Center for Biologics Evaluation and
Research
Division of Cytokine Biology

Annual report
1991-1992

DIVISION OF CYTOKINE BIOLOGY

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

ANNUAL REPORT OF RESEARCH

October 1, 1991 through September 30, 1992

DIVISION OF CYTOKINE BIOLOGY

OFFICE OF BIOLOGICS RESEARCH

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

ANNUAL REPORT OF RESEARCH

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DIVISION OF CYTOKINE BIOLOGY

DIRECTOR'S SUMMARY

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DIVISION OF CYTOKINE BIOLOGY

Annual Report for October 1, 1991 through September 30, 1992

The Division of Cytokine Biology has continued to facilitate research, development, review and compliance of cytokine and growth factor products. The staff consists of 80 individuals including full time equivalent professional positions, staff fellows, visiting scientists, postdoctoral and Fogarty fellows and support staff. The Division laboratories are:
The Laboratory of Cytokine Research (LCR), the Laboratory of Cellular Immunology (LCI), the Laboratory of Cell Biology (LCB) and the Laboratory of Immunology (LI). There are a total of 39 Ph.D.'s, 18 M.D.'s and 5 M.D./Ph.D.'s.

Research programs in the Division continue to expand in the areas of immunology, gene therapy, molecular biology, protein chemistry and AIDS. The focus of the Laboratory of Cytokine Research is the role of cytokines in host defense inflammation and disease. The Chemical Biology Section continues to make major advances in the areas of IFN and Interleukin 2 structure and biological characterization, as well as receptors structure and function (i.e., more than one IFN-alpha receptor component). Interferon-alpha gene expression and secretion in monocytes is being investigated along with the characterization of the interferon-alpha receptor. The physicochemical analysis of interferon-alpha species continues to further the understanding of structure/function relationships. Techniques are being developed to isolate biologically active cytokines by non-denaturing PAGE. The Immunobiology Section has studied stimuli for the m-CSF message and secretion by human monocytes. Studies to identify the LPS binding sites on monocytes are ongoing. The Section of Biochemical Immunology continues its studies on the IFN-gamma receptor and the synthesis of soluble IL-4 receptor by macrophages. Studies on IFN-induced gene activation are being performed to understand the signal transduction signaling of IFN. The Hematopoietic Growth Factor Section has characterized the expression of GM-CSF gene in lymphocytes 1B posttranscriptional regulation.

The Laboratory of Cellular Immunology (LCI) has continued to progress in its studies in the areas of cytotoxic T lymphocyte development, cellular therapy (including gene therapy), and transplantation immunology. The Immune Regulation Section continued its studies on T lymphocyte activation and proliferation with an emphasis on IL-2, IL-3, IL-4 and IL-6. Studies continue on the regulation of the cytotoxic T lymphocyte CTL development and activity, especially the roles of IL-4 and IL-2. Also, the program in transplantation immunology continues to make progress in that it was demonstrated that CD4+ can play a suppressive role in the rejection of MHC class I disparate skin grafts. In addition, studies continue with restriction endonucleases and their ability to inhibit infection in mammalian cells of both double stranded DNA viruses and retroviruses. The Cell and Molecular Biology Section continues its studies on the cytotoxic lymphocyte response with an emphasis on the effects of aging and also the roles of adhesion molecules in the regulation of NK activity.

The Laboratory of Cell Biology has made significant contributions in the areas of wound healing and neurotrophic growth factors. The Growth Factor Section has purified and characterized a new class of lymphocyte growth factors called oncoimmunins which show myeloid differentiation induction activity and, in addition, work continues on AIDS vaccine development using peptide tertiary structural templates. The Cellular Pathology Section continues its studies on purifying and characterizing amphiregulin, and the expression of the EGF family of ligands in ovarian cells and tissues. This section is continuing its studies on the potential role of transforming growth factor-alpha to function as an autocrine growth factor in ovarian carcinoma cell lines.

The Laboratory of Immunology has made several major new discoveries in the area of molecular immunology and AIDS: The Gene Regulation Section has discovered two unusual forms of human IgE encoded by alternative RNA splicing of ϵ heavy chain membrane exons. The Retrovirology Section continued its programs examining the role of anti-HIV antibodies that block the interaction between HIV gp120 and CD4. The Immunology Section has focused on the identification of new autocrine growth factors for EBV-immortalized B cells, i.e., lactic acid, and has identified IL-10 as having structural and functional homology with a poorly characterized open reading frame in the EBV genome.

The Division currently has responsibility for greater than 600 INDs. and greater than 50 PLA/ELA original submissions and amendments.

This year the Division of Cytokine Biology was primarily responsible for the licensing of one new biotechnology-derived product, Interleukin-2, for metastatic renal cell carcinoma. In addition, DCB led the approval of interferon alpha-2b for Hepatitis B and the approval of GM-CSF for treatment of bone marrow transplantation engraftment failure.

The Division published 58 papers and book chapters this year. Many were in major journals e.g., Proceedings National Acadamy of Sciences, USA, Journal of Immunology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Virology, Science and Blood. The staff was involved in international cytokine standardization, international cytokine and immunology meetings, and served as members of editorial boards and reviewers for major scientific journals.

Scientific collaboration continued with NIH, USUHS, Walter Reed Army Research Institute, NCTR and numerous of academic institutions.

The Laboratories of Immunology and Cell Biology (Dr. Akira Komoriya only) had their site visits by the Biological Response Modifier Committee members and expert consultants on June 18, 1992.

Mary K. Mellinger received the Outstanding Achievement Award in May 1992 for providing invaluable secretarial services to the Division of Cytokine Biology.

Michael Norcross, M.D. was awarded the Commendation Medal in May 1992 for his review of the treatment IND for Ampligen.

DIVISION OF CYTOKINE BIOLOGY

STAFF

LABORATORY OF CYTOKINE RESEARCH

Theresa L. Gerrard, Ph.D., Chief

Immunobiology Section

Theresa L. Gerrard, Ph.D., Section Head	Supervisory Microbiologist
Raymond Donnelly, Ph.D.	Staff Fellow
Deborah Webb, Ph.D.	Staff Fellow
Marion Gruber, Ph.D.	Staff Fellow
Doreen Dyer	Biologist
Claudia Williams	Microbiologist
Kathleen Clouse-Strebel, Ph.D.	Senior Staff Fellow
Karis Weih Faust	Biologist

Chemical Biology Section

Kathryn C. Zoon, Ph.D.	
Dorothea Miller	Biologist
Joseph Bekisz	Microbiologist
Joan Enterline	Biologist
Mark Hayes , Ph.D.	Staff Fellow
Ren Qui Hu, Ph.D.	Visiting Scientist

Biochemical Immunology Section

David Finbloom, M.D., Section Head	Senior Investigator (Comm. Corps)
Gerald Feldman, Ph.D.	Staff Fellow
Karen Winestock	Biologist
Kenishi Igarashi, MD	Fogarty
Andrew Larner, M.D., Ph.D.	Research Investigator (Comm. Corps)
Rebecca Hackett, Ph.D.	Staff Fellow
Emmanual Petricoin, Ph.D.	NRC Fellow
Michael David, Ph.D.	Visiting Scientist
Nga Nguyen, Ph.D.	Chemist
Yaffa Rubinstein	Microbiologist
Mario Hirata, Ph.D.	Guest Worker
Rosario Hirata, Ph.D.	Guest Worker

Hematopoietic Growth Factors

Dov H. Pluznik, Ph.D.	Supervisory Microbiologist
Kouichi Akahane, Ph.D.	Guest Worker
Sharon B. Midura	Microbiologist
Suanne F. Dougherty	Biologist
Yoshitaka Iwai, M.D., Ph.D.	Fogarty Fellow
Roger Cohen, M.D.	Senior Staff Fellow
Shu-Mei Liang, Ph.D.	Chemist
Yang Rong, M.D.	Fogarty Fellow
Nancy Lee	Chemist
Yang Yang Chen	Biologist

LABORATORY OF CELL BIOLOGY

Dr. Susan Vargo, Ph.D., Acting Director, Division of Cytokine Biology

Growth Factors Structure and Function Section

Akira Komoriya, Ph.D.	Senior Staff Fellow
Susumu Shinagawa, Ph.D.	Visiting Scientist
Song Yuan Liu, Ph.D.	Fogarty Fellow
Beverly Packard, Ph.D.	Senior Staff Fellow
Fred Varricchio, M.D., Ph.D.	Senior Staff Fellow

Cellular Pathology Section

Kurt Stromberg, M.D., Section Head	Staff Medical Officer (Comm. Corps)
Gibbes Johnson, Ph.D.	Staff Fellow
Alfred Gordon, Ph.D.	NRC
Bhanu Kannan	Technician

LABORATORY OF CELLULAR IMMUNOLOGY

Jay P. Siegel, M.D., Chief

Immune Regulation Section

Jay P. Siegel, M.D.	Senior Surgeon
Jim Crim	Biologist
Howard Mostowski	Biologist
Priti Mehrotra, Ph.D.	Senior Staff Fellow
Raj Puri, M.D., Ph.D.	Senior Investigator
Pamela Leland	Biologist
Nicholas Obiri, Ph.D.	NRC Fellow
Amy Rosenberg, M.D.	Senior Staff Fellow
Joan Sechler	Microbiologist
Robert W. Kozak, Ph.D.	Research Microbiologist
Linda A. Jones	Microbiologist
S.H. Kim, Ph.D.	Guest Worker

Molecular and Cell Biology Section

Eda T. Bloom, Ph.D., Section Chief	Research Biologist
Judy A. Horvath	Biologist
Hisanori Umehara, M.D.	Visiting Scientist
D'Anna Hohe	Guest Worker
Akira Yamauchi, M.D., Ph.D.	Fogarty Fellow

LABORATORY OF IMMUNOLOGY

Giovanna Tosato, M.D., Chief

Immunobiology Section

Giovanna Tosato, M.D., Section Head	
Sandra Pike	Microbiologist
Karen Jones	Microbiologist
Kazuyuki Taga, M.D.	Visiting Scientist
Leyla Diaz	Technician
Barry Cherney, Ph.D.	Staff Fellow
Jerome Tanner, Ph.D.	Staff Fellow
Cecelia Sgadari, M.D.	Guest Worker

Retrovirology Section

Michael Norcross, M.D., Section Head	Senior Investigator (Comm. Corps)
Gregory Rodriquez	Microbiologist
Richard Weber, Ph.D.	Guest Worker
Mahesh Patel, Ph.D.	Guest Worker
Tamas Oravecz, Ph.D.	Fogarty Fellow
Dumith Bou-Habib	Visiting Scientist

Gene Regulation Section

Edward E. Max, M.D., Ph.D.	Senior Investigator (Comm. Corps)
Fred Mills, Ph.D.	Staff Fellow
Jean Gabriel Judde, Ph.D.	Fogarty Fellow
Mary Malarkey-Briggs	Biologist
Mary Mitchell	Biologist

DIVISION OF CYTOKINE BIOLOGY

SUMMARY OF CURRENT RESEARCH FINDINGS

OFFICE OF THE DIRECTOR

Susan A. Vargo, Acting Director

Jay Siegel, M.D., Senior Surgeon	Acting Deputy Director
Michelle R. Naylor	Secretary to the Acting Director
Patricia Hacker	Secretary
Mary Archi	Secretary
Linda K. Burbank	Applications Examiner
Nancy Carpenter	Editorial Assistant

Laboratory of Cell Biology

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The Laboratory of Cell Biology has been reorganized to consist of two sections: Growth Factor Structure Section, Susan A. Vargo, Acting Section Chief; Cellular Pathology Section, Kurt Stromberg, Section Chief. The research and regulatory studies for this laboratory continue to involve wound healing and, more recently, neurotropic growth factors. More specifically, studies include the areas of a) the structure and function of growth factors and their receptors, b) the characterization of immunostimulatory soluble factor(s) secreted by tumor cells, and c) the involvement of transforming growth factor-α in ovarian cancer.

