did not form free radicals when added to L1210 cells, and was a much less effective inhibitor of $^{3}$H-thymidine incorporation by L1210 cells than was AZQ. These interesting metabolic and pharmacologic differences between parent compound and the simple derivative are undergoing further investigation.

Combinations of heat (HT), DOX and cyclophosphamide (CYC) have been studied for clinical efficacy against tumors in man. Alterations in human CYC metabolism and elimination during HT have been reported. In vitro metabolism of CYC by rat liver slices and microsomes is reduced by HT. We examined in vitro uptake and metabolism of DOX by liver slices obtained from male Sprague-Dawley rats. Slices (0.5 mm) were incubated in Krebs-Ringer-$PO_4$-$HCO_3$ buffer, under 95% $O_2$/5% $CO_2$, at 37°, 39°, 41°, and 43°C, with DOX, 7-8 μM, in 3 ml reaction volumes. Reactions, assayed in triplicate at each time point, were terminated by addition of acidified isopropanol or chloroform-isopropanol extractants, and DOX and metabolites were determined. DOX was rapidly accumulated by the slices for 30 min, with decreased accumulation thereafter. No significant

differences occurred among the four temperatures (temps.) in rate or total uptake of DOX. 50-75% of added DOX appeared in the slices by 90 min. The principal intracellular species was parent DOX. The only metabolite which could be quantified was a polar conjugate (1-2% of total DOX). The initial rates of conjugate appearance were reduced with progressively higher temps., but by 45 min there were no significant differences in conjugate content of slices. We concluded that: A) DOX uptake by liver slices is not influenced by HT, and B) the rate but not the extent of DOX metabolism is altered during HT. Further studies of DOX metabolism by isolated hepatic microsomes under aerobic and anaerobic conditions are in progress.

Adriamycin and daunorubicin, anthracycline antibiotics, have previously been shown to be localized in cell nuclei and lysosomes. However, certain analogs of these drugs were found in the cytoplasm. Since nuclear localization may be explained by the high affinity of anthracyclines for DNA, preferential cytoplasmic localization of certain analogs may be due to their affinity for a specific cytoplasmic receptor. To determine if this receptor exists, we synthesized a photoaffinity analog (N-(p-azidobenzoyl)-$^{3}$H -daunorubicin) (NABD) which also is localized in the cytoplasm. $^{3}$H -NABD was added to the 105,000 x g supernatant of DBA/2 mouse liver homogenates and the mixture was activated with UV light. The mixture was electrophoresed on sodium dodecyl sulfate gels, the gels cut into slices and the radioactivity of each slice counted. This analysis showed a major band of radioactivity which corresponded to a major protein present in cytoplasmic but absent in nuclear extracts. Excess nonradioactive cytoplasmically localized anthracycline analogs competitively reduced $^{3}$H -NABD labeling of this cytoplasmic protein to a greater degree than unlabeled nuclear localized daunorubicin. These results suggest the presence in the cytoplasm of a protein with high affinity for certain anthracycline analogs. This binding protein may explain the specific subcellular localization of various anthracycline analogs.

Publications:

Clawson, R.E., Egorin, M.J., Fox, B.M., Ross, L.A., and Bachur, N.R.: Hyperthermic modification of cyclophosphamide metabolism in rat hepatic microsomes and liver slices. Life Sci. 28: 1133-1137, 1981.

Egorin, M.J., Clawson, R.E., Ross, L.A., Chou, F.E., Andrews, P.A., and Bachur, N.R.: Disposition and metabolism of adriamycin octanoyl-hydrazone (NSC 233853) in mice and rabbits. Drug. Metab. Disp. 9: 240-245, 1981.

Andrews, P.A., Egorin, M.J., May, M.E., and Bachur, N.R.: Reversed-phase high performance liquid chromatography analysis of 6-thioguanine applicable to pharmacologic studies in humans. J. Chromatog., 227: 83, 1982.

Andrews, P.A., Callery, P.S., Chou, F.E., and Bachur, N.R.: Qualitative analysis of trimethylsilylated daunosamine and N-alkylated analogues by gas chromatography/mass spectrometry. Anal. Biochem. In press, 1982.

| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 CM 06241-12 LCB |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Clinical Pharmacology of Antineoplastic and Other Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---|---|---|---|
| PI: | N.R. Bachur | Chief | LCB, NCI |
| OTHER: | R.D. Friedman | Chemist | LCB, NCI |
| | J.M. Collins | Research Chemist | BEIB, NIH |
| | M.J. Egorin | Associate Professor | UMCC, U of Md |
| | C.E. Riggs | Assistant Professor | UMCC, U of Md |
| | D.A. Van Echo | Associate Professor | UMCC, U of Md |
| | M. Whitacre | Chemotherapy Nurse | UMCC, U of Md |

COOPERATING UNITS (if any)
Johns Hopkins Cancer Center

LAB/BRANCH
Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland

| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.2 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We studied the plasma and CSF pharmacokinetic of AZQ in five patients with Ommaya reservoirs. AZQ had a T 1/2 α of 2-6 min and T 1/2 β of 25-35 min. We showed effectiveness of AZQ delivery to CSF. Probenecid did not effect the plasma pharmacokinetics of cis platinum (DDP) but did effect the renal clearance of ultrafilterable DDP. Probenecid did not mitigate the DDP toxicities in these patients. Whole body heat (hyperthermia) was studied with doxorubicin and cytoxan pharmacokinetics. Hyperthermia affected cytoxan pharmacokinetics. Successive doxorubicin administration shows effect on C x T of doxorubicin metabolites.

Project Description:

The search for antitumor agents to cross the blood brain barrier joined with the antitumor activity of quinone containing drugs lead to the development of the aziridinyl benzoquinone AZQ. We initiated a phase I pharmacokinetic study in patients (pts) with implanted Ommaya reservoirs to obtain CSF pharmacokinetics. Five informed pts were selected for study because they already had indwelling Ommaya reservoirs which had been placed for therapy of meningeal leukemia or glioma. At the time of study the leukemia pts had normal CSF. The brain tumor pts had received whole brain irradiation, chemotherapy and re-exploration. Pts received 16 mg/$M^2$/day to 24 hrs/$M^2$/day AZQ as 30 min infusions. Venous blood and CSF samples were extracted and assayed by the HPLC method of Kelly & Sin Chong with diaminodichlorobenzoquinone as the internal standard. No AZQ was detected in pre-infusion plasma or CSF. By 4 hrs after infusion, the plasma AZQ was below detectable limits. The T 1/2 $\alpha$= 2-6 min and the T 1/2 $\beta$ was 25-35 min. CSF AZQ rose following the infusion to peak at 100-250 ng/ml by 30-70 min post infusion before declining at a rate slower than plasma disappearance to give a concentration > 5ng/ml for at least 4 hrs. One pt with the Ommaya in a tumor cyst showed persistance of AZQ. Plasma protein binding indicated only 21% AZQ was unbound. Plasma disappearance, half-life and low clearance yield a small volume of distribution, 2.0-10.0 l/n. Comparison of area under curve for plasma and CSF indicates a high degree of drug availability to the CSF, 22-42% of plasma exposure. Our study demonstrates the effectiveness of AZQ delivery to CSF through the blood brain barrier when the drug is administered intravenously.

Probenecid is claimed to ameliorate the nephrotoxicity of DDP in rats and not to reduce the activity of DDP against L1210 leukemia. We have investigated the effects of Probenecid on the plasma pharmacokinetics, the urinary excretion, and toxicities of DDP in patients with advanced neoplasms. Patients received 500 mg. Probenecid p.o. q6h starting 24 hrs before DDP therapy and continuing for 5 days after DDP therapy. Prehydration of 1 liter of 5% dextrose in 0.45% NaCl. and 1 liter of 0.9% saline were given over 9 hours. Then 375 mg mannitol and DDP at doses of 60-120 mg/$m^2$ were administered as a 6 hr infusion in 2 liters of 5% dextrose in 0.45% NaCl. DDP doses were escalated in a Phase I fashion. Elemental platinum (Pt) in urine, plasma, and plasma ultrafiltrate was measured by flameless atomic absorption spectrometry. When compared to previous studies in patients receiving similar DDP therapy without Probenecid, Probenecid did not alter the peak plasma concentration of Pt, the terminal plasma T 1/2 of Pt, the peak plasma concentration of ultrafilterable Pt, the plasma T 1/2 of ultrafilterable Pt, or the 24 hr urinary excretion of Pt. However, Probenecid did significantly reduce the renal clearance of ultrafilterable Pt during the first 6 hrs of treatment. Also during these 6 hrs, the ratio of clearance of ultrafilterable Pt to creatinine clearance (Ccr) was significantly reduced when compared to similar data from patients not receiving Probenecid. Toxicities observed in patients receiving Probenecid and DDP included azotemia, reduced Ccr, and myelosuppression. Thus although Probenecid alters the renal handling of DDP, it may not mitigate its toxicity and further studies are needed to determine if this combination of drugs enhances DDP toxicity.