In the Growth Factor Structure Function Section, there were two major research accomplishments. Dr. Packard has continued her work on the new class of lymphocyte growth factors, the oncoimmunins. Two oncoimmunins (Oncoimmunin-L and Oncoimmunin-M) have been identified and purified to homogeneity by SDS-PAGE analysis from tumor cell supernatants. Oncoimmunin-L is a human T-lymphocyte mitogen and oncoimmunin-M inhibits the growth of at least three human myeloid leukemic cell lines. Identification of secreted factors from tumor cells as a source of mitogenic activity for lymphocytes supports the idea that the tumor immunoenvironment, as recognized by lymphocytes, is defined by soluble mediators in addition to the well-studied cell-cell contact interactions exemplified by MHC antigens and T-cell receptors. This work was presented at FASEB (April, 1992).

The second accomplishment is that Dr. Komoriya, Senior Staff Fellow and Dr. Shinagawa, Visiting Scientist have continued their studies in the AIDS vaccine development area by the synthesis of conformationally constrained peptide tertiary structural templates (PTST). The CSg-A sequence of fibronectins IIICS domain is grafted into a helical PTST molecule, the observed biological activity was less than CSg-A alone, indicating that the PTST molecule would stabilize the grafted sequence's conformation. Various synthetic peptides of RSV related proteins were synthesized to determine the monoclonal antibodies' antigenic epitopes. This RSV system will be used to further test the concept of the PTST molecule for an AIDS vaccine development. This work was presented at the AIDS Conference held in Amsterdam (July, 1992).

Dr. Fred Varricchio joined the laboratory as a Senior Staff Fellow in November of 1991. He completed and published his work (in Neurology) on zidovudine myopathy. His work indicated that zidovudine alone can cause mitochondriopathy in hamster skeletal muscle. Dr. Varricchio also is working on the protein composition of ovarian cyst fluid. Fluid from both benign and malignant cysts/tumors was analyzed by SDS-PAGE revealing a predominant 65 KD band. Studies are underway to further characterize and identify this protein.

The Growth Factor Structure Function Section is responsible for the scientific review of wound healing and neurotropic growth factors. Major studies in assessing the long-term safety issues of growth factors have been initiated by Drs. Komoriya and Cavagnaro (OBR) in collaboration with NIH and NCTR.

The regulatory responsibility of the Growth Factor Structure Function Section includes the review of manufacturing and chemical issues for neurotrophic factors (NF). Three original IND submissions for NFs were received for the treatment of

neurological disorders and chemotherapy induced neuropathy.

The role of ligands of the EGF family (particularly Transforming Growth Factor-α and Amphiregulin) in the regulation of ovarian cell growth is the research focus of the Cellular Pathology Section. Dr. Stromberg's emphasis has been the potential of transforming growth factor-α (TGF-α) to function as an autocrine growth factor in numerous ovarian carcinoma cell lines. The potential involvement of the EGF family and c-erbB-2 in the initiation and progression of human ovarian epithelial cancer was investigated by comparison of the mRNA and protein level of these genes in carcinoma cell lines and normal ovarian surface epithelial (OSE) cells. All 17 ovarian carcinoma cell lines which were examined expressed EGF-R (Kd equals 0.21 to 5.3 nM) and 16 cell lines, in addition, concomitantly secreted TGF-α (16 to 197 pg/ml). The growth of 8 carcinoma lines was stimulated in a dose-dependent manner when grown in the presence of exogenous TGF-α (30 to 88 percent). Growth in 4 of 5 of the cell lines capable of serum-free propagation was inhibited (28 to 56 percent) when cultured in medium containing a TGF-α neutralizing monoclonal antibody. Concentrations of AR ranging from 1-5 nM stimulated the growth of 3 OSE samples (20 to 50 percent) and 4 of 6 carcinoma cell lines (28 to 210 percent). Differential immunohistochemical detection of AR and cripto was documented in normal human colon and colorectal tumors. Whereas normal colon did not express cripto, 86 percent of the tubulo adenomas, but only 43 percent of the tubular adenomas were positive for cripto expression. AR expression appeared to be associated with both normal and malignant colonic epithelial cells that were more differentiated. AR localizes in both nucleus and cytoplasm by immunohistochemistry of ovarian carcinoma and normal ovarian surface epithelial cells, and functions in an autocrine manner in a colon carcinoma cell line.

Dr. Johnson, Senior Staff Fellow, continues to concentrate on amphiregulin (AR), a polypeptide growth regulator which has sequence homology to the epidermal growth factor-related family of ligands and contains putative nuclear targeting sequences. Using antibodies directed against amphiregulin, the in vivo expression and localization of amphiregulin was studied in normal human colon, human colon carcinomas and normal human placenta. In all normal colon specimens, amphiregulin was detected and localized to the terminally-differentiated, non-proliferative epithelial cells of mucosal surface. In 18 of 36 cancers, amphiregulin was detected in the proliferative malignant epithelial cells of the tumors and was more commonly detected in the well-differentiated carcinomas (71%). Amphiregulin was detected in the terminally-differentiated syncytiotrophoblasts of human placenta but was not detectable after 18 weeks of gestation. In all three instances, amphiregulin was detected in the nucleus as well as cytoplasm of the cells in vivo. Thus, amphiregulin expression in normal tissues is related to differentiation, but amphiregulin may function as autocrine growth stimulator in colonic carcinomas. An evaluation of the mechanism of action of amphiregulin in a human colon carcinoma cell line reveals that the cells secrete amphiregulin and that it acts via an extracellular autocrine loop to drive the proliferation of the cells. The data suggests that amphiregulin acts through the EGF receptor. Expression of the cDNA for various oncogenes result in the transformation of normal human mammary epithelial cells and the concomitant elevated expression of amphiregulin mRNA and protein, suggesting that amphiregulin may be a key intermediate in oncogene-mediated malignant transformation. Dr. Johnson's work was published in the Journal of Cell Biology.

Dr. Alfred Gordon, an NRC Fellow, left the laboratory in March, 1992. He was replaced by Joyce Pegues, a Senior Staff Fellow, in June 1992.

The regulatory responsibility of the Cellular Pathology Section includes scientific review of manufacturing and clinical issues for wound healing indications. In all, eight different IND's are active with three approaching pivotal trial stage.

Laboratory of Cellular Immunology

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During this year, tenured scientific staff of the Laboratory of Cellular Immunology expanded considerably. Upon the recommendations of FDA and NIH peer review groups, Dr. Eda Bloom converted from term to tenured status, and Drs. Amy Rosenberg and Raj Puri converted from staff fellow to tenured status. They join Dr. Robert Kozak and Dr. Jay Siegel, Laboratory Chief, as tenured staff directing the research and regulatory programs of the laboratory. The laboratory has regulatory responsibility for a variety of cytokines, activated lymphocytes and gene therapies, and provides expert review and consultation in several areas: biological therapies for sepsis and for HIV infection, use of cell substrates to manufacture biological products, clinical trial design, transplantation related issues in biological therapy, and computer sciences. These efforts are supported by a research program spanning a broad range of cellular immunology.

Continuing their studies of IL-4 receptors on murine tumor cells, Dr. Puri's group found that tumor necrosis factor alpha upregulated their expression. They have also documented the presence of IL-4 receptors on a variety of fresh and cultured human tumors and AIDS-associated Kaposi's sarcoma cells. Various receptors were shown to be functional in regulating cell growth, cytokine secretion, and/or MHC expression. HIV-1 tat gene was found to regulate IL-4 receptor expression on B lymphoblastoid cells.

In their ongoing studies of cellular function in transplant rejection, Dr. Rosenberg's group has found that CD4+ lymphocytes can play a suppressive role in the rejection of MHC class I disparate skin grafts. In order to develop mouse-human chimeras which could be a valuable tool for research and and development, and testing of biologicals, they have elucidated some of the mechanisms of murine immune responses against human cells. In other studies, they found that restriction endonucleases can inhibit infection in mammalian cells of both double stranded DNA viruses and retroviruses.

Dr. Kozak's group has furthered studies in the areas of regulation of receptor expression during lymphocyte activation. Studies of IL-4 effects in this area are ongoing. Their other major area of study is IL-2 receptor directed immunotherapy. They are evaluating radionuclide and toxin conjugates and fusion proteins directed by IL-2 and/or anti-IL-2 receptor antibodies for specificity, stability and toxicity in various animal models.

Dr. Bloom's group found numerous aspects of cell signalling through LFA-1 cross-linking which differ between lymphokine activated killer (LAK) cells and natural (NK) cells. With their earlier observation that IL-2 induces phosphorylation of LFA-1 while activating NK cells to become LAK cells, these data suggest that changes in LFA-1 structure and function may be important in accounting for differences between NK and LAK cells. In ongoing studies of aging, they have found that alloantigen stimulated CD8+ cells from older mice express less perforin per cell. Having earlier found that CD4+ cells from aged mice have diminished function in transplant rejection, they have recently observed that these cells have altered T cell receptor expression.

In their continuing studies of cytokine regulation of cytotoxic T lymphocyte

(CTL), Dr. Siegel's group found that IL-4 can act directly on CD8+ CTL precursors, if and only if present during the initial exposure to activation stimuli, resulting in complete block of maturation of a subset of these CTL precursors. Additionally, presence of IL-4 during activation of CD8+ cells was found to result in growth of non-cytotoxic CD8+ clones which are presently under investigation. Studies of IL-12 have demonstrated significant effects on the growth and differentiation of human CTL.

Laboratory of Cytokine Research

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The scientific research goals of the Laboratory of Cytokine Research center on the understanding of the complex interactions involved in the mechanism of action of cytokines and their role in disease processes. Investigators in this laboratory have examined cytokine action at the cellular, biochemical and molecular level.

In the Immunobiology Section, Dr. Gruber has studied stimuli for M-CSF message and secretion by human monocytes and has observed that two distinct signals are required for optimal M-CSF secretion. Dr. Webb has analyzed the cellular release mechanisms for IL-1. In addition, she is also studying LPS binding proteins on human monocytes. Dr. Donnelly has delineated the transcriptional and post transcriptional mechanisms of IL-4 inhibition of inflammatory monokines and the effects of IL-4 on IL-1 receptor antagonist. Dr. Clouse-Strebel is studying binding sites on monocytes other than CD4 for the HIV envelope protein, gp120. She is also studying inhibition of HIV activation by soluble TNF receptor. Working with Dr. Ehrenreich, Dr. Clouse-Strebel has observed that HIV gp120 is a stimulus for monocyte production of endothelin-1 and that thrombin is a regulator of astrocytic endothelin-1.

In the Chemical Biology Section, Dr. Hayes has investigated interferon-alpha (IFN-α) gene expression and secretion in monocytes and he is characterizing the IFN-α receptor. Dr. Hu is investigating the chemical and biological properties of a unique interferon component, species o. Ms. Miller and Mr. Bekisz are involved in the physicochemical analysis of interferon species in order to understand structure and function relationships. Dr. Nguyen has developed techniques to isolate biologically active cytokines by non-denaturing PAGE. She has also cloned the receptor for IFN-α. Dr. Liang has delineated the effects of glutathione on the biological activity of IL-4 and IL-2. In addition, she has been involved in structure/function studies of IL-2 using site directed mutagenesis.

In the Biochemical Immunology Section, Dr. Finbloom has identified 2 new subunits of the interferon-gamma receptor. Dr. Feldman has examined the regulation of expression of IL-4 receptors and soluble IL-4 receptors. Drs. Larner, Hackett, Petricoin and David are involved in studying several interferon induced genes, their modulation by phorbol esters, and involvement of protein kinases.

In the Hematopoietic Growth Factor Section, Dr. Pluznik has studied integrin expression, their role in differentiation and modulation by cytokines. In addition, they have characterized the expression of the GM-CSF gene in lymphocytes and its posttranscriptional regulation.

Laboratory of Immunology

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The Laboratory of Immunology is composed of three senior investigators, each directing independent research programs, administratively identified as sections. These include an Immunobiology section, a Retrovirology Section and a Gene Regulation Section.

Much has been achieved by the Laboratory of Immunology during the past year both from a research and regulatory viewpoint.

The Gene Regulation Section, headed by Dr. Edward Max, has made major contributions in critical areas of immunology. The highlight of these recent achievements includes the discovery of two unusual forms of human IgE encoded by alternative RNA splicing of ϵ heavy chain membrane exons (J. Exp. Med., in press). This finding is likely to provide means for better understanding of immediate hypersensitivity reactions and other IgE mediated disorders. In addition, Dr. Max's laboratory has developed a novel PCR-based technique that permits detection of immunoglobulin heavy chain switch at a single cell level (Proc. Natl. Acad. Sci., in press). Using this and other techniques, the laboratory has demonstrated that μ-ϵ isotype switch can involve sequential switch in EBV-infected B cells (J. Immunol., in press).

The Immunobiology Section, headed by Dr. Giovanna Tosato, has continued productive research work in the area of EBV infection and immunoregulation. The major discovery relates to the identification of lactic acid as the predominant autocrine growth factor molecule in EBV-immortalized cells grown in serum free conditions (Proc. Natl. Acad. Sci., 1991). This finding, together with the information linking tumorigenesis with biochemical dysfunctions leading to increased production of lactic acid, provides an important link between autocrine growth and tumorigenesis. Additional work on this topic showed that high level expression of the human IL-6 gene in EBV-immortalized cells by retroviral mediated transduction leads to significant increase in tumorigenicity of the EBV-infected B cells (J. Clin. Invest., 1991) Recently, the novel cytokine IL-10 was found to have structural and functional homology with a poorly characterized open reading frame in the EBV genome. This suggested the possibility that EBV may have acquired a cellular gene for its advantage during evolution. Dr. Tosato's laboratory found that recombinant human IL-10, as well as the homologous viral product, BCRF-1, profoundly inhibits T cell growth and IFN-γ production. This indeed suggests that the EBV has acquired, through BCRF-1 production, an ability to suppress immunity in the host (J. Immunol., 1992).

The Retrovirology Section, headed by Dr. Michael Norcross, has continued characterization of anti-CD-4 autoantibody production during the course of HIV infection. After HIV infection, membrane CD4 processing is abnormal. As a consequence, cryptic epitopes on CD4 become exposed and recognized as foreign antigens leading to autoimmune antibody production (J. Immunol., in press). In other experiments, Dr. Norcross's laboratory had examined the possibility that molecules other than CD4 may contribute to HIV infection. A cell surface sulfated polysaccharide, heparan sulfate proleoglycan, was identified as a novel HIV binding site on the T-cell surface. Together with CD4, this compound serves to mediate HIV entry into T cells. This finding may prove to be extremely important in the understanding of HIV infection and the development of novel

rational approaches to treatment of HIV infection.

The Laboratory of Immunology has utilized its strong scientific base to perform important regulatory activities. For example, Dr. Max has provided critical input in the review of gene therapy products, particularly the α1-antitrypsin gene complexed with liposomes. He also serves as PLA committee chairman for Burroughs Wellcome α-interferon. Dr. Norcross, because of his scientific expertise on HIV infection, has reviewed many of the biological products for treatment and prevention of HIV infection, particularly CD4-based products. Dr. Tosato, because of her scientific expertise on IL-6 and tumorigenesis, has reviewed applications relating to IL-6 and a variety of oncology-related products. She has also helped to define surrogate endpoints for bone marrow stimulatory growth factors and has served as a liaison with NCI.

DIVISION OF CYTOKINE BIOLOGY

October 1, 1991 through September 30, 1992

PUBLICATIONS

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PUBLICATIONS

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CBER, FDA
PUBLICATIONS
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DIVISION OF CYTOKINE BIOLOGY

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DIVISION OF CYTOKINE BIOLOGY

ABSTRACTS

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Shinagawa, S, Humphries, MJ, Yamada, KM and A Komoriya. Structure-function study of the alternatively spliced IIICS domain of fibronectin:functional roles of the CS1 N-terminal domain in cell adhesion. Sixth Symposium of the Protein Society. San Diego, CA, July 25-29, 1992.

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DIVISION OF CYTOKINE BIOLOGY

CONTRACTS

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

DIVISION OF CYTOKINE BIOLOGY

CONTRACT REPORT

October 1, 1991 through September 30, 1992

Contractor: National Cancer Institute,
Frederick Cancer Research Facility

Title: NIH Biotechnology Service: Preparation of Monoclonal Antibodies

Contractor's Project Officer: Henry J. Hearn, Ph.D.

Contract Number: 224-88-1100

Contract Project Officer: Dr. Akira Komoriya

Objectives and Methods Employed:

(a) Dr. Akira Komoriya's research project requires several different monoclonal antibodies. A total of 8 different monoclonal antibodies against cytokines will be needed. Also, a new procedure of "single-shot intrasplenic immunization" will be tried. Serum samples will be drawn for testing for 3 antigens by CBER laboratories. The remaining 5 antigens will be screened by FCRF staffs. In addition, a whole cell immunization will be carried out to identify cellular viral receptor for hepatitis A virus and other viruses. When a potential HIV peptide vaccine candidate becomes available, preparation against this antigen will be performed.

Major Findings:

Several positive clones for hepatitis A virus cell surface receptor are being screened for the specificity.

Significance to Program:

The monoclonal antibodies will be used for establishing RIA assays for regulatory projects and for structure/function study of ligand and cytokine receptors. In addition, a potentially new antibody vaccine against hepatitis A virus cellular receptor will enhance our regulatory responsibility. Our efforts to prepare a better peptide vaccine for HIV is a part of the FDA's AIDS regulatory/research efforts.

Date Contract Initiated: 1988

Current Annual Level: $55,000 ($147,000 total contract funding for FY 91 includes 10 other projects)

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

DIVISION OF CYTOKINE BIOLOGY

CONTRACT REPORT

October 1, 1991 through September 30, 1992

Contractor: National Institutes of Health
Bethesda, MD (ATCC. as a subcontractor)

Title: "Storage and Handling of WI-38 Diploid Cell Cultures".

Contractor's Project Officer: Dr. Robert Hay (ATCC)

Contract Number: 224-79-1104

Contract Project Officer: Dr. Akira Komoriya

Objectives: To support an NIH contract with the ATCC to store the WI-38 cell line.

Significance to Program: WI-38 cell line is a useful cell line for vaccine production

Date Contract Initiated: 1075

Current Annual Level: $20,000

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

DIVISION OF CYTOKINE BIOLOGY

CONTRACT REPORT

October 1, 1991 through September 30, 1992

Contractor: Center for Biologics Evaluation and Research, FDA
Bethesda, MD

Title: "Acquired Immunodeficiency Syndrome, Intramural AIDS Targeted Antiviral Program" Development of AIDS drug peptide vaccine: design and synthesis of conformationally constrained peptide tertiary structural templates (PTST) for AIDS vaccine development.

Contractor's Project Officer: Dr. William A. Eaton

Contract Number: 224-91-1104

Contract Project Officer: Dr. Akira Komoriya

Objectives: The recent identification of virus neutralizing epitopes on the GP120 HIV coat protein and identification of the cellular receptor for the AIDS virus itself have provided the opportunity to develop potentially effective vaccines against the AIDS virus. A number of problems that must be addressed before successful preparation of a peptide containing the neutralizing epitopes as a useful vaccine against HIV can occur. The peptide vaccine must be able to elicit antibodies that can recognize and bind with sufficiently high affinity for the native protein. Also, peptide vaccine may not elicit antibodies against a neutralizing determinant of the virus. And, the vaccine must induce active immunity. We have focused on the first two problems of how to prepare antibodies using a small peptide antigen that would cross react with native proteins and have the desired antigenic determinant. If it was possible to design and synthesize conformationally constrained tertiary structural template (TST) molecules, then the dichotomy of reduction in the size of the peptide for limiting possible sites with increase in the size of the peptide for stabilizing the conformation of the peptide

antigen could be resolved. This TST molecule will also be useful in a structure-function study of other biologically active molecules.

When the CSg-A sequence of fibronectins IIICS domain is grafted into a helical PTST molecule, the observed biological activity was less than CSg-A alone, indicating that the PTST molecule would stabilize the grafted sequence's conformation. Various synthetic peptides of RSV related proteins were synthesized to determine the monoclonal antibodies' antigenic epitopes. This RSV system will be used to further test the concept of the PTST molecule for AIDS vaccine development.

Date Contract Initiated: January 1990

Current Annual Level: $ $75,000

DIVISION OF CYTOKINE BIOLOGY

INDIVIDUAL PROJECT REPORTS

DIVISION OF CYTOKINE BIOLOGY

OFFICE OF BIOLOGICS RESEARCH

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

ANNUAL REPORT OF RESEARCH

October 1, 1991 through September 30, 1992

I INDIVIDUAL PROGRESS REPORTS

DIVISION OF CYTOKINE BIOLOGY

LABORATORY OF CELL BIOLOGY

GROWTH FACTORS STRUCTURE & FUNCTION SECTION

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

PROJECT NUMBER
Z01 BD 01001-04 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Activity of Growth Factors: Effects on Cellular Adhesion and Cellular Activity

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

Susumu Shinagawa, Ph.D., Visiting Scientist, LCB, DCB, CBER
PI: Akira Komoriya, Ph.D., Senior Staff Fellow

COOPERATING UNITS (if any)

Dr. M. Humphries, Dept. of Biochemistry and Molecular Biology, University of Manchester, United Kingdom; Dr. K. Yamada, NIDR, NIH

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Growth Factors Structure and Function Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

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

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

An area of cytokine research that has received relatively little attention is the cytokine-induced cellular responses that are not directly related to mitosis. An example of a cytokine capable of inducing these types of cellular activities is TGF-β. Earlier, we have shown that TGF-β induces cellular hypertrophy in cultured, vascular, smooth muscle cells and, concomitantly, the stimulated cells exhibited an enhanced level of f-actin. This observation led to a series of studies on the cellular adhesion molecule, fibronectin, and its interactions with its receptor.