WBH is an experimental adjunct to combination chemotherapy treatment of human malignancy. Hyperthermia reduces metabolism of CYC to alkylating activity in man and in vitro. We examined the pharmacokinetics of CYC and DOX in 6 patients (pts) treated with and without WBH. DOX 45 mg/$M^2$ was given by I.V. bolus, followed 6 hrs later by CYC, 1 gm/$M^2$, as a 1 hr infusion. Pts were studied at 37$^0$ during the first course of

therapy. In all subsequent treatments, DOX was given at the start of a 4 hr course of WBH (41.8°C). Plasma elimination of CYC was not influenced by WBH, but increased urinary CYC and decreased urinary alkylating activity were observed, suggesting reduced activation of CYC. DOX and 2 major metabolites 13-OH-DOX (DOXOL) and a polar aglycone (AAM) were consistently measured in all studies. Plasma C x T ( M-hr) of DOX, without and with WBH, were 3.29 ± 1.41, and 3.32 ± 1.96 (p 0.95). Similar C x T's for DOXOL, without and with WBH, were 2.43 ± 1.46 and 2.87 ± 2.01 (p< 0.6). C x T's for AAM were 2.09 ± 1.11 without WBH and 4.4 ± 1.89 with WBH (p< 0.005). C x T of AAM in non-CYC-containing combination regimens historically has averaged 5-6 μM-hr. We propose that microsomal metabolism of DOX to AAM, is inhibited by CYC, which also must be activated by microsomes. Formation of DOXOL, which proceed via soluble enzymes, is unaffected by CYC. The decreased activation of CYC which accompanies WBH, is associated with increased concentrations and C x T of AAM. Whether WBH itself affects AAM kinetics, and the precise mechanism of the CYC effect on AAM formation and elimination, cannot be elucidated from these data. These findings dictate the need for study of other DOX-CYC combination regimens, especially those in which liver dysfunction or concomitant administration of hepatoxins may be factors.

Previous pharmacologic studies have demonstrated lower plasma DOX concentrations in successive course of DOX therapy. This suggested that repeated exposure to DOX might accelerate its own metabolism and/or elimination. We performed 26 detailed pharmacokinetic studies on 8 patients who were treated with DOX, 45 mg/M$^2$ (I.V. bolus), cyclophosphamide (CYC), 1 gm/M$^2$ (1 hr infusion), and whole-body hyperthermia. Treatment scheme did not vary between courses. Peak plasma CYC concentration (±S.D.) was 44.2 + 12.5 μg/ml, and plasma elimination half-time for CYC was 7.3 ± 1.6 hrs. These did not vary appreciably between patients or from course to course. Plasma total anthracyclines (DOX plus metabolites), DOX, DOXOL, and an aglycone metabolite (AAM) were quantified as areas under concentration time plots (C x T, μ M hr). C x T's (±S.D.) for total anthracyclines were reduced from 16.9 ± 8.5 during the first course to 11.1 ± 0.06 by the fourth course. The C x T's for DOX fell similarly, from 4.8 ± 2.4 during the first course to 1.0 ± 0.2 by the fourth course. DOXOL C x T's fell from 3.4 ± 3.1 to 2.2 ± 0.4. The C x T's for AAM did not change consistently. In most cases of C x T decline, the greatest decrements occurred between the third and fourth course of therapy. We could not rule out DOX-CYC interaction, or some other feature of treatment, as partial explanation for our findings. We conclude that acceleration of DOX disappearance from plasma may occur by virtue of prior DOX treatment, and this may be an issue of concern in long-term DOX therapy in patients.

Publications:

Ostrow, S., Van Echo, D.A., Egorin, M., Whitacre, M., Grochow, L., Aisner, J., Colvin, M., Bachur, N., and Wiernik, P.H.: Cyclophosphamide pharmacokinetics in patients receiving whole body hyperthermia. J. Natl. Cancer Inst. (in press).

Riggs, C.E., Jr., Egorin, M.J., Fuks, J.Z., Schnaper, N., Duffey, P., Colvin, O.M., Aisner, J., Wiernik, P., and Bachur, N.R.: Initial observations on the effects of delta-9-tetrahydrocannabinol on the plasma pharmacokinetics of cyclophosphamide and adriamycin. J. Clin. Pharmacol 21 (Suppl): 905-985, 1981.

Fuks, J.Z., Egorin, M.J., Aisner, J., Ostrow, S., Klein, M.E., Bachur, N.R., Colvin, M., and Wiernik, P.H.: Cyclophosphamide and dimethylsulfoxide in the treatment of squamous carcinoma of the lung: Therapeutic efficacy, toxicity and pharmacokinetics. Cancer

Chemother. Pharmacol. 6: 117-120, 1981.

Konits, P.H., Egorin, M.J., Van Echo, D.A., Aisner, J., Andrews, P.A., May, M.E., Bachur, N.R., and Wiernik, P.H.: Phase II evaluation and plasma phamracokinetics of high-dose intravenous 6-thioguanine in patients with colorectal carcinoma. Cancer Chemother. Pharmacol.

Bachur, N.R., Collins, J.M., Kelley, J.A., Van Echo, D.A., Kaplan, R.S., and Whitacre, M.: Diaziquone, 2,5-diaziridinyl-3,6-biscarboethyoxyamino-1,4-benzoquinone, plasma and cerebrospinal fluid kinetics. Clinical Pharmacol. Therap. 31: 650-655, 1982.

Egorin, M.J., Van Echo, D., Andrews, P.A., Fox, B.M., Nakazawa, H., Whitacre, M., and Bachur, N.R.: The clinical Pharmacology of aclacinomycin A. In Antibiotics in Cancer Therapy. Ed. Muggia, F., Martinus Nijhoff, in press, 1981.

SUMMARY REPORT
CLINICAL ONCOLOGY BRANCH
BALTMORE CANCER RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
OCTOBER 1, 1981 - SEPTEMBER 30, 1982

The BCRP has been undergoing the final aspects of its conversion from an intramural program of DCT to one which is fully integrated into the University of Maryland at Baltimore. The following represents a brief synopsis of some of the major clinical research projects conducted during the past year. These projects are divided into those which are tumor specific and those which represent supportive care. The annual report of the Laboratory of Clinical Biochemistry, which has now moved to Bethesda, has been submitted separately.

Tumor Specific Studies

Acute Leukemia
Hodgkin's Disease
Lung Cancer
Head and Neck Cancer
Breast Cancer
New Drug Development

Supportive Care Studies

Diagnosis and Epidemiology of Infections
Treatment of Infections
Prevention of Infection
Prevention of Alloimmunization
Indium-labelling of Granulocytes
Cryopreservation of Platelets

## CLINICAL TRIALS IN ACUTE LEUKEMIA

A major component of this section is the ongoing Phase III study for the treatment of newly diagnosed patients with acute non-lymphocytic leukemia (ANLL). Induction therapy is with daunorubicin (3 days) and Cytosine Arabinoside (Ara-C) (7 days). Following remission, patients are treated with intensive maintenance chemotherapy (Ara C/6 thioguanine) to marrow aplasia every 3 months and are randomized to 1) chemotherapy alone 2) splenectomy plus Ara C/6 TG or 3) immunotherapy with neuraminidase treated allogeneic blasts plus chemotherapy. As of June 1982, 132 patients have been entered in this study with 83 (63%) achieving complete remission. The 83 patients were eligible for maintenance randomization and twenty-eight patients have been randomized to chemotherapy alone, 27 to splenectomy and 23 to immunotherapy. Median remission duration continues to be substantially longer for the group receiving immunotherapy with neuraminidase-treated cells than those receiving chemotherapy alone or splenectomy. A similar trend appears to exist for the patients who underwent splenectomy compared with chemotherapy alone. Fifteen percent of all treated patients are currently alive longer than 3 years after beginning treatment, and 30% have survived greater than 2 years from beginning treatment.

A second major area of investigation has been and continues to be the use of new agents for treatment of relapse in ANLL. The following Phase I-II studies in acute leukemia have been completed:

Aziridinylbenzoquinone (AZQ) was studied as a dose-finding pilot study in 22 patients at doses of 8-28 mg/m$^2$/day for 7 days. This study is now being expanded into a cooperative group (CALGB) study for phase II confirmation. In addition, this agent has some very interesting pharmacologic features such as very short half life, the necessity of prolonged exposure for cell entry and the necessity of an equilibrium to maintain intracellular concentrations. A new study at UMCC will utilize a continuous infusion AZQ with pharmacokinetic studies to investigate these properties and attempt to optimize therapeutic response.