With our collaborators, we have shown that the minimum essential amino acid sequence for the CS1 activity has been shown to be Leu-Asp-Val. In addition, we found that a weak adhesive activity shown by CS1-E and CS1-C peptides is due to GPEIL C-terminal sequence. The amino terminal domain of CS1 enhances the CS1-A activity by stabilizing the conformation of the C-terminal GPEILDVPST sequence. A study is underway to verify this prediction by NMR method.

Komoriya, A, Green, LJ, Mervic, M, Yamada, SS, Yamada, KM and MJ Humphries. The minimal essential sequence for a major cell type adhesion site (CS1) within the alternatively spliced IIICS domain of fibronectin is Leu-Asp-Val. J Biol Chem, 266:15075, 1991.

Shinagawa, S, Humphries, MJ, Yamada, KM and A Komoriya. Structure-function study of the alternatively spliced IIICS domain of fibronectin:functional roles of the CS1 N-terminal domain in cell adhesion. Sixth Symposium of the Protein Society. San Diego, CA, July 25-29, 1992.

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

PROJECT NUMBER
Z01 BD 01003-04 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanisms of Growth Factor Signal Transduction: EGF Receptor

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

Song Yuan Liu, Ph.D., Fogarty Fellow, LCB, DCB, CBER
PI: Akira Komoriya, Ph.D., Senior Staff Fellow, LCB, DCB, CBER

COOPERATING UNITS (if any)
A. Danishefsky, R. Rubin and A. Wlodawer, NCI/FCRT; T. Williams, Naval Medical Research Institute, Bethesda, MD; Y.C. Lee, Dept. of Biology, The Johns Hopkins University, Baltimore, MD

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Growth Factors Structure and Function Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Cells of the human adenocarcinoma line A431 secrete a truncated form of the EGF receptor (sEGF-R). This soluble receptor with a molecular mass of ca. 110 kDaltons has been isolated from different culturing time points ranging from 40 to 710 days. Carbohydrate analysis of sEGF-R indicates the presence of one to two O-linked oligosaccharides and at least 6 N-linked oligosaccharides per mole of protein. IEF gel analysis of the sEGF-R from various culture time-points suggests significant changes in pI occurred at about 200 days as indicated by an acidic shift, i.e., from a pI range of pH 6.3 to 6.8 to a pI range of 4.9 to 6.5. The determined ligand-receptor equilibrium dissociation constants using the equilibrium dialysis method for the receptor with acidic and near neutral pIs are 315 plus/minus 40nM respectively. Although the sEGF-R is observed to be refractory toward protease digestion by trypsin, SV8, and Chymotrypsin at 1:20 enzyme substrate ratio at 37 degrees C up to 24 hrs, the receptor becomes protease sensitive in the presence of 2M GuHCl, condition under which the sEGF-R is known to undergo a denaturation transition as determined by CD.

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

PROJECT NUMBER
Z01 BD 01002-04 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Synthesis of Peptide Tertiary Structural Templates for AIDS Vaccine Development

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

PI: Akira Komoriya, Ph.D., Senior Staff Fellow, LCB, DCB, CBER
Susumu Shinagawa, Ph.D., Visiting Scientist, LCB, DCB, CBER

COOPERATING UNITS (if any)

Judy Beeler, M.D., Senior Staff Fellow, DV, CBER; K. Yamada, M.D., LDB, NIDR

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INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

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

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

The recent identification of virus neutralizing epitopes on the GP120 HIV coat protein as well as the identification of the cellular receptor for the AIDS virus itself have provided us with the opportunity to develop potentially effective vaccines against the AIDS virus. However, there are a number of problems that must be addressed before one can successfully prepare a synthetic peptide containing the neutralizing epitopes as a useful vaccine against HIV. (1) The first problem is that the peptide vaccine must be able to elicit antibodies which can recognize and bind with sufficiently high affinity for the native protein. (2) The second problem is that the peptide vaccine may not elicit antibodies against a neutralizing determinant of the virus. (3) Lastly, the vaccine must induce active immunity. We have focused on the first two problems, namely, how to prepare antibodies using a small peptide antigen that would cross react with native proteins and have the desired antigenic determinant. If one could design and synthesize conformationally constrained tertiary structural template (TST) molecules, then the dichotomy of reduction of the size of the peptide for limiting possible antigenic sites with increase in the size of the peptide for stabilizing the conformation of the peptide antigen could be resolved. This TST molecule will also be useful in a structure-function study of other biologically active molecules.

As predicted, when the CSg-A sequence of fibronectins IIICS domain is grafted into a helical PTST molecule, the observed biological activity was less than CSg-A alone, indicating that the PTST molecule would stabilize the grafted sequence's conformation. Various synthetic peptides of RSV related proteins were synthesized to determine the monoclonal antibodies' antigenic epitopes. This RSV system will be used to further test the concept of the PTST molecule for an AIDS vaccine development.

PHS 6040 (Rev. 5/92)

Mervic, M, Moody, TW and A Komoriya. A structure function of C-terminal extensions of bombesin. Peptides, 12:1149, 1991.

Komoriya, A and S Shinagawa. Design and synthesis of conformationally constrained peptide tertiary structural templates (PTST) for AIDS vaccine development:II. Presented at the VIII International Conference on AIDS/III STD World Congress, Amsterdam, The Netherlands, July 19-24, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 BD 01011-02 LCB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Animal Models for the Long-term Safety Assessment of CSFs

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

PI: Akira Komoriya, Ph.D., Senior Staff Fellow, LCB, DCB, CBER
Beverly S. Packard, Ph.D., Senior Staff Fellow, LCB, DCB, CBER
Joy Cavagnaro, Ph.D., Office of Center Director, CBER

COOPERATING UNITS (if any)

Dr. Dan Sheehan, Chief, Div. of Reproductive and Developmental Toxicology, Dr. Lionel Poirier, Dir. and Dr. N. Littlefield, Div. of Comparative Toxicology, NCTR

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Growth Factors Structure and Function Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

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

Recently developed pharmaceutical manufacturing processes based on recombinant molecular biology and biotechnology methods present us with unresolved critical concerns related to the long-term effects of biotechnology-derived products. One concern is the issue of whether growth factors such as G-CSF, GM-CSF, M-CSF, IL-3, IL-1 and stem cell growth factor have any carcinogenic potential by themselves or more likely potentiate the carccinogenicity of other known drugs such as chemotherapeutic agents. Currently available in vitro data suggest this latter possibility may be a genuine concern. There has been no animal study conducted for the long-term safety assessment of the hemopoietic colony stimulating factors (CSFs).

The main regulatory objective of this study is, therefore, to gather sufficient in vivo data for safety information in selecting what carcinogenic chemotherapeutic agents may be safely used with CSFs. Potential results from the proposed animal study are expected to provide relevant information for a more complete risk-benefit evaluation of a given combination of CSFs and chemotherapeutic agents.

Hence, we designed 3 separate experimental approaches for the above objectives. One is to examine the potential modulation by colony stimulating factors on the carcinogenicity of a chemotherapy agent in mice. The second approach is to develop an in vivo murine long-term bone marrow stromal cell and stem culture using the xenotransplant methodology. With this long-term culturing system, one can examine the long-term effects of a chemotherapeutic agent.

The third experimental approach is to evaluate the effects of CSFs on the mutagenic potential of a known mutagen using a recent recently developed transgenic mouse (Big Blue mouse). A control experiment using this short term in vivo mutagenic assay system is being carried out.

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

PROJECT NUMBER
Z01 BD 01025-01 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Oncoimmuning: New Tumor-derived Immunoregulatory Factors

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

PI: B. Packard, Ph.D., Senior Staff Fellow, LCB, DCB, CBER
A. Komoriya, Ph.D., Senior Staff Fellow, LCB, DCB, CBER
L. Harvath,. Ph.D., Senior Microbiologist, LCH, DH, CBER
S. S.-Y. Lee, FAES Guest Worker, LCB, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cell Biology

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INSTITUTE AND LOCATION
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TOTAL STAFF YEARS: 1.25	PROFESSIONAL: 1.25	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)

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

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

Two new cytokines which have been designated as Oncoimmunin-L (MW 45kD) and Oncoimmunin-M (MW 36 kD) were identified and purified to homogeneity by SDS-PAGE analysis from a tumor cell supernatant. To achieve this end, combinations of ion exchange, gel filtration, hydrophobic interaction, and reverse phase chromatography were implemented.

Oncoimmunin-L is a human T-lymphocyte mitogen and Oncoimmunin-M inhibits the growth of at least three human myeloid leukemic cell lines. Concomitantly with the inhibition of proliferation, the latter factor induces the cell surface expression of the integrin CD11 and migration of cells toward a chemotactic gradient.

Results from this project which was aimed at exploring the possible presence of soluble immunoregulatory factors of tumor origin support the idea that the tumor immunoenvironment as recognized by immunocytes is defined by soluble mediators in addition to the well-studied cell-cell contact interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 01031-01 LCB

PERIOD COVERED

November 3, 1991 through September 30, 1992

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

Zidovudine Myopathy

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

PI: F. Varricchio, Ph.D., M.D., Senior Staff Fellow, LCB, DCB, CBER

COOPERATING UNITS (if any)

M. Reyes, M.D., Cook County Hospital, Chicago, IL

LAB/BRANCH

Laboratory of Cell Biology

SECTION

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DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Although mitochondrial abnormalities have been found in the muscle of some zidovudine-treated HIV+ patients with clinical myopathy (New England J. of Med., 1990, 322:1098), it is not clear if the myopathy results from zidovudine, the HIV infection, or both. To determine if zidovudine is myotoxic, the quadriceps muscle of Syrian hamsters given zidovudine 150 mg/kg body weight intraperitoncally twice daily was compared with control animals.

Frozen sections showed an equivocal increase of formazan deposits after reduced nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase histochemistry but no ragged-red fibers after modified trichrome stain. Electron microscopic examination revealed slight increases in the number and size of mitochondria of treated animals compared with controls. This finding was confirmed by the higher volume fraction of mitochondria in eight treated animals compared with six controls:9.2 plus or minus 1.5 volume percent versus 4.7 plus or minus 0.6 (p less than 0.05). SDS polyacrylamide gel electrophoresis of mitochondrial proteins showed several changes including a relative reduction of a 45-kd protein in the treated animals. These results show that zidovudine alone can cause mitochondriopathy in hamster skeletal muscle.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 01032 -01 LCB

PERIOD COVERED

November 3, 1991 through September 30, 1992

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

Protein Secretory Patterns of Ovarian Epithelium In Vivo and In Vitro

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

PI: F. Varricchio, Ph.D., M.D., Senior Staff Fellow, LCB, DCB, CBER

COOPERATING UNITS (if any)

Kurt Stromberg, M.D., LCB, DCB, CBER, FDA

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Growth Factors Structure and Function Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The protein composition of ovarian cyst fluid has not been studied extensively. Ovarian cyst fluid from 13 benign serous or mucinous cystadenomas, 1 dermoid cyst and 2 ovarian carcinomas were analyzed by SDS polyacryamide gel electrophoresis (SDS PAGE). In each case, 90 percent or more of the total protein was a 65,000 KD band. There was no consistent difference in the minor protein bands among the various types of cyst fluids analyzed. Conditioned serum free medium from 4 ovarian carcinoma cell lines was analyzed similarly. The protein pattern obtained was closely similar to that of the cyst fluids. Especially remarkable was the predominant 65 KD band present in vivo in the cyst fluids and in vitro in the media of the carcinoma cell lines. The migration rate on SDS PAGE of the 65 KD band is identical to human serum albumin (66 KD). Studies are in progress to further characterize and identify this protein. It is noteworthy that the ovarian carcinoma cell lines secrete the same or similar protein that is found in ovarian cyst fluids. To our knowledge, secretion of an abundant albumen-like protein by ovarian cyst lining cells in vivo and ovarian carcinoma cells in vitro has not been previously reported. Secretion of a large amount of protein by cyst lining cells may be instrumental in ovarian cyst fluid accumulation and therefore cyst enlargement.