A study of Dihydroxyanthiacenedione (DHAD) using 8-10 mg/m$^2$/day X 7 days has been completed with 26 patients entered. Among 16 patients with ANLL, there were 2 CR's and 1 PR. One of two patients with ALL achieved a CR lasting 2+ months. Among 8 patients with chronic myelogenous leukemia in blast crisis (CML-B), there was one CR. This study demonstrated activity of this drug in 20% of patients with relapsed leukemia which is comparable to that seen with other second-line drugs when used as single agents in acute leukemia. Furthermore, no cardiac toxicity was noted in these previously treated patients. Additional studies are warranted using DHAD as both a single agent and in combination.

An initial dose finding study of Anthracenedicarboxaldehyde (ADC) has been completed, with 11 patients treated at doses of 100-400 mg/m$^2$/day x 3 days. In an attempt to minimize the substantial cardiac and renal toxicity seen, with little therapeutic response, addition evaluation of this drug will be at a midrange dose of 200 mg/m$^2$ for five days.

Hyperthermia - A Phase I-II study to assess the antileukemic potential of whole body hyperthermia was initiated, but has been discontinued.

## CLINICAL TRIALS IN HODGKIN'S DISEASE

The major project is a randomized comparison of 3 treatment approaches to stages IB through III A ("early") Hodgkin's disease. This project, BCRP protocol 7824, is a comparison of a) extended field radiation (mantle + periaortic node irradiation + hepatic radiation for patients with splenic involvement); b) MOPP chemotherapy x 6 courses, or 2 courses past clinical CR; and c) combined modality therapy with radiation (as in a but no hepatic field) plus MOPP. This study tests whether MOPP chemotherapy alone will prove adequate initial treatment for these stages. If so, its use would obviate the need for staging laparotomy in many patients and the omission of radiation would presumably decrease the late cardiac, thyroid and other complications discussed below.

BCRP 7824 is being performed as a joint study of BCRP, University of Maryland Department of Radiation Oncology, and the NCI - Medicine Branch. Forty-nine patients have been randomized as of 4/1/82. Referral of newly-diagnosed HD patients to NIH and to University programs in recent years has decreased so that protocol 7824 has been reduced to a two arm comparison of Radiation and MOPP, eliminating the combined modality arm. Current results of the study, with 24 months median follow-up, are as follows:

| | ENTERED | EVALUABLE | CR | RELAPSED | DIED |
|---|---|---|---|---|---|
| RT | 20 | 20 | 20 | 6 | 2 |
| MOPP | 20 | 15 | 15 | 0 | 0 |
| | | (2 TETE, 1 Refused, 1 Lost, 1 acute MI) | | | |
| RT + MOPP | 9 | 3 | 3 | 0 | 0 |
| | | (6 pts switched to RT alone, not included above) | | | |

These preliminary data and those of an earlier pilot study, support the concept that MOPP alone may be adequate therapy for most stages of HD. However, in addition to further accrual, much longer follow-up will be required to assess the durability of initial CR's, results of salvage treatment and, most importantly, the relative rates of delayed complications of therapy.

A comprehensive analysis of fertility in 215 potentially fertile patients, from more than 350 treated for HD, has been completed. The major conclusions were that combined modality therapy suppressed fertility more than radiation or chemotherapy as single modalities, especially when pelvic irradiation was part of the combined treatment program. However, fertility overall was greater than expected and even severely oligospermic men successfully sired children. Pregnancy outcomes (i.e. stillbirths, birth defects, etc.) were not different from those of the general population.

In advanced disease, BCRP 7808 is a comparison of MOPP alone versus MOPP alternating with SCAB (Streptozotocin, CCNU, Adriamycin, Bleomycin - a highly successful combination developed at BCRP) for untreated stages $III_2A$, IIIB and IV Hodgkin's. This study, also being carried out in collaboration with the NCI Medicine Branch, is the only ongoing test of an alternating non-cross-resistant combination other than ABVD in Hodgkin's disease. As such, it represents an important test of the principles of Goldie & Coldman as they seem to apply to the recently presented MOPP/ABVD trial of Bonadonna & colleagues.

As of 5/1/82, 54 patients have been randomized to BCRP 7808. The median follow-up is 30 months with a range of 1 month to 4 years. Present data are as follows:

| | MOPP | | MOPP/SCAB | |
|---|---|---|---|---|
| # Entered | 24 | | 30 | |
| # Disqualified | 0 | | 3 | |
| # Too early to evaluate | 3 | | 6 | |
| # Currently Evaluable | 21 | | 21 | |
| Age M (range) | 26 | (15-58) | 28 | (17-63) |
| # 40 years | 3 | (14%) | 5 | (24%) |
| # male | 12 | (57%) | 17 | (81%) |
| B sx | 17 | (81%) | 15 | (71%) |
| MC or LD histology | 5 | (24%) | 8 | (38%) |
| # Achieving CR | 17 | (81%) | 17 | (81%) |
| # Achieving PR | 2 | (10%) | 1 | (5%) |
| No Response | 2 | (10%) | 3 | (14%) |
| # Relapsed | 4 | (23%) | 4 | (23%) |
| # Dead | 3 | (14%) | 1 | (5%) |

Clearly, no conclusion can as yet be drawn from these data or from the data on salvage of relapsing or non-responding patients. Further follow-up time will be required, as well as further accrual.

A number of studies have been concluded in this project including the completion of an alternating non-cross resistant combination treatment program for small cell. This showed that alternating therapy was not more efficacious than the sequential use of the treatments. Serial studies of our combination of doxorubicin, cyclophosphamide and etoposide have shown a high response percentage. A large number of patients have been accrued so that identification of evaluation of changes in natural history and complications of treatment can occur This aggressive combination chemotherapy alone can "cure" a fraction of these and the three serial studies have shown that this therapy represents one of the best available treatments for this common disease. A multivariate analysis showed that the goal for treatment is the achievement of complete remission. These randomized studies have also demonstrated the value of prophylactic cranial irradiation when given to patients who achieve complete remission and the value of trimethoprim/sulfamethoxazol in reducing both infection and hospitalization time in patients receiving induction treatment. Since many patients still relapse, this patient pool has allowed for the identification of second-line treatment programs as well as the testing of new agents. Vindesine and AMSA have both been shown to be ineffective (<20% activity, >90% confidence) in previously treated patients. Analysis of sequential biomarkers has shown that several biomarkers (CEA, NSE and LDH) correlate with disease activity but none are more sensitive in predicting progression or relapse than the routine technology used for clinical evaluation.

The 2 drug combination cisplatinun and cyclophosphamide has been evaluated in patients with head and neck cancer recurring after surgery and radiotherapy. Cyclophosphamide 600 mg/m$^2$ IV and cisplatinum 60 mg/m$^2$ IV were repeated every 3 weeks until disease progression was documented. The objectives of this study were to evaluate the duration of response and toxicity. A total of forty two patients have been entered. Currently, 4 are too early to evaluate and 3 patients have been lost to follow-up. Of the remaining 35 evaluable patients with measurable disease there have been 3 complete responses (CR) and 10 partial responses (PR) (overall response rate of 37%), 5 minor responses (MR) and 17 progressions. The median duration of response (MDR) was 16 months with a range of 10-17 + months for complete responders and 4 months, range of 2-18 + months for partial responders. The major toxicity was nausea and vomiting in 81% of patients. Hematologic toxicity was manageable, WBC nadir < 2,000 occurred in 22 patients, platelet nadir < 50,000 occurred in 2 patients. There were no episodes of bleeding or infection directly related to the chemotherapy. Mild creatinine elevation (1.5-2.0 mg/dl) occurred in 4 patients. One patient experienced significant renal toxicity with a creatinine > 2.0 mg/dl. Neurotoxicity, peripheral neuropathy and high frequency hearing loss, was documented in 1 patient after receiving a total dose of 480 mg/m$^2$ cisplatinum. This study has been closed to further patient entry since the results have not shown improvement in overall response rate over what one would expect from cisplatinum used alone. However, the combination did show that patients achieving complete disease regression had a significant improvement in median duration of response and survival.

## CLINICAL TRIALS IN BREAST CANCER

Several combinations have been piloted for antitumor activity in advanced breast cancer. A combination of vinblastine and mitomycin produced a 40% response rate (complete plus partial responses) in 30 patients. Toxicity included moderate to severe myelosuppression. This response rate is noteworthy in view of the extensive prior treatment that most of the patients had received before this chemotherapy regimen. Several patients treated with this combination developed pulmonary reactions which appeared to be temporally related to vinblastine. In one case the reaction was associated with a rechallange of vinblastine. These works suggest that this two drug combination adds further treatment possibilities to patients who have undergone extensive chemotherapy. The observation that vinblastine may cause pulmonary toxicity should alert physicians to this possibility. The relatively high level of response in these extensively treated patients has led to a new protocol (see below).

An additional combination with activity in patients with advanced breast cancer has been the combination of doxorubicin and VP16-213. This combination produced a 40% response rate among 30 evaluable patients with a median response duration of 146 days. Toxicity included moderate to severe myelosuppression. This two drug combination appears to be active as second line therapy but this response rate is within the 95% confidence limits of other responses to other combinations such as doxorubicin and vincristine. It is, however, no more toxic than doxorubicin alone in full dose. The significance of this combination is that it may be particularly worthwhile in patients who have received prior vinca drugs.