PHS 6040 (Rev. 5/92)

PUBLICATIONS

Reyes, MG, Casanova, J, Varricchio, F, Sequeira, W and K Fresco. Zidovudine myopathy. Neurology, 42:1252, 1992.

CELLULAR PATHOLOGY SECTION

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

PROJECT NUMBER
Z01 BD 01005-04 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Autocrine Role of the EGF Supergene Family in Ovarian Carcinoma

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

PI: K. Stromberg, M.D., Staff Medical Officer, LCB, DCB, CBER
G.R. Johnson, Ph.D., Staff Fellow, LCB, DCB, CBER
A.W. Gordon, Ph.D., NRC Fellow, LCB, DCB, CBER
B. Kannan, M.S., Technician, LCB, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Cellular Pathology Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.2	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

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

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

The potential involvement of Epidermal Growth Factor (EGF), Transforming Growth Factor-α, Amphiregulin (AR), Epidermal Growth Factor-Receptor (EGF-R), and c-erbB-2 in the initiation and progression of human ovarian epithelial cancer was investigated by comparison of the mRNA and protein level of these genes in carcinoma cell lines and normal ovarian surface epithelian (OSE) cells. All 17 ovarian carcinoma cell lines which were examined expressed EGF-R (Kd equals 0.21 to 5.3 nM) and 16 cell lines, in addition, concomitantly secreted TGF-α (16 to 197 pg/ml). The growth of 8 carcinoma lines was stimulated in a dose-dependent manner when grown in the presence of exogenous TGF-α (30 to 88 percent). Growth in 4 of 5 of the cell lines capable of serum-free propagation was inhibited (28 to 56 percent) when cultured in medium containing a TGF-α neutralizing monoclonal antibody. Concentrations of AR ranging from 1-5 nM stimulated the growth of 3 OSE samples (20 to 50 percent) and 4 of 6 carcinoma cell lines (28 to 210 percent).

Differential immunohistochemical detection of AR and cripto was documented in normal human colon and colorectal tumors. Whereas normal colon did not express cripto, 86 percent of the tubulo adenomas, but only 43 percent of the tubular adenomas were positive for cripto expression. AR expression appeared to be associated with both normal and malignant colonic epithelial cells that were more differentiated.

AR localizes in both nucleus and cytoplasm by immunohistochemistry of ovarian carcinoma and normal ovarian surface epithelial cells, and functions in an autocrine manner in a colon carcinoma cell line.

PHS 6040 (Rev. 5/92)

PUBLICATIONS

Stromberg, K, Collins, TJ, Gordon, AW, Jackson, CL, and GR Johnson. Transforming growth factor-α acts as an autocrine growth factor in ovarian carcinoma cell lines. Cancer Res., 52:341, 1992

Johnson, GR, Saeki, T, Auersperg, N, Gordon, AW, Shoyab, M, Salomon, DS and K Stromberg. Response to and expression of amphiregulin by ovarian carcinoma and normal ovarian surface epithelial cells: nuclear localization of endogenous amphiregulin. Biochem. Biophys. Res. Comm., 180:481, 1991.

Saeki, T, Stromberg, K, Qi, C-F, Gullick, WJ, Tahara, E, Normanno, N, Ciardiello, F, Kenney, N, Johnson, GR and DS Salomon. Differential immunohistochemical detection of amphiregulin and cripto in human normal colon and colorectal tumors. Cancer Res., 52:3467, 1992.

Johnson, GR, Saeki, T, Gordon, AW, Shoyab, M, Salomon, DS and K Stromberg. Autocrine action of amphiregulin in a colon carcinoma cell line and immunocytochemical localization of amphiregulin in human colon. J. of Cell Biol., August issue, 1992.

ABSTRACTS

Salomon, DS, Normanno, N, Kenney, N, Saeki, T, Qi, C-F, Kim, N, Ciardiello, F, Stromberg, K, Johnson, G, Gullick, WJ, Plowman, G, Tahara, E and G Todaro. Epidermal growth factor (EGF)-related peptides in breast and colorectal cancer. J. Cell. Biochem. Suppl., 15A, 1992.

Gordon, AW, Johnson, GS, Auersperg, N and K Stromberg. Does mRNA phenotyping of the EGF supergene family in ovarian carcinoma cells support the autocrine hypothesis of growth control? NCI Symposium on Investigational Strategies for Detection and Intervention in Early Ovarian Cancer, Annapolis, MD, April 12-15, 1992.

Stromberg, K, Gordon, AW, Kannan, B and GR Johnson. The Autocrine role of the EGF supergene family in human ovarian carcinoma. FDA Science Expo '92, May 21-22, 1992.

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

PROJECT NUMBER
Z01 BD 01010-02 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Role and Mechanism of Action of Amphiregulin in Biological Processes

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

PI: Gibbes R. Johnson, Ph.D., Sr. Staff Fellow, LCB, DCB, CBER
K.J. Stromberg, M.D., Medical Officer, LCB, DCB, CBER
A.W. Gordon, NRC Fellow, LCB, DCB, CBER
B. Kannan, M.S., Technician, LCB, DCB, CBER

COOPERATING UNITS (if any)

Dave Salomon, Ph.D., NCI, NIH
P.K. Lala, M.D., Ph.D., University of Western Ontario

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Cellular Pathology Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

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

Using our antibodies directed against amphiregulin, we have studied the in vivo expression and localization of amphiregulin in normal human colon, human colon carcinomas and normal human placenta. In all normal colon specimens, amphiregulin was detected and localized to the terminally-differentiated, non-proliferative epithelial cells of mucosal surface. In 18 of 36 cancers, amphiregulin was detected in the proliferative malignant epithelial cells of the tumors and was more commonly detected in the well-differentiated carcinomas (71%). Amphiregulin was detected in the terminally-differentiated syncytiotrophoblasts of human placenta but was not detectable after 18 weeks of gestation. In all three instances , amphiregulin was detected in the nucleus as well as cytoplasm of the cells in vivo. Thus, amphiregulin expression in normal tissues is related to differentiation, but amphiregulin may function as autocrine growth stimulator in colonic carcinomas.

An evaluation of the mechanism of action of amphiregulin in a human colon carcinoma cell line reveals that the cells secrete amphiregulin and that it acts via an extracellular autocrine loop to drive the proliferation of the cells. The data suggests that amphiregulin acts through the EGF receptor.

Expression of the cDNA for various oncogenes result in the transformation of normal human mammary epithelial cells and the concomitant elevated expression of amphiregulin mRNA and protein, suggesting that amphiregulin may be a key intermediate in oncogene-mediated malignant transformation.

PUBLICATIONS

Johnson, GR, Saeki, T, Auersperg, N, Gordon, AW, Shoyab, M, Salomon, DS and K Stromberg. Response to and expression of amphiregulin by ovarian carcinoma and normal ovarian surface epithelial cells: Nuclear localization of endogenous amphiregulin. Biochemical and Biophysical Research Communications, 180:481, 1991.

Stromberg, K, Collins, TJ, Gordon, AW, Jackson, CL and GR Johnson. Transforming factor-α acts an autocrine growth factor in ovarian carcinoma cell lines. Cancer Research, 52:341, 1992.

Saeki, T, Stromberg, K, Qi, C-F, Gullick, WJ, Tahara, E, Normanno, N, Ciardiello, F, Kenny, N, Johnson, GR and DS Salomon. Differential immunohistochemical detection of amphiregulin and cripto in human normal colon and colorectal tumors. Cancer Research, 52:3467, 1992.

PUBLICATIONS IN PRESS

Johnson, GR, Saeki, T, Gordon, AW, Shoyab, M, Salomon, DS and K Stromberg. Autocrine action of amphiregulin in a colon carcinoma cell line and immunocytochemical localization of amphiregulin in human colon. Journal of Cell Biology, (cover article), 118:3, August 1st issue. (in press)

ABSTRACTS

Normanno, N, Saeki, T, Johnson, G, Kenney, N, Kim, N, Ciardiello, F and DS Salomon. Differential expression of amphiregulin in oncogene transformed human mammary epithelial cells. Presented at the First Joint Conference on Concepts and Molecular Mechanisms of Multistage Carcinogenesis, Santa Margherita, Italy, November 6-9, 1991.

Salomon, DS, Normanno, N, Kenney, N, Saeki, T, Qi, C-F, Kim, N, Ciardiello, F, Stromberg, K, Johnson, G, Plowman, G, Tahara, E and G Todaro. Epidermal growth factor (EGF)-related peptides in breast and colorectal cancer. Presented at the Keystone Symposium on Breast and Prostate Cancer, Lake Tahoe, CA, March 7-13, 1992. J. of Cellular Biochemistry. (in press)

Gordon, AW, Johnson, GR, Auersperg, N and K Stromberg. Does mRNA phenotyping of the EGF supergene family in ovarian carcinoma cells support the autocrine hypothesis of growth control? Proceedings of the National Cancer Institute Symposium:Investigational strategies for detection and intervention in early ovarian cancer, Annapolis, MD, April 13-14, 1992. (in press).

Stromberg, K, Gordon, AW, Kannan, B and GR Johnson. The autocrine role of the EGF supergene family in human ovarian carcinoma. Presented at the 1992 FDA Science Expo, Rockville, MD, May 21-22, 1992.

Normanno, N, Saeki, T, Bianco, C, Johnson, G, Kenney, N, Kim, N, Ciardiello, F and DS Salomon. Expression of amphiregulin (AR) in oncogene transformed human mammary epithelial cells. Proceedings of the American Association for Cancer Research, 33:271, 1992.

Normanno, N, Panneerselvam, M, Bianco, C, Johnson, G, Kim, N, Kenney, N, Ciardiello, F and DS Salomon. Amphiregulin as a potential autocrine growth factor for human colon carcinoma cells. Proceedings of the American Association for Cancer Research, 33:354, 1992.

Lysiak, JJ, Graham, CH, Riley, SC, Johnson, GR and PK Lala. Localization of transforming growth factor β (TGFβ) and amphiregulin in the human placenta and decidua throughout gestation. American J. of Reproductive Immun., 27:46, 1992.

Normanno, N, Panneerselvam, M, Saeki, T, Johnson, G, Kenney, N, Kim, N, Ciardiello, F, Shoyab, M, Todaro, G and DS Salomon. Amphiregulin (AR) as an autocrine growth factor for c-Ha-ras and c-erbB-2 transformed human mammary epithelial cells. Presented at the 8th Annual Oncogene Meeting, Frederick, MD, June 23-27, 1992.

Salomon, DS, Normanno, N, Saeki, T, Kenney, N, Kim, N, Ciardiello, F, Johnson, G, Gullick, W, Plowman, G, Shoyab, M and G Todaro. Biology of epidermal growth factor (EGF)-related peptides in breast cancer. Presented at the Genetics of Human Breast Cancer Meeting at Cold Spring Harbor, NY, September 2-6, 1992.

DIVISION OF CYTOKINE BIOLOGY

LABORATORY OF CELLULAR IMMUNOLOGY

IMMUNE REGULATION SECTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 02001-06 LCI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Cytokine Regulation of Lymphocyte Activation

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

PI: Jay P. Siegel, M.D., Senior Surgeon, LCI, DCB, CBER
Jim Crim, Biologist, LCI, DCB, CBER
Howard Mostowski, Biologist, LCI, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Immunology

SECTION

Immune Regulation Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Studies of the regulation of human cytotoxic T lymphocyte (CTL) development were continued.

a) The high efficiency cloning system for human CTL precursors developed last year was employed for limiting dilution analysis of CTL differentiation. Using this technique and others, we furthered our studies regarding IL-4 suppression of CTL development. Four principal findings developed. First, the suppressive effect of IL-4 was found to be exerted directly on the CD8 plus CTL precursor. Second, the partial suppression due to IL-4 was found to be due to complete blockage of CTL development in a subset of CTL precursors rather than partial block in all precursors. The nature of the subset is under study. Third, suppression occurred if and only if IL-4 was present during activity, not earlier or later. Fourth, presence of IL-4 during activation of CD8 plus lymphocytes resulted in an increase in the frequency of proliferating, noncytotoxic CD8 plus cells. These cells are now under study.

b) Human CTL lines have been developed and grown in the presence of IL-4 rather than IL-2. Their growth and differentiation have been shown to be IL-2 independent. The specificity and range of their cytotoxicity differs from lines grown in IL-2.

c) IL-12 has been found to have significant effects upon the growth and differentiation of human CTL. These effects are under active investigation.

d) RT-PCR techniques for analyzing key molecular events in CTL development are under development.

PUBLICATIONS

Otani, H, Siegel, JP, Erdos, M, Gnarra, JR, Toledano, MB, Sharon, M, Mostowski, H, Feinberg, MB and JH Pierce. Interleukin (IL)-2 and IL-3 induce distinct but overlapping responses in murine IL-3-dependent 32D cells transduced with human IL-2 receptor β chain:Involvement of tyrosine kinase(s) other than p56(lck). Proc. Natl. Acad, Sci. USA, 89:2789, April 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 02002-04 LCI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Immunoregulation In Vivo and In Vitro by Cytokines

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

PI: Raj Puri, Ph.D., M.D., Senior Investigator, LCI, DCB, CBER
Pamela Leland, Biologist, LCI, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Immunology

SECTION

Immune Regulation Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Previously, we have demonstrated that IFN-α synergizes with IL-2 in the augmentation of lymphokine activated killer (LAK) cells in various compartments of mice. Both proliferation and augmentation of LAK cell activity on a per cell basis was observed in response to IFN-α and IL-2 therapy. These observations suggest that anti-tumor effects of IFN-α with IL-2 may be mediated through activation of LAK cells. The administration of IFN-γ with IL-2 increased LAK activity while it has not been shown to cause synergistic anti-tumor effects. Other effectors such as CTL, macrophages and unknown cell population may be responsible for differences observed with the two interferons. Additionally, we have observed that IL-6 causes the proliferation of lymphoid cells in the spleen of mice. These cells appear to be T lymphocytes because no proliferation was observed in the irradiated and nude mice. Proliferating cells did not bear NK or LAK activity, however, secondary CTL activity was induced by IL-6.

This project is now complete and no further studies are planned for the immediate future.

PUBLICATIONS

Puri, RK and P Leland. In vivo treatment with interferon-α causes augmentation of IL-2-induced lymphokine activated killer cells in the organs of mice. Clin. Exp. Immunol., 85:317, 1991.

Puri, RK. MHC class I H-2(b) antigen expression on MCA-102 tumor cells and their upregulation by recombinant interferon alpha and gamma. [Letter], Cancer Res., 51:6209, 1991.

Fujita, S, Puri, RK, Yu, Z, Travis, WD and VJ Ferrans. Ultrastructural study of in vivo interactions between lymphocytes and endothelial cells in the pathogenesis of the vascular leak syndrome induced by Interleukin-2. Cancer, 68:2169, 1991.

Ansher, SS, Puri, RK, Thomson, WC and WH Habig. The effects of Interleukin-2 and Interferon-α administration on hepatic drug metabolism in mice. Cancer Res., 52:262, 1992.

Puri, RK and P Leland. Systemic administration of recombinant Interleukin-6 in mice induces proliferation of lymphoid cells in vivo. Lymphokine and Cytokine Res., 11:133, 1992.

Puri, RK, FitzGerald, D, Leland, P, Kozak, RW and I Pastan. In vitro and in vivo suppression of IL-2 activated killer cell activity by chimeric proteins between IL-2 and pseudomonas exotoxin. Cellu, Immunol., 1992. (in press)

ABSTRACTS

Puri, RK and P Leland. Interferon-α augments Interleukin-2-induced cytotoxic T lymphocytes response in peritoneal exudate cells of mice in vivo. Proc. J. Interferon Res., Abstract No. 4.12, 1991.

Puri, RK, Loberbaum-Galski, H, Leland, P, Kozak, RW and I Pastan. In vitro and in vivo suppression of Interleukin-2 activated killer cell activity by chimeric protein between Interleukin-2 and pseudomonos exotoxin. Proc. Amer, Assoc, Cancer Res. 33, Abstract 1940, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 02003-04 LCI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

IL-4 Receptors on Murine Solid Tumors and Tumor Infiltrating Lymphocytes

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

PI: Raj Puri, Ph.D., M.D., Senior Investigator, LCI, DCB, CBER
Pam Leland, Biologist, LCI, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Immunology

SECTION

Immune Regulation Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

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

We have previously reported the presence of the Interleukin-4 receptor (IL-4R) on methylcholanthrene (MCA-106, MCA-102 and MC-38) and viral DNA (G-2TS and 14-2TS) induced murine sarcoma cells. These receptors are similar in characteristics to that observed by us on TIL cells and by others on T and B lymphocytes, mast cells and macrophages. These receptors are internalized after binding to a chimeric protein between IL-4 and pseudomonas exotoxin (IL-4-PE40). Using IL-4PE40, we observed that IL-4-PE40 was cytotoxic (determined by inhibition of protein synthesis by [(3)H]-Leucine uptake) to MCA-106 tumor cells in a dose dependent manner. IL-4-PE40 asp(553), a chimeric mutant protein which can bind to IL-4 receptors but does not have the capability to inhibit protein synthesis was not cytotoxic to tumor cells. These studies suggest that IL-4R on murine MCA-106 sarcoma cells is internalized when occupied by IL-4-PE40 and may be functional. taken together, these data suggest that IL-4 receptor may be a target for IL-4-toxin therapy.

Now we have found that TNF-α upregulates IL-4 receptors on tumor cells. We are actively pursuing our research to investigate the mechanism and possible role of augmented expression of IL-4 receptors on tumor cells. This project is currently active.

Puri, RK and P Leland. Tumor necrosis factor-α upregulates Interleukin-4 receptors on murine sarcoma cells. 8th International Congress of Immunology. Budapest, Hungary, August 23-28, 1992.

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

PROJECT NUMBER
Z01 BD 02004-04 LCI

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization of Tumors and TIL from Tumors Induced by HHV-6

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

PI: R.K. Puri, Ph.D., M.D., Senior Investigator, LCI, DCB, CBER
P. Leland, Biologist, LCI, DCB, CBER

COOPERATING UNITS (if any)
A. Razzaque, Ph.D., DV, CBER

LAB/BRANCH
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DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

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

We reported that tumors induced by HHV-6 DNA are tumorigenic in immunocompetent NIH-Swiss mice and tumor specific TIL can be generated from these tumors. The metastatic potential of NIH Swiss derived tumor and tumorigenic potential of another clone, 14-2T DNA, was investigated in this fiscal year. We found that 14-2T clone was also tumorigenic and these tumors metastasized to lungs in immunocompetent mice after tail vein injection. TILs could also be generated from these tumors, however, they were not tumor specific. We have published an additional paper from this project. This project is now closed because of lack of personnel and resources.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 02012-02 LCI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Expression of IL-4 Receptors on Human Tumors

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

PI: Raj Puri, Ph.D., M.D., Senior Investigator, LCI, DCB, CBER
Nicholas Obiri, Ph.D., NRC Fellow, LCI, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Immunology

SECTION

Immune Regulation Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In this project, we are studying the expression of IL-4R and the role of IL-4 on solid human tumors. We have observed that human tumors of different histology also express IL-4R. Human breast carcinoma, ovarian carcinoma, melanoma and renal tumor lines, as well as fresh tumors obtained from the NCI, express high affinity IL-4R. The characteristics of these receptors appear to be similar to that reported on hematopoetic cells such as T cells, B cells, mast cells and macrophages. Human tumors also express a single species of mRNA of IL-4R. We are currently investigating the function of IL-4 on tumor cells. Our preliminary data indicate that IL-4 is directly growth inhibitory to some IL-4R positive tumor lines as tested by MTT assay. Furthermore, IL-4 appear to upregulate HLA-DR antigen on some tumor cells.

We have two manuscripts from this project which are being revised for publication. Experiments are underway which will examine the regulation of IL-4R on tumor cells. This project is currently active.

Razzaque, A and RK Puri. Human herpes virus (HHV-6):Tumorigenicity and tumor infiltrating lymphocytes. Cancer Letters, 61:111, 1992.

ABSTRACTS

Hillman, GG, Obiri, NI, Haas, GP, Sud, S and RK Puri. Expression of Interleukin-4 receptors (IL-4R) on human renal cell carcinoma (RCC) tumor cells and IL-4 inhibition of tumor cell growth in vitro. American Urological Association, 1992.

Obiri, NI and RK Puri. Expression of Interleukin-4 receptors on human breast and ovarian carcinoma cell lines. The FASEB J. 6, Abstract 4518, 1992.

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

PROJECT NUMBER
Z01 BD 02019-01 LCI

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
HIV-1 tat Regulation of IL-4 Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: R.K. Puri, Ph.D., M.D., Senior Investigator, LCI, DCB, CBER

COOPERATING UNITS (if any)
Bharat Aggarwal, Ph.D., Professor of Medicine, MD Anderson Hospital, University of Texas, Houston, TX.