The identification of new agents or new schedules are of importance in advanced breast cancer and phase II studies continue. A study of AZQ has been completed and thus was shown to be an ineffective agent (less than 20% level of activity with greater than 95% confidence). Another phase II study of anthracenedicarboxaldehyde (ADC) is currently being completed with no responders among the patients who received prior anthracyclines (less than 20% activity with greater than 95% confidence) and no responders among 10 patients entered without prior anthracycline exposure.

## CLINICAL TRIALS IN THE APPLICATIONS OF NEW TREATMENT MODALITIES

Biologic Response Modifiers have been studied in two diseases; acute leukemia and colo-rectal carcinoma. In acute leukemia, 75 relapsed patients over the last 30 months have been given thymidine (TdR) combined the cytosine arabinoside (Ara-C). This study has investigated many dose changes of both agents given as simultaneous continuous infusions until bone marrow aplasia (10-14 days). This has resulted in a fairly constant 40-50% response rate for acute nonlymphocytic leukemia patients (ANLL) and occasional responses in blastic phase chronic myelogenous leukemia (CML-B) patients. Most recently the schedule has been fixed to 7 days of treatment with TdR at 40 $GM/M^2/d$ and Ara-C at 430 $mg/m^2/d$. This modification delivers the same Ara-C cumulative dose per induction course over a shorter period of time with a high daily dose of the modifier TdR. The intent is to preserve the encouraging therapeutic response rate, decrease nonhematologic toxicities and days in hospital. This intent has been achieved in the first 3-4 patients so treated but needs confirmation with a larger cohort.

This study now has collaborative laboratory studies investigating mechanisms of synergistic cytotoxicity between TdR, guanosine and Ara-C in human derived leukemia cells HL60 and K562. In addition studies of leukemic cells taken from patients are being studied to see whether effects of synergy in Ara-C uptake and incorporation into the DNA in the presence of TdR and other nucleosides correlates with clinical response. This may eventually allow for a predicative test to select patients best able to respond to such treatment. These studies have the potential for developing another approach to the variety of neoplasms.

Whole body hyperthermia (WBH) has been combined with cyclophosphamide (CTX) and Adriamycin (Adr) in 11 patients with soft-tissue sarcomas. Six of these patients have achieved stabilization or shrinkage of tumor and 5 had rapid progression of disease. The survival of the stable or responding patients is very similar (median 12+ months) but markedly different from non-responding patients (median 3 months). Pharmakinetic data indicate that WBH affects metabolism of CTX and Adr in an adverse manner and the combination is probably not warranted in future studies. A new study of cisplatin (CDDP) plus WBH has been started in patients with refractory malignancies, especially sarcomas. The study is too early to evaluate but will also explore the effects of WBH on the pharmacokinetics of CDDP.

Staphylococcus epidermidis: Infection by this organism has been found to be increasingly frequent among granulocytopenic patients who have severe mucosal damage. Whereas S. epidermidis bacteremias were rare prior to 1977, the incidence was 7.6/1000 hospital days through 1979, a significant increase. Infection with this organism generally does not respond to therapy with cephalosporins, despite in vitro susceptibility, but responses have been observed when treated with vancomycin. The frequency of recovery from blood cultures has increased during June, July and August, 1981. Characterization of the strains by enzyme profiles and sensitivity to lysostaphin has been undertaken to investigate the epidemiological implications of these recoveries.

Implications of Hepatitis in Patients with Acute Nonlymphocytic Leukemia: Review of the development of infectious hepatitis, primarily nonA/nonB hepatitis, indicates that this infection is exceedingly common in patients with acute nonlymphocytic leukemia during and following induction chemotherapy (67%) and that the median survival for patients who develop hepatitis is significantly longer (549 days) than is survival of those patients who do not develop hepatitis (322 days). This increase in survival is primarily related to an increase in the duration of complete remission. Two additional large cohorts of patients have now been analyzed with similar results to the first review.

In an initial evaluation of 612 microorganisms tested by the microtiter technique, moxalactam plus amikacin was generally synergistic against most organisms as was piperacillin plus amikacin. Of particular importance, the combination of moxalactam plus piperacillin was never found to be antagonistic when tested against any of these organisms and was synergistic in approximately 50%. Hence, these two antibiotics can be used together in appropriate patient trial situations.

Empiric Antibiotic Therapy for Suspected Infection in Granulocytopenic Cancer Patients: A Prospective, Randomized Trial of Moxalactam Plus Amikacin Versus Moxalactam Plus Piperacillin (UMCC Protocol 8107): Piperacillin and moxalactam have very broad spectra so that most bacterial pathogens are susceptible to both agents and thus the double agent coverage that is usually required when treating granulocytopenic patients is provided with this combination. The presence of synergism should enhance this activity. A combination of this nature would be devoid of the nephro- and ototoxicity inherent in aminoglycoside containing regimens.

Clinical Trial: Approximately 163 episodes have been studied in 11 months. Efficacy of the two combinations appears to be similar. Patients who received moxalactam plus piperacillin had significantly less ototoxicity and a trend towards less nephrotoxicity than patients in the other arm of the study.

Pharmacokinetics of Moxalactam and Piperacillin: The half-life of moxalactam and piperacillin when given by rapid intravenous infusion was evaluated in six patients receiving each regimen studied with serial serum samples.

Serum Bactericidal Activity: Serum samples obtained 1 hour after antibiotic administration are tested against the patient's own infecting organism. Results will be correlated with clinical outcome and synergy data (see below). Serum samples of selected patients were tested against a battery of gram-negative rods and gram-positive cocci to determine the geometric mean bactericidal titer against each species for the two antibiotic combinations.

Synergy Assays: The patient's own infecting organism is tested against the two antibiotic combinations in a checkerboard fashion to determine antibiotic synergism. Results will be correlated with efficacy of the combination and serum cidal activity.

## PREVENTION OF INFECTION IN PATIENTS WITH CANCER

Comparative Evaluation of LAF Environment: The LAF environment is being studied with the particle air sampler, the large volume air sampler, the standard Anderson air sampler and the collection of Rodac plate samples, large volume water samples, drain samples, and food samples. Particle sampling and air sampling is done with the room empty, with a patient in bed but quiet, and with the patient in bed or near the bed but during room activity such as bedmaking. At least 6 horizontal and vertical surfaces are sampled with Rodac plates, sink drains are sampled on a volumetric basis, and foods are sampled on a quantitative and qualitative basis. Water samples of at least one liter are obtained. All the above is done in a single laminar air flow room housing a patient with ANLL, a regular inpatient UMCC room (9th floor) housing a patient with ANLL, a room on the 11th floor (general medicine) of the North Hospital housing a patient with ANLL and a room in the South Hospital on a medical floor preferably housing a patient with ANLL. Extensive sampling continues to assure that microbiological contamination does not occur especially in the LAF environment.

Microbiologic Evaluation of Complete Versus Limited Reverse Isolation in LAF Rooms (UMCC Protocol 8211): Considering that the primary sources of new organism acquisition are food, water, hand contact and air, it follows that many of the procedures characteristically associated with complete LAF isolation may be superfluous. In this evaluation, patients are either isolated under complete LAF technique or a more limited form which includes complete housekeeping, complete control of food and water access and water egress, limited gowning procedures and the use of clean but not sterile materials within the LAF room. The measure of success of this program will be through an evaluation of environmental microbiologic cultures, the acquisition of new organisms as judged by patient surveillance cultures during the period of isolation, and patient outcome reflected by remission rate and survival. The mean total number of organisms acquired/patient/week was similar for the first 11 patients in limited and 13 patients in full LAF reverse isolation and the majority of these were only transiently acquired. The difference in cost/patient/week, however, was $2,175.00, indicating that although both these techniques of protected isolation are effective, limited LAF reverse isolation is far less expensive.

## PREVENTION OF ALLOIMMUNIZATION WITH LEUKOCYTE POOR PLATELETS

Alloimmunization to histocompatibility antigens remains the major problem remaining in the field of platelet transfusion therapy. This is a prospectively randomized study designed to determine whether rendering platelet concentrates "leukocyte poor" by an additional centrifugation will prevent the development of alloimmunization. Only newly diagnosed patients with acute nonlymphocytic leukemia receiving identical chemotherapy are randomized on this study with the end point of the study being the development of lymphocytotoxic antibody and/or the requirement for HLA matched platelets. A total of 95 patients have been randomized to date including 49 patients randomized to receive leukocyte depleted platelets and 46 to receive conventional platelet concentrates. Thirty-nine of the patients randomized are inevaluable because of the following reasons: 10 patients required granulocyte transfusions as part of their supportive care; 12 patients randomized had lymphocytotoxic antibody present on admission; 4 patients died within the first two weeks of therapy; 2 protocol violations; 1 lost to followup. Most of the 12 patients who had antibody present on admission were females who were alloimmunized because of previous preganancies. This rate of prior sensitization is not appreciably different from our previous experience with this population of patients but does decrease the number of patients suitable for study on this protocol. In addition, because we administer granulocyte transfusions according to our usual clinical criteria, a number of patients who require this type of supportive care because of severe infectious episodes during induction also have to be eliminated from study. The rate of granulocyte transfusions useage in this study is approximately the same as our past experience (10 to 15% of patients receiving initial induction therapy). Except for these issues the study is progressing well. At the moment there is no difference in the alloimmunization rate between the two groups of patients. The groups are not entirely homogenous however in terms of prior transfusion history. Sixteen patients in the "leukocyte poor" have neither received prior transfusions nor had previous pregnancies; 13 patients receiving standard platelet support have had no prior exposure to histocompatibility antigens. Five patients in each group have become alloimmunized to date.

## INDIUM LABELED GRANULOCYTES IN THE STUDY OF FACTORS AFFECTING GRANULOCYTE TRANSFUSION

$^{111}$Indium labeled granulocytes were used to study the effects of histocompatibility factors on the migration of transfused granulocytes to infected sites. Fourteen alloimmunized and 20 non-alloimmunized patients received approximately $10^8$ $^{111}$Indium labelled granulocytes from ABO compatible, non HLA-matched donors and scans were performed over known infected sites. All 14 alloimmunized patients had lymphocytotoxic antibody (LCTAb) and required HLA-matched platelet transfusions. 20/20 non-alloimmunized patients had positive scans at sites of infection. None of the 20 had LCTAb, 0/17 had a positive lymphocytotoxic crossmatch (LCTXM) with the donor and 3/18 had a positive leukoagglutinin crossmatch (LAXM). Thus, histocompatibility testing was not found to be important in non-alloimmunized patients. In contrast, only 3/14 alloimmunized patients had positive scans at sites of infection (p=.00001 compared to non-alloimmunized patients). One of three had a positive LCTXM and 2/3 had a positive LAXM. 10/11 alloimmunized patients with negative scans had a positive LCTXM and 8/11 had a positive LAXM. Labelled granulocytes failed to reach sites of infection in 11/14 (78%) alloimmunized patients demonstrating that histocompatibility factors can be of major importance in affecting the outcome of granulocyte transfusions. Granulocytes from random donors are unlikely to be effective in alloimmunized patients.

An assessment has been made of the persistence of granulocyte-associated pulmonary radioactivity during the first 30 minutes after injection, comparing alloimmunized and non-alloimmunized patients. The number of patient studies is small due to technological problems with the computer system. However, the initial analysis suggests that the rate of decline of pulmonary activity is quite different in these two groups of patients. Nine alloimmunized and four non-alloimmunized patients were evaluated by analyzing the ratio of lung to heart radioactivity during the first thirty minutes of scanning. Alloimmunized patients had significantly longer retention of granulocytes in their lungs (p .02). Evaluation of a patient who received only platelets prepared in this same way, showed minimal retention, verifying that what is seen in granulocyte scans indeed represents granulocytes. Alloimmunized patients had significantly greater retention compared to 2 patients studied with autologous granulocytes (p .05) which were similar to non-alloimmunized patients. This finding is compatible with clinical observations of pulmonary reactions following granulocyte transfusions and provides objective evidence of pulmonary granulocyte retention in humans. This could suggest potential significant toxicity from the use of random donor granulocytes in alloimmunized recipients.

## PLATELET CRYOPRESERVATION

Autologous platelets obtained from patients with leukemia in remission and cryopreserved in 5% dimethylsufoxide have become a major component of our transfusion program. This procedure has produced consistent posttransfusion platelet recoveries with evidence of hemostatic function as documented by shortening of the bleeding time and cessation of hemorrhage. The results have been quite consistent through the years with posttransfusion recoveries which average approximately 60% of that achieved with fresh platelets. Most of the patients plateletpheresed were alloimmunized and for some patients, frozen autologous platelets represented the only source of histocompatible platelets. There are no side effects following transfusion of the small amounts of residual dimethylsulfoxide and patient acceptance of this program is excellent. It has been demonstrated by our laboratory that the technique is relatively simple, reproducible and cost effective and suitable for use in most blood banks. Although the overall results of the platelet cryopreservation program have been quite satisfactory there is variation in results from patient to patient. An analysis of platelet collection method revealed similar results whether the platelets were collected using manual multiple bag plateletpheresis techniques (mean one hour posttransfusion corrected increment of $11,700/mm^3$, 211 transfusions to 44 patients) or the mechanical Haemonetics Model 30 Blood Separator (mean increment 12,300, 66 transfusions to 17 patients). The "surge pump" adaptation of the Haemonetics machine described in the Cell Component Research Core has also been used with similar results. This adaptation has the advantage of producing red cell free platelets thereby eliminating a post collection centrifugation and considerably simplifying the freezing process. We were unable to complete a project aimed at separating platelets of different sizes in order to determine whether different sized platelets have a different susceptibility to freeze injury. Although we could separate different populations of platelet size by differential centrifugation or on a density gradient, there was considerable overlap amongst the platelet populations and it was also difficult to collect a sufficient number of platelets by differential centrifugation without multiple centrifugations. The multiple centrifugations in and of themselves can produce considerable injury making subsequent assessment of the amount of injury from freezing difficult to determine.

## BIBLIOGRAPHY

### CLINICAL TRIALS IN ACUTE LEUKEMIA

Van Echo DA, Schulman P, Ferrari A, Budman D, Wiernik PH: A phase I trial of aziridinylbenzoquinone (AZQ, NSC-182986) in patients with previously treated acute leukemia. Cancer Clin Trials (in press).

Bachur NR, Collins JM, Kelley JA, Van Echo DA, Kaplan RS, Whitacre MY: Human 2,5-Diaziridinyl-3,-6-Biscarboethoxy Amino-1,4-Benzo Quinone (AZQ) plasma and cerebrospinal fluid pharmacokinetics. Cancer Chemotherapy and Pharmacology (in press).

Van Echo DA, Shulman PN, Ferrari A, Budman D, Markus SD, Wiernik PH: A phase II trial of Mitozantrone (DHAD, NSC301739) in adult acute leukemia (AL). Proceedings of American Society of Clinical Oncology. 23: 1982.

Van Sloten K, Wiernik PH, Schiffer CA, Schimpff SC, Aisner J: Evaluation of Levamisole as an adjuvant to chemotherapy for treatment of ANLL. Cancer (in press).

Wiernik PH: Leukemias and Myeloma. In Pinedo HM (Ed): Cancer Chemotherapy 1981, Amsterdam, Excerpta Medica, pp. 203-235, 1981.

Dutcher JP and Wiernik PH: Adult Leukemia: How to recognize the early signs. Your Patient & Cancer 1:37-40, 1981.

Dutcher JP and Wiernik PH: Adult Leukemia: Treatment of Choice, Maintenance Therapy, Supportive Care. Your Patient and Cancer 1:32-46, 1981.

Wiernik PH: Adult Acute Leukemia. In DeVita VT, Hellman S, Rosenberg S (Eds): Cancer: Principles and Practice of Oncology, Philadelphia, J. B. Lippincott Co., pp. 1402-1426, 1982.

Wiernik PH: Acute Nonlymphocytic Leukemia in Young People. In Levine AS (Ed.): Cancer in Children and Adolescents. CRC Press, New York, pp. 461-472, 1982.

Esterhay RJ Jr., and Wiernik PH: The Therapy of Adult Acute Lymphoblastic Leukemia. In Bloomfield CD (Ed): Acute Leukemia, in press, 1982.

Schiffer CA, DeBellis R, Kasdorf H, Wiernik PH: Treatment of blast crisis of chronic myelogenous leukemia with 5-azacytidine and VP16-213. Cancer Treat Rep 66:267-271, 1982.

Esterhay RJ Jr, Wiernik PH, Grove WR, Markus SD, Wesley MN: Moderate dose methotrexate, vincristine, asparaginase and dexamethasone for treatment of adult acute lymphocytic leukemia. Blood 59:334-345, 1982.

Yates G, Glidewell OH, Wiernik PH, et al: Cytosine arabinoside with daunorubicin or adriamycin for therapy of acute myelocytic leukemia: A CALGB Study. Blood, in press, 1982.

Aisner J, Konits PH, Wiernik PH, Rulenz C: Fertility, pregnancy and progeny in patients treated for Hodgkin's disease. (submitted to Cancer)

Espana P, Kaplan R, Smith F, Robichaud K, Lichtenfeld L, Wiernik P, Schein P: A phase II trial of spirogermanium in lymphoma patients. (submitted to Cancer)

CLINICAL TRIALS IN LUNG CANCER

Small Cell Publications

Aisner J: Is combined modality treatment of small cell carcinoma of the lung necessary. In: Controversies in Oncology, P.H. Wiernik (ed), Wiley and Sons, New York, N.Y., 1982, pp. 155-173.