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Immune Regulation Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

We have observed that human B lymphoblastoid cell lines express high affinity IL-4 receptors. Because tat and IL-4 can be immunosuppressive agents, we examined the regulation of IL-4 receptors on these cells in response to tat gene. We found that constitutive expression of HIV-1 tat gene in Raji cells caused elevated expression of IL-4 receptors. This upregulation was observed at both protein and gene levels. In the presence of cycloheximide, no upregulation of IL-4 receptors was observed indicating that receptor protein synthesis is required for this upregulation to take place. Our manuscript describing this work is in press and will appear in the July issue of Cancer Research. This project is currently active.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 02020-01 LCI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Expression and Function of IL-4 Receptors on AIDS-associated Kaposi's Sarcoma

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

PI: R.K. Puri, Ph.D., M.D., Senior Investigator, LCI, DCB, CBER

COOPERATING UNITS (if any)

Parakash Gill, M.D., Associate Professor, University of Southern California, Los Angeles, California

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DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have demonstrated that AIDS-associated Kaposi's sarcoma (KS) cells express IL-4 receptors. These receptors are functional because IL-4 inhibited the growth of these cells in tissue culture. KS cells are known to produce IL-6 and Oncostatin-M and they proliferate in an autocrine manner to these cytokines. IL-4 inhibited the production of both cytokines. These observations suggest that IL-4 can be a useful lymphokine for the treatment of KS. A manuscript describing this work has recently been submitted for publication. This project is currently active.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 02021-01 LCI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Cellular Mechanisms Involved in Allograft and Xenograft Rejection

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

PI: A. Rosenberg, M.D., Senior Staff Fellow, LCI, DCB, CBER
J. Sechler, Microbiologist, LCI, DCB, CBER

COOPERATING UNITS *(if any)*

Kathleen Clouse, Ph.D., Karis (Weih) Faust, DCB, CBER; Klaus Strebel, Sandy Morse, Janet Hartley, NIAID; Ralph Quinones, Children's Hospital.

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Laboratory of Cellular Immunology

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Immune Regulation Section

INSTITUTE AND LOCATION

DCB, CBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

The main objective of our research is to understand the cellular mechanisms and interactions involved in in vivo mediated tissue destruction as exemplified by allograft and xenograft rejection. An additional project, considerably removed from the field of transplantation, has examined the anti-viral activity of restriction endonucleases.

Over the past year, our studies have focused on the role of CD4(+) T cells in rejection of MHC class I disparate skin grafts and on the cellular mechanisms underlying the mouse anti-human xenograft response. We have found that, in the absence of additional alloantigenic disparities, CD4(+) T cells slow the rejection rate of MHC class I disparate skin grafts, mediated by CD8(+) T cells. We are currently exploring whether CD4(+) T cells exposed to class I alloantigen in the absence of additional cellular populations are capable of mediating complete suppression of the rejection response. In xenografting experiments, we are investigating the cellular requirements for generation of mouse CTL to human tumor cells in an effort to generate tumor specific CTL which do not depend on multiple accessory molecules. We are further characterizing the murine cellular responses to human hematopoeitic progenitor cells, and thymic epithelium with the goal of establishing stable mouse-human chimeras. We have found that murine CTL responses to human alloantigens expressed on tumor cells require in vivo priming and that both CD4(+) Th cells and CD8(+) Tk cells are required. Preliminary studies have further revealed that primed effector cells may not require mouse antigen presenting cells for their activation. We are further investigating the dependency of such CTL on various accessory molecules, both human and mouse. Lastly, studies using restriction endonucleases as anti-viral agents, have revealed that restriction endonucleases with sites on dS DNA viruses (or RNA viruses whose replicative cycle involves a dsDNA form) appear to specifically inhibit infection in infected mammalian cells.

PUBLICATIONS

Rosenberg, A. Skin allograft rejection. In: Current Protocols in Immunology. J.E. Coligan et. al., eds. Greene Publishing Associates/Wiley Interscience, New York, 1991.

Rosenberg, AS and A Singer. Cellular basis of skin allograft rejection: an in vivo model of immune mediated tissue destruction. Annual Rev. Immunol., 10:333, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03016-04 LCI

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cytokines and the Cascade of Receptors: Lymphocyte Activation

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

PI: R.W. Kozak, Ph.D., Research Microbiologist, LCI, DCB, CBER
L.A. Jones, M.S., Microbiologist, LCI, DCB, CBER
S.H. Kim, Ph.D., Guest Worker, LCI, DCB, CBER

COOPERATING UNITS (if any)

Metabolism Branch, Lab of Molecular Biology and Radiation Oncology Branch, NCI, NIH

LAB/BRANCH
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SECTION
Immune Regulation Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 5	PROFESSIONAL: 4	OTHER: 1

CHECK APPROPRIATE BOX(ES)

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

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

Human lymphocytes, upon activation, express on their cell surfaces a number of new receptors for cytokines. The cascade of early appearing activation antigens include receptors for: IL-2, transferrin, insulin, IL-4, and insulin-like growth factors types I and II. The effects of these cytokines on their own and other cytokine receptors is being studied through radioligand binding, receptor enumeration by Scatchard analysis, receptor structure identified by chemical crosslinking of iodinated ligand-receptor complexes and the level of receptor messenger RNA induction by Northern blot analysis. IL-4 and IL-2 along with other immunomodulators are being examined for their mechanism of enhancement or suppression of cell-mediated immune responses by assessing their effects on growth factor production and receptor expression. Delineating the events that occur during lymphocyte activation and understanding the consequences of perturbing these events, should help us pinpoint defects in regulation of cell-mediated immunity and provide a model for analyzing the effects of other cytokines on the immune response. In addition, we are also studying the IL-2 receptor as a target for immunotherapy. Therapeutic reagents being studied include toxin-conjugated IL-2 and anti-IL-2 receptor monoclonal antibodies along with radionuclide (alpha and beta particle emitters) chelate coupled anti-IL-2 receptor monoclonal antibodies. The design of cytokine-toxin recombinant fusion proteins along with cytolytic conjugates of MAb are tested for specificity, stability and toxicity in animal models for evaluation as potential therapeutic reagents for human use in treatment of cancer, transplantation and autoimmune diseases.

PUBLICATIONS

Johnson, EW, Jones, LA and RW Kozak. Expression and function of insulin-like growth factor receptors on anti-CD3 activated human T lymphocytes. J. Immunol., 148:63, 1992.

PUBLICATIONS IN PRESS

Puri, RK, FitzGerald, DJP, Leland, P, Kozak, RW and I Pastan. In vitro and in vivo suppression of interleukin-2 activated killer cell activity by chimeric proteins between interleukin-2 and Pseudomonas exotoxin. Cell Immun., 1992. (in press)

Kozak, RW, Durfor, CN and CL Scribner. Regulatory considerations when developing biological products. Cytotechnology, 1992. (in press)

CELL & MOLECULAR BIOLOGY SECTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 02017-02 LCI

PERIOD COVERED: October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Molecular and Cellular Regulation of Natural Killer Cells

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

PI: Eda T. Bloom, Ph.D., Section Chief, Research Biologist, LCI, DCB, CBER
Hisanori Umehara, M.D., Visiting Scientist, LCI, DCB, CBER
D'Anna Hohe, M.S., Guest Worker, LCI, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Cellular Immunology

SECTION: Molecular and Cell Biology Section

INSTITUTE AND LOCATION: DCB, CBER, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Infusion of ex vivo activated killer cells is currently a popular modality of immunotherapy, especially for the treatment of cancer. A significant proportion of the activated killer cells have been stimulated by culture with rIL-2, and are termed lymphokine activated killer (LAK) cells. The predominant cellular precursor of LAK cells are natural killer (NK) cells. We have continued our ongoing interest in NK cell biology.

The mechanism by which LAK cells acquire a broader lytic capacity than their precursors, NK cells, is poorly understood. LFA-1 has been shown to function for adhesion of NK and LAK effector cells to their target cells. It is a member of a superfamily of adhesion molecules consisting of covalently linked heterodimers containing unique α chains (CD11a for LFA-1) and a common β chain (CD18). Based on published evidence and our own observations, we generated and explored the hypothesis that utilization of an LFA-1 signal transduction pathway functionally distinguishes human LAK from NK cells. Using NK cells isolated by negative selection with monoclonal antibodies (approximately 90% CD16-positive), we found that antibodies to the LFA-1 β chain strongly inhibited LAK activity while only moderately suppressing NK activity, suggesting a differential role for LFA-1 β in LAK compared to NK mediated lysis. LFA-1 β was strongly phosphorylated in LAK but not NK cells. Crosslinking of the LFA-1 β chain stimulated calcium-dependent release of cytoplasmic granules containing lytic molecules and induced phosphatidyl inositol turnover in LAK but not NK cells. We concluded that the IL-2-induced phosphorylation of the LFA-1 β chain in LAK cells and associated alteration in signal transduction, may represent an important functional distinction between NK and LAK cells.

Preliminary results have suggested that the turnover of LFA-1 is more rapid in LAK compared to NK cells at the protein level.

PUBLICATIONS

Umehara, H, Minami, Y, Takashima, A and ET Bloom. LFA-1 β chain (CD18) in human LAK cells: phosphorylation and signal transduction. 7th International Rinshoken Conference, Tokyo, October, 1992. (in press)

Umehara, H, Bloom, ET, Minami, Y and N Domae. Signal transduction via phosphorylated adhesion molecule LFA-1β (CD18) distinguishes human LAK from NK cells. Japanese Cancer Association, 1992. (in press)

Umehara, H, Minami, Y, Takashima, A, Bloom, ET and N Domae. Signal transduction via phosphorylated adhesion molecule LFA-1β (CD18) distinguishes NK from LAK cells. Japanese Immunology Association, November 1992. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 02018-02 LCI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Molecular and Cellular Regulation of Cytolytic T Cells and Effects of Age

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

PI: E.T. Bloom, Ph.D., Section Chief, Research Biologist, LCI, DCB, CBER
J.A. Horvath, M.S., Biologist, LCI, DCB, CBER

COOPERATING UNITS *(if any)*

Dr. A.S. Rosenberg, Immunoregulation Section, LCI, DCB

LAB/BRANCH

Laboratory of Cellular Immunology

SECTION

Molecular and Cell Biology Section

INSTITUTE AND LOCATION

DCB, CBER, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Aging alters the individual's physiological and functional responses to external stimuli, including the responses to infectious agents and therapeutics including biological response modifiers. We therefore examined the effect of aging on immunity. As a model, we utilized the cytolytic T lymphocyte (CTL) response generated against alloantigens.

Although we have observed a reduction in the number of alloantigen-stimulating cells producing perforin or pore-forming protein (Pfp) with age, the reduction was small compared to the reduced cytolytic activity. We found that the Pfp level was reduced on a per cell basis, suggesting that (1) a threshold level of Pfp may be required for potency of effector cell function, and (2) aging may affect most or all potential effector cells rather than a "mosaic" of cells.

We have examined allogeneic CTL generated in response to antigens stimulating either CD4-positive or CD8-positive T helper and T effector cell function. We found that age-related effect was most evident in the CD4-positive T cell-dependent system in vitro and in vivo. We have observed that the decline in CD4-positive cell activity correlates with an alteration in expression of T cell receptors. Current efforts are focused on defining the difference in T cell antigen receptor expression in CD4-positive cells from young and aged mice.

PUBLICATIONS

Makinodan, T, Bloom, ET, James, SJ and J Lubinski. Immunity and aging. In: Principles and Practices of Geriatric Medicine, 2nd. edition. M.S.J. Pathy (ed.), John Wiley and Sons, Ltd., Sussex, England, pp. 3-12, 1991.

Bloom, ET and JA Horvath. Age-related decline in expression of pore-forming protein (Pfp) in individual cells: A probe for understanding the CTL lytic mechanism. FASEB J., 6:A2006, 1992.

Horvath, JA, Mostowski, HS, Okumura, K and ET Bloom. Pore-forming protein (Pfp) in individual CTL. The effect of senescence provides a probe for understanding the lytic mechanism. Eur. J. Immunol. (in press)

Bloom, ET and JA Horvath. The immunosenescence of cytolytic T lymphocytes (CTL). Reduction of pore-forming protein and granzyme levels. In "Cytotoxic Cells: Generation, Recognition, Effector Functions, Methods", M. Sitkovsky and P. Henkart, eds., Birkhauser, Boston. (in press)

Bloom, ET. Mixed lymphocyte culture for the generation of allo-specific CTL. In "Cytotoxic Cells: Generation, Recognition, Effector Functions, Methods", M. Sitkovsky and P. Henkart, eds. Birkhauser, Boston. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 03002-03 LCR

PERIOD COVERED: October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Studies on Properties of Lymphoblastoid Interferon-α Component O

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

PI: R.-Q. Hu, Ph.D., Visiting Scientist, LCR, DCB, CBER
D. Miller, Biologist, LCR, DCB, CBER
J. Enterline, Biologist, LCR, DCB, CBER
K.C. Zoon, Director, CBER

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Cytokine Research

SECTION: Chemical Biology Section

INSTITUTE AND LOCATION: DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Component o is one of 22 components purified from human lymphoblastoid interferon-alpha (IFN-α) by monoclonal antibody affinity and high performance liquid chromatography. The apparent molecular weight of this component on reducing SDS-PAGE is 19,000 KD. Component o exhibits very high antiproliferative activity on Daudi and AU937 cells; the concentration that yielded 50 percent inhibition of cell growth was 0.003 ng/ml on Daudi cells and 0.05 ng/ml on AU937 cells. Binding studies using radiolabelling recombinant IFN-α2b indicated that component o competes very poorly for IFN-α2b binding to these cells. The partial amino acid sequence of component o is similar to that of the deduced amino acid sequences from IFN-α88 and IFN-αF. Western blotting analysis of component o and IFN-α88 with anti-IFN-α monoclonal antibodies revealed that their immunoreactivities are different. Component o bound to 4F2 antibody but not to NK2 and III/21 antibodies. IFN-α88 bound to NK2 antibodies but not to the III-21 antibody. Cross-linking experiments using Daudi cells revealed a 134-kD complex with component o.

DIVISION OF CYTOKINE BIOLOGY

LABORATORY OF CYTOKINE RESEARCH

CHEMICAL BIOLOGY SECTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03030-02 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

cDNA Cloning and Expression of Human IFN-α Component O and F

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

PI: R.-Q. Hu, Ph.D., Visiting Scientist, LCR, DCB, CBER
D. Miller, Biologist, LCR, DCB, CBER
J. Enterline, Biologist, LCR, DCB, CBER
K.C. Zoon, Director, CBER

COOPERATING UNITS (if any)

K.J. Lei, Ph.D., Visiting Scientist, HGB, NICHD, NIH

LAB/BRANCH

Laboratory of Cytokine Research

SECTION

Chemical Biology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

An IFN-α2 cDNA and the monoclonal antibodies NK2 and 4F2 were used as probes to screen a human Namalwa cDNA library to find the cDNA coding for component O.21 positive clones which strongly hybridize with IFN-α2 cDNA were obtained. Due to the strong sequence similarity of the human IFN-α family, a specific oliganucleotide was applied to the 21 positive clones for component o to find cDNA coding for component o.

We have performed the partial amino acid sequence of IFN-α component o isolated from human IFN-α results from sequence analysis of component o. revealed that it is very similar to IFN-αF. In order to know the relationship between component o and IFN-αF, we cloned the gene for IFN-α into several expression vectors. Analysis by western blotting, SDS PAGE and antiviral activity revealed IFN-αF gene was expressed well in an pKK 223-3 expression system. We are now preparing sufficient quantities of IFN-αF to perform further studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 03005-04 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Purification and Characterization of Natural Human Interferon Alpha

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

PI: D.M. Miller, Biologist, LCR, DCB, CBER
J.C. Enterline, Microbiologist, LCR, DCB, CBER
J.B. Bekisz, Microbiologist, LCR, DCB, CBER
R.Q. Hu, Ph.D., Visiting Scientist, LCR, DCB, CBER
K.C. Zoon, Ph.D., Director, CBER

COOPERATING UNITS (if any)

I.K. Hewlett, Ph.D., LR, DTS, CBER
M. Meltzer, M.D., Cellular Immunology, Walter Reed Medical Center

LAB/BRANCH
Laboratory of Cytokine Research

SECTION
Chemical Biology Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.5	PROFESSIONAL: 3.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Our research studies are directed toward understanding the structure and function of human interferon alphas (IFN-α) and their receptors. The objective of these studies is to delineate the rational for the existence of this family of structurally-related proteins and to understand the mechanism by which they elicit their pleiotropic biological activities. To date, 18 major and 4 minor components of human IFN-α derived from Sendai virus-induced human lymphoblastoid cells (Namalwa) were isolated by sequential monoclonal antibody affinity chromatography using four different monoclonal antibodies, ultrafiltration and reverse-phase high performance liquid chromatography. Many biological properties of these components have been examined including their antiviral activity on human, bovine and murine cell lines, antiproliferative activity on U937 and Daudi cells, their relative affinities for IFN-α 2b binding sites on U937 and Daudi cells, enhancement of the expression of Class I histocompatibility antigens, induction of IL-1, induction of monocyte tumoricidal activity and enhancement of natural killer cell activity. We are currently examining their antiretroviral properties. Chemical characterization of these IFN-α components has also been a major program in our laboratory. Their apparent molecular weights, amino acid compositions and N-terminal amino acid sequences have been determined for all of the isolated components. We are currently studying the carbohydrate structure of the IFN-α components as we have identified 3 major glycosylated components and 11 components with low levels of glycosylation.

Our group is responsible for the regulatory physicochemical testing of all cytokine products as well as the lot release testing and review of 2 recombinant and 1 natural preparations of IFN-α and one recombinant IFN-γ. Potency assays for these products are performed on a routine basis. Our group participates in the inspection program and in the review of cytokine and growth factor INDs, PLAs and ELAs.

PUBLICATIONS

Zoon, KC, Miller, DM, Bekisz, J, zur Nedden, D, Enterline, J, Nguyen, NY, and R-Q Hu. Purification and characterization of multiple components of human lymphoblastoid interferon-α. J. Biol. Chem., 267(21):15210, 1992.

Zoon, KC, Bekisz, J and DM Miller. Interferon structure and function. Interferon: Principles and Medical Application. Baron, S, Coppenhaver, DH, Dianzani, F, Fleischmann, Jr., WR, Hughes, Jr., TK, Klimpl, GR, Niesel, DW, Stanton, GJ and SK Tyring, eds. ISIR. University of Texas Medical Branch at Galveston, Galveston, TX, publishers, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 03004-03 LCR

PERIOD COVERED: October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Antibodies to Human IFN in J.L.P. Patients Treated with Human IFN

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

PI: J. Bekisz, Microbiologist, LCR, DCB, CBER
K.C. Zoon, Ph.D., Director, DCB, CBER
J.C. Enterline, Microbiologist, LCR,DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Cytokine Research

SECTION: Chemical Biology Section

INSTITUTE AND LOCATION: DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

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

Six hundred and sixty serum samples from 135 patients treated with human leukocyte α interferon (Hu IFN (Le) (New York Blood Center, Melville, N.Y.) for Juvenile Laryngeal Papillomatosis (J.L.P.) were tested for the presence of neutralizing antibodies to the above-mentioned α interferon. Eleven samples were found to contain neutralizing antibodies to α interferon from the N.Y.B.C. Ten of these contained neutralizing antibodies to human leukocyte α interferon (Alferon; Interferon Sciences, New Brunswick, N.J.) and nine contained neutralizing antibodies to recombinant α interferon (Intron A, Schering-Plough, Kenilworth, N.J.). None of the samples were seen to bind to either Alferon or Intron in ELISA assays. Six, however, did bind to Wellferon (Burroughs Wellcome, Beckenham, U.K.) which is a natural lymphoblastoid interferon.

Since Wellferon is the starting material used in the purification of interferon components in our laboratory, we intend to determine if the samples which bound to Wellferon will also bind to the purified components.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03037-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Tryptic Digestion of Interferon Components

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

PI: J. Bekisz, Microbiologist, LCR, DCB, CBER
K.C. Zoon, Ph.D., Director, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cytokine Research

SECTION

Chemical Biology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

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

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

Twenty two interferon components which have been purified from Wellferon in our laboratory will be digested using trypsin TPCK. All digestion will be carried out at 37 degrees C for a period of 16 to 18 hours. The digested material will then be passed over a C-18 HPLC column to separate the individual fragments. This will enable us to generate a tryptic digest map (chromatogram) for each component. The fragments from each component will then be collected and assayed for protein sequence and amino acid analysis. To date, we have only been able to partially characterize the components. By sequencing the digestion fragments, we should be able to determine the full protein sequence for each component. The amino acid analysis will provide a compositional evaluation of each component.

Characterization of the entire molecule is important if we are to develop hydrophobicity and antigenicity profiles as well as determining the secondary structure of the component.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03009-03 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Isolation of Biologically Active Cytokines by Non-denaturing PAGE

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

PI: N.Y. Nguyen, Ph.D., LCR, DCB, CBER
P.D. Witte, Ph.D., LCR, DCB, CBER
J. Bekisz, M.S., LCR, DCB, CBER
J. Enterline, LCR, DCB, CBER
K.C. Zoon, Ph.D., Director, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cytokine Research

SECTION

INSTITUTE AND LOCATION

DCB, CBER, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 2	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

One of the most difficult steps in the study of biological macromolecules is the isolation of sufficient amounts of pure species with retention of native configuration. Reverse phase high pressure liquid chromatography in both conventional and narrow bore format and electrophoresis in SDS buffer systems followed by electroelution or electroblotting of resolved components represent the most efficient methods whenever affinity or other gel chromatography techniques are not applicable. However, many macromolecules of interest are not amenable to these approaches due to low recovery and loss of biological activity during separation in the presence of organic solvents or sodium dodecyl sulfate. We are developing techniques to purify biologically active molecules by multiphasic zone electrophoresis in polyacrylamide gel under non-denaturing conditions, in conjunction with HPEC and elution under carefully controlled temperature and buffer composition. The buffer systems are designed to encompass operative pH's ranging from 7.5 to 11.0 and to provide enough mobility for proteins with acidic to basic isoelectric points. The high resolving power of polyacrylamide gel electrophoresis and the ability to separate molecules on the basis of differences in size, net charge and hydrophobicity will be applied to the isolation of native cytokines, human goiter proteins and tetanus toxins, and recombinant interferon-α receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03045-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Expression and Isolation of a Functional IFN-α Receptor from Inclusion Bodies

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

PI: N.Y. Nguyen, Ph.D., Chemist, LCR, DCB, CBER
M. Hirata, Ph.D., LCR, DCB, CBER
R. Hirata, Ph.D., LCR, DCB, CBER
M. Hayes, Staff Fellow, LCR, DCB, CBER
J. Enterline, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

A. Karpas, Ph.D., DBP, CBER; G. Uze, Ph.D., LOV, France; D. Levy, Ph.D., NYU Medical Center.

LAB/BRANCH

Laboratory of Cytokine Research

SECTION

Chemical Biology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

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

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

The gene coding for the entire extracellular domain of the IFN-α receptor (lacking the signal peptide) has been cloned in plasmid pGEX-2T and expressed in E. coli DH5 as a fusion protein with bacterial glutathione-S-transferase. A DNA fragment encoding amino