Aisner J, Whitacre M, Van Echo DA, Slawson R, Wiernik PH: Combination chemotherapy for small cell carcinoma of the lung: Continuous vs. alternating non-cross resistant combinations. Cancer Treat Rep 66: 221-230, 1982.

Rosen ST, Aisner J, Makuch RW, Matthews MJ, Ihde DC, Whitacre M, Glatstein EJ, Wiernik PH, Lichter AS, Bunn PA Jr: Carcinomatous leptomeningitis in small cell lung cancer: A clinicopathologic review of the National Cancer Institute experience. Medicine (Baltimore) 61: 45-53, 1982.

Fuks JZ, Aisner J, Carney DN, Van Echo DA, Ostrow S, Ihde D, Wiernik PH: A phase II trial of vindesine in patients with refractory small cell carcinoma of the lung. Cancer Clin Trials 5: 49-52, 1982.

Aisner J, Van Echo DA, Whitacre M, Wiernik PH: A phase I trial of continuous infusion VP16-213 (etoposide). Cancer Chemo Pharmacol 7: 157-160, 1982.

Aisner J, Whitacre M, Van Echo DA, Wesley M, Wiernik PH: Doxorubicin, cyclophosphamide and VP16-213 (ACE) in the treatment of small cell lung cancer. Cancer Chemother Pharmacol 7: 187-193, 1982.

Fuks JZ, Aisner J, Van Echo DA, Levitt M, Carney D, Wiernik PH: A phase II trial of aziridinylbenzoquinone (AZQ) in patients with refractory small cell carcinoma of the lung. Cancer Clin Trial (in press).

Poplin EA, Aisner J, Van Echo DA, Whitacre M, Wiernik PH: CCNU, vincristine, methotrexate and procarbazine treatment of relapse small cell lung carcinoma. Cancer Treat Rep (in press).

Aisner J, Alberto P, Bitran J, Comis R, Daniels J, Hansen HH, Ikeyami H, Smyth J: Role of chemotherapy in small cell lung cancer. Cancer Treat Rep (in press).

de Jongh C, Wade J, Finley R, Joshi J, Aisner J, Schimpff SC, Wiernik PH: Double blind randomized comparison of trimethoprim/sulfamethoxazole versus placebo for prophylaxis of infection in patients with oat cell carcinoma. Proc Am Soc Clin Oncol 1: 55, 1982 (manuscript submitted).

Aroney RS, Aisner J, Wesley MN, Whitacre MY, Van Echo DA, Wiernik PH: The value of prophylactic cranial irradiation (PCI) given at complete remission in small cell lung carcinoma (SCLC). Am Fed Clin Research 32: 414A, 1982 (manuscript submitted)

Wiernik PH: Leukemias and myeloma, in Pinedo HM (Ed), Cancer Chemotherapy 1982, Amsterdam, Excerpta Medica, in press, 1982.

Gallagher RE and Wiernik PH: Acute leukemia: New perspectives on pathogenesis; Progress in therapy of children and adults, in Fairbanks VF (ed), Hematology 1981, in press, 1982.

Testa JR, Oguma N, Pollak A, Wiernik PH: Near-tetraploid clones in acute leukemia. Blood (In Press), 1982.

Testa JR, Oguma N, Misawa S, Pollak A, Wiernik PH: Association of an 8;21 translocation with a specific subtype of acute non-lymphocytic leukemia. Amer Soc of Human Genetics (Abstract), 1982.

CLINICAL TRIALS IN HODGKIN'S DISEASE

Applefeld MM, Cole JF, Pollock SH, Sutton F, Slawson RG, Singleton RT, Wiernik PH: The late appearance of chronic pericardial disease in patients treated by radiotherapy for Hodgkin's disease. Ann Int Med 94: 338-341, 1981.

Applefeld MM, Slawson RG, Hall-Craigs M, et al: Delayed pericardial disease after radiotherapy. Amer J Cardiol 47: 210-213, 1981.

Applefeld MM, Slawson RG, Spicer KM, et al: Long-term cardiovascular evaluation of patients with Hodgkin's disease treated by thoracic mantle radiation therapy. Cancer Treat Rep 66: 1003-1013, 1982.

Wiernik PH and Slawson RG: Hodgkin's disease with direct extension into pulmonary parenchyma from a mediastinal mass: A presentation requiring special therapeutic considerations. Cancer Treat Rep 66: 711-716, 1982.

Stein RS, Golomb HM, Wiernik PH, et al: Anatomic substages of stage IIIA Hodgkin's disease: follow-up of a collaborative study. Cancer Treat Rep 66: 733-741, 1982.

Wiernik PH, Longo D, Duffey PL, Young RC, DeVita VT: MOPP vs MOPP alternating with streptozotocin, CCNU, adriamycin and bleomycin (SCAB) for advanced Hodgkin's disease. Proc Am Assoc Cancer Res 22: 159, 1981.

Konits PH, Wiernik PH, Aisner J, Rulenz CA: Pregnancy outcome in patients treated for Hodgkin's disease (HD). Proc Am Soc Clin Oncol 22: 381, 1981.

Coker DD, Morris DM, Coleman JJ, Wiernik PH, Elias EG: Restaging laparotomy for Hodgkin's disease. Annals of Surgery (in press).

Duffey P, Campbell EW, Wiernik PH: Hydrocele following treatment for Hodgkin's disease. Cancer (in press).

Coker DD, Morris DM, Coleman JJ, Schimpff SC, Wiernik PH, Elias EG: Infection among 210 patients with surgically staged Hodgkin's disease. Amer J Med (in press).

Aroney RS, Dermody WC, Aldenderfer P, Parsons R, McNitt K, Marangos PJ, Whitacre MY, Aisner J, Ruddon RW, Wiernik PH: Multiple sequential biomarkers in monitoring patients with small cell lung carcinoma (SCLC). Clin Res 32: 414A, 1982 (manuscript in preparation).

Non-Small Cell Publications

Aisner J, Hansen HH: Commentary: Chemotherapy for non-small cell carcinoma of the lung: current status. Cancer Treat Rep 65: 979-986, 1981.

Fuks JZ, Eogrin MJ, Aisner J, Ostrow S, Klein ME, Bachur NR, Colvin M, Wiernik PH: Cyclophosphamide and dimethylsulfoxide in the treatment of squamous carcinoma of the lung: Therapeutic efficacy, toxicity and pharmacokinetics. Cancer Chemother Pharmacol 6: 117-120, 1981.

Aroney RS, Dermody WC, Aldenderfer P, Parsons R, McNitt K, Marangos PJ, Whitacre MT, Aisner J, Ruddon RW, Wiernik PH: Multiple sequential biomarkers in monitoring patients (pts) with carcinoma of the lung. Proc of the Endocrine Lung in Health and Disease, George Washington University Medical Center, Washington, D.C. 1982.

Konits PH, Aisner J, Wiernik PH: Lung cancer as a complication of prolonged survival in patients with lymphoma. Med Ped Oncol (in press)

Fuks JZ, Van Echo DA, Aisner J, Schipper H, Levitt M, Ostrow S, Klein M, Wiernik PH: Cyclophosphamide (c), adriamycin (a) and etoposide (e) with or without cis-platinum (p) (CAEP vs. CAE) in advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 1: 145, 1982 (manuscript submitted).

Fuks JZ, Egorin MJ, Aisner J, Van Echo DA, Ostrow S, Bachur NR, Wiernik PH: Therapeutic efficacy and pharmacokinetics of vindesine and vindesine-platinum in previously treated patients with non-small cell lung carcinoma (manuscript submitted).

Mesothelioma Publications

Whitley NO, Brenner DE, Antman KH, Grant D, Aisner J: Computed tomographic evaluation of peritoneal mesothelioma: An analysis of eight cases. Am J Roentgen 138: 531-535, 1980.

Antman K, Pomfert L, Aisner J, et al: Peritoneal mesothelioma: natural history and response to chemotherapy. (submitted for publication).

CLINICAL TRIALS IN HEAD AND NECK AND ESOPHAGEAL CANCER

Forastiere AA, Crain SE, Callahan K, Van Echo D, Mattox A, Thant M, Von Hoff DD, Wiernik PH: A phase II trial of AZQ (NSC 183986) in head and neck cancer (submitted).

Perry DJ, Crain SE, Weltz MD, Wilson JP, Davis RK, Woolley PV, Forastiere AA, Taylor HG, Weiss RB: Phase II trial of mitoguazone (methylglyoxal bis guanylhydrazone) in patients with recurrent or metastatic squamous cell carcinoma of the head and neck (submitted).

Forastiere AA, Crain SM, Coker DD, Elias EG, Amornmarn R, Wiernik PH: Cisplatin (P) and cyclophosphamide (C) combination chemotherapy in advanced head and neck squamous cell cancer (HNSCC). Proceedings of American Society of Clinical Oncology, 1:96, 1982.

Forastiere AA: Management of Advanced Stage Epidermoid Carcinoma of the Head and Neck. The American Journal of the Medical Sciences (in press).

CLINICAL TRIALS IN BREAST CANCER

Aisner J, Morris D, Elias EG, Wiernik PH: Mastectomy as an adjunct to chemotherapy for locally advanced or metastatic breast cancer. Arch Surg 117: 882-887, 1982.

Barr L, Morris D, Goldman L, Aisner J: Chemotherapy and adjuvant mastectomy for locally advanced breast cancer. Proc Am Soc Clin Oncol 1: 88, 1982.

Budman DR, Forastiere A, Perloff M, Perry M, Aisner J, Weinberg V, Wood W: AZQ (NSC 182986) in advanced breast cancer. Cancer Treat Rep (in press).

Konits PH, Aisner J, Van Echo DA, Lichtenfeld K, Wiernik PH: Mitomycin C and vinblastine chemotherapy for advanced breast cancer. Cancer 48: 1295-1294, 1981.

Konits PH, Aisner J, Sutherland JC, Wiernik PH: Possible pulmonary toxicity secondary to vinblastine. Cancer (in press).

Konits PH, Van Echo DA, Aisner J, Morris D, Wiernik PH: Doxorubicin plus VP16-213 for the treatment of refractory breast carcinoma. Am J Clin Oncol (in press)

CLINICAL TRIALS IN THE APPLICATIONS OF NEW TREATMENT MODALITIES

Akman SA, Ross DD, Rosen H, Salinger C, Andrews PA, Chou FE, and Bachur NR: Growth inhibition by thymidine of leukemic HL-60 and normal human myeloid progenitor cells, 41:2141-2146, 1981, Cancer Res.

Ross DD, Akman SA, Schrecker AW, and Bachur NR: Deoxynucleoside effects on cultured human leukemic cells. I. Effects on growth and deoxynucleotide pools, 41:4493-4498, 1981, Cancer Res.

Akman SA, Ross DD, and Bachur NR: Synergistic inhibition of human leukemic cell growth by deoxygaunosine and 1-#-D arabinofuranosylcytosine. (Submitted to Cancer Res.).

Ross DD, Akman SA, Bachur NR and Groth DP: 5-Iododeoxycytidine kinase activity of human acute myelogenous leukemic blast cells. (Submitted to Exper. Hematol.).

Akman SA, Ross DD, Joneckis C, Schrecker AW and Bachur NR: mechanism of synergistic cytotoxicity between nucleosides and arabinofuranosyl cytosine (ara-C). Proc Am Assoc Cancer Res., 22:219, 1982.

Ostrow S, Van Echo DA, Egorin M, Whitacre M, Grochow L, Aisner J, Colvin M, Bachur NR, Wiernik PH: Alteration of cyclophosphamide (CY) pharmacokinetics by whole body hyperthermia (WBH). J National Cancer Institute, 1981, (in press).

Gerad H, Van Echo DA, Ostrow S, Whitacre MY, Aisner J, Wiernik PH: Adriamycin (ADR), cytoxan (CY) and whole body hyperthermia (WBH) for soft tissue sarcoma (STS). Proceedings of American Society of Clinical Oncology, 1:175, 1982,

Riggs CE, Jr., Egorin MJ, Akman SR, Angelou JE, Van Echo DA, Whitacre M, Bachur NR: Doxorubicin (DOX) metabolism is altered by combination therapy with cyclophosphamide (CYC) and whole body hyperthermia (WBH). Proceedings of American Society of Clinical Oncology, 1:26, 1982.

Riggs CE, Jr., Egorin MJ, Whitacre M, Akman SR, Angelou JE, Van Echo DA, Aisner J, Bachur NR: Cyclophosphamide (CYC) and doxorubicin (DOX) pharmacokinetics in patients with sarcomas - evidence for altered doxorubicin metabolism. Clinical Research, 30:423A, 1982.

Broadwell RD, Salcman M, Kaplan RS: Morphologic Effect of Dimethyl Sulfoxide on the Blood-Brain Barrier. Science, 217:164-166, 1982.

Leavitt RD, Duffey PL, Wiernik PH, Fein S, Sherwin S, Scogna D, and Oldham R: A phase I trial of twice daily recombinant Leukocyte A Interferon (IFL-rA) in cancer patients. Proceedings of American Society of Clinical Oncology, 1:41, 1982.

Maluish AE, Conlon J, Ortaldo JR, Sherwin SA, Leavitt RD, Fein S, Wiernik PH, Oldham RK, Herberman RB: Modulation of NK and monocyte activity in advanced cancer patients receiving interferon. Proceedings of the ICN-UCLA Symposium, 1982 (in press).

## INFECTIOUS DISEASES AND MICROBIOLOGY

### Abstracts

Viollier AF, De Jongh DE, Newman K, Schimpff S: Aspergillus sinusitis in cancer patients. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, 1981.

Joshi J, Wang KP, Hornedo J, De Jongh C, Newman K, Wiernik S: Sensitivity, specifity and safety of fiberoptic bronchoscopy (FOB) in febrile, myelosuppressed acute leukemia patients with possible fungal pneumonia. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Joshi JH, Wang KP, De Jongh CA, Newman KA, Wiernik PH, Schimpff SC: A comparative evaluation of two fiberoptic bronchoscopy catheters: The plugged telescoping catheter versus the single sheathed nonplugged catheter. American Federation for Clinical Research, 1981.

de Jongh C, Newman K, Drusano G, Moody M, Wharton R, Joshi J, Schimpff S: Serum levels, bactericidal activity and in vitro synergism of two broad spectrum antibiotic combinations. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Moody M, Warton R, Sprecker S, Schimpff SC: In vitro activity of six extended spectrum beta lactam antibiotics in combination with each other or with amikacin. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Caplan ES, Moody MM: Ticarcillin levels in human cerebrospinal fluid. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, 1981.

Viollier AF, Standiford H, Klastersky J, Moody M, Tatem B, Schimpff S: Antibiotic combinations against gram-negative bacilli and s. aureus in cancer patients: comparative in vitro and in vivo activity of cefoperazone and mezlocillin singly or combined together. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, 1981.

Viollier AF, Standiford H, Drusano G, Tatem B, Moody M, Schimpff S: Comparative pharmacokinetic and serum bactericidal activity of mezlocillin and ticarcillin with and without gentamicin. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, 1981.

Viollier AF, Standiford H, Drusano G, Moody M, Tatem B, and Schimpff S: Comparative in vivo and in vitro activities of cefoperazone and mezlocillin alone and combined together or with gentamicin. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

de Jongh C, Joshi J, Newman K, Danhauer F, Finley R, Moody M, Wiernik P, Schimpff S: Moxalactam plus piperacillin or amikacin: Empiric antibiotic therapy for febrile, neutropenic cancer patients. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Viollier AF, Standiford H, Drusano G, Tatem B, Moody M, Schimpff SC: Comparative pharmacokinetic and serum bactericidal activity of mezlocillin (M), ticarcillin (T), piperacillin (P) with and without gentamicin (G) - A new outlook on the granulocytopenic host. 2nd International Conference on Infection in the Immunocompromised Host, Stirling, Scotland, 1982.

Fitzpatrick B, Standiford H, Drusano G, Tatem, B, Moodv M, Schimpff S: Comparative serum bactericidal titers produced by ceftazidine, moxalactam and the ticarcillin-amikacin combination in normal volunteers. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Moody M, Wharton R, Sprecher S, de Jongh C: An assessment of moxalactam and piperacillin resistance in gram negative bacilli during empiric antibiotic therapy. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Moody M, Wharton R, Sprecher S: In vitro activity of ceftazdime-aminoglycoside combinations on multiply-resistant bacteria. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Drusano G, Ryan P, Fitzpatrick B, Standiford H, Moody M, Schimpff S: Ceftazidime & Moxalactam: A computer assisted comparison. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Drusano G, Ryan P, Fitzpatrick B, Standiford H, Moody M, Schimpff S: Ceftazidime & Moxalactam: A computer assisted comparison. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Joshi J, de Jongh C, Newman K, Schimpff S: Discontinuation of antibacterial therapy in persistently febrile, granulocytopenic cancer patients without a demonstrable infectious etiology. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

de Jongh C, Finley R, Joshi J, Newman K, Wiernik P, Schimpff S: A comparison of ketoaconazole to nystatin: Prophylaxis of fungal infection in neutropenic patients. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

de Jongh C, Wade J, Finley R, Joshi J, Aisner J, Schimpff S, Wiernik P: Double blind, randomized comparison of trimethoprim/sulfamethoxazole versus placebo for prophylaxis of infection in patients with oat cell carcinoma. American Society of Clinical Oncology, St. Louis, Missouri, 1981.

Newman KA, Oken HA, Moody MR, Wharton R, Schimpff SC: Isolation of P. stutzeri from phenolic disinfectant system in laminar air flow rooms. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, 1981.

Joshi J, Newman K, de Jongh C, Allen M, Moody M, Thomas G, Wiernik P, Schimpff S: A comparative microbiological evaluation of two techniques for protected isolation in laminar air flow (LAF) rooms-limited versus complete reverse isolation. 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Miami Beach, Florida, 1982.

Manuscripts Submitted for Publication

Wade JC, de Jongh CA, Newman KA, Crowley J, Wiernik PH, Schimpff SC: A comparison of trimethoprim/sulfamethoxazole to nalidixic acid: Selective decontamination as infection prophylaxis during granulocytopenia. Ann Int Med (submitted).

de Jongh CA, Finley R, Joshi JH, Schimpff SC: Trimethoprim/sulfamethoxazole versus placebo for infection prophylaxis in patients with small cell lung carcinoma. NEJM (submitted).

Newman KA, Schnaper N, de Jongh CA, Schimpff SC: An evaluation of the effects of the Hickman catheter on the self-esteem and body image of the acute leukemia patient. Cancer (submitted).

Newman KA, Reed W, de Jongh CA, Schimpff SC: Hickman catheter related infections. Cancer (submitted).

de Jongh CA, Moody MR, Finley RS, Newman KA, Wiernik PH, Schimpff SC: Serum cidal activity and antibiotic levels of three amikacin-beta lactam combinations. J Infect Dis (submitted).

Danhauer FJ, Fortner CL, Schimpff SC, de Jongh CA, Wesley MN, Wiernik PH: Ototoxicity in granulocytopenic cancer patients associated with pharmacokinetically dosed amikacin. Clinical Pharm (submitted).

Wade JC, Wiernik PH, Schimpff SC, Hoofnagle JH, Schiffer CA: Hepatitis among patients with acute nonlymphocytic leukemia. Ann Intern Med (submitted).

Manuscripts Accepted for Publication/Published

Schimpff SC: Infections in patients with acute leukemia. In Leukemia, F Gunz and ES Henderson (eds), Academic Press, New York (in press) 1981.

Schimpff SC, Wiernik PH: Infection in cancer patients (predisposing factors, sites, organisms and general approach to diagnosis). In: Topics in Cancer RT Silver (ed), Physician Programs, Inc, New York (in press).

Schimpff SC, Wiernik PH: Therapy of infection in granulocytopenic patients. In: Topics in Cancer RT Silver (ed), Physician Programs, Inc, New York (in press).

Schimpff SC, Wiernik PH: Infections in the patient with cellular immune dysfunction. In: Topics in Cancer RT Silver (ed), Physician Programs, Inc, New York (in press).

Schimpff SC, Wiernik PH: Infection prevention in patients with cancer. In: Topics in Cancer RT Silver (ed), Physician Programs, Inc, New York (in press).

Schimpff SC: Infection in the abnormal host. In: Textbook of Infectious Diseases HL DuPont and L Pickering (eds), Wesley Publ Co., Menlow Park, California (in press).

Tenney JH, Schimpff SC: Non-enteric infections due to gram-negative bacilli (Enterobacteriaceae). In: Clinical Medicine S Finegold (ed), Harper and Row, Publishers, Inc, Hagerstown, Maryland (in press).

de Jongh CA, Schimpff SC: Prevention and management of infectious complications of cancer. In: Supportive Care of the Cancer Patient PH Wiernik (Ed), Futura Publishing Company, New York (in press).

de Jongh CA, Schimpff SC, Wiernik PH: Resistant E. coli septicemia in leukemic patients receiving trimethoprim/sulfamethoxazole or nalidixic acid as prophylaxis. (Letter to the Editor) Ann Intern Med (in press).

Schimpff SC: Empiric antibiotic therapy for granulocytopenic patients. Bull NY Acad Med (in press).

de Jongh CA, Wade JC, Schimpff SC, Newman KA, Finley RS, Salvatore PC, Moody MR, Standiford HC, Fortner CL, Wiernik PH: Empiric antibiotic therapy for suspected infection in granulocytopenic cancer patients: A comparison between the combination of moxalactam plus amikacin and ticarcillin plus amikacin. Am J Med (in press).

Moody MR, Young VM, Glor DE: Type associated differences in alternate pathway activation by Pseudomonas aeruginosa. Rev Infect Dis (in press).

Joshi JH, Wang KP, De Jongh CA, Newman KA, Wiernik PH, Schimpff SC: A comparative evaluation of two fiberoptic bronchoscopy catheters: The plugged telescoping catheter versus the single sheathed nonplugged catheter. Am Rev Resp Dis (in press).

Moody MR, de Jongh CA, Schimpff SC, Tillman GL: The effects of long-term amikacin usage on aminoglycoside susceptibility patterns of gram negative bacilli. JAMA (in press).

Finley RS, Fortner CL, de Jongh CA, Wade JC, Newman KA, Caplan E, Britten J, Wiernik PH, Schimpff SC: A comparison of standard vs pharmacokinetically adjusted amikacin dosing in granulocytopenic cancer patients. Antimicro Ag Chemother (in press).

Coker DA, Morris DM, Colemen JJ, Schimpff SC, Wiernik PH, Elias G: Infection among 210 patients with surgically staged Hodgkin's disease. Am J Med (in press).

Schimpff SC: Gram-negative bacteremia. In: Clinical Medicine JA Spittell, Jr (ed), Harper and Row, Publishers, Inc, Philadelphia, 1981, Chapter 54.

Aisner J, Schimpff SC: Aspergillosis. In: Clinical Medicine JA Spittell, Jr (ed), Harper and Row, Publishers, Inc, Philadelphia, 1981, Chapter 116.

Newman KA, Schimpff SC, Wade JC: Antibiotic prophylaxis of infection for patients with granulocytopenia. In: Infections in the Immunocompromised Host-Pathogenesis, Prevention and Therapy J Verhoef, PK Peterson and PG Quie (eds), Elsevier/North-Holland Biomedical Press, Amsterdam, 1980, pp. 187-204.

Schimpff SC, Protective environments for the treatment of high risk cancer patients. In: Controversies in Oncology PH Wiernik (ed), John Wiley and Sons, Inc, Publ, New York pp 247-265, 1980.

Wade JC, Schimpff SC: Approaches to therapy of bacterial infections in the granulocytopenic patient. In: EORTC Monograph Series of the European Organization for Research on Treatment of Cancer—Volume 10. Infections in Cancer Patients J Klastersky (ed), Raven Press, New York, 1982 pp. 105-129.

Schimpff SC: Intravenous trimethoprim-sulfamethoxazole plus ticarcillin as empiric antibiotic therapy for granulocytopenic patients. Arch Intern Med 141:844-846, 1981.

Remington JS, Schimpff SC: Please don't eat the salads. N Engl J Med 304:433-435, 1981.

Schimpff SC: Surveillance cultures. J Infect Dis 144:81-84, 1981.

Anonymous: Antimicrobial prophylaxis and treatment with granulocytopenia. Med Let Drugs Ther 23:55-56, 1981.

Bishop JF, Schimpff SC, Diggs CH, Wiernik PH: Infections in non-Hodgkin's lymphoma patients during intensive chemotherapy. Ann Intern Med 95:549-555, 1981.

Standiford HC, Viollier AF, Moody M, Klastersky J, Tatem B, Schimpff SC: Antibiotic combinations against gram-negative bacilli and Staphylococcus aureus in cancer patients: Comparative in-vitro and in-vivo activity of cefoperazone and mezlocillin singly or combined together. J Antimicrobial Chemotherapy 9:(suppl) 47-49, 1982.

Moody MM, Wharton RC, Schnaper N, Schimpff SC: Do water pipes prevent transmission of fungi from contaminated marijuana? New Engl J Med 306:1492-1493, 1982.

INDIUM LABELED GRANULOCYTES IN THE STUDY OF FACTORS AFFECTING GRANULOCYTE TRANSFUSION

Dutcher JP, Schiffer CA, Johnston GS: Rapid migration of $^{111}$Indium-labelled granulocytes to sites of infection. New Eng J Med 304:586-589, 1981.

Dutcher JP, Schiffer CA, Johnston GS, Papenberg D, Daly PA, Aisner J, Wiernik PH: The effect of histocompatibility factors on the migration of transfused $^{111}$Indium-labeled granulocytes. Blood 57(Suppl): 181a, 1981 (manuscript submitted for publication)

PREVENTION OF ALLOIMMUNIZATION WITH LEUKOCYTE POOR PLATELETS

Schiffer CA, Aisner J, Dutcher JP, Reilly J, Wiernik PH: A clinical program of autologous frozen platelet transfusion. Proceedings American Association of Blood Banks, 1981 (Abstract S60).

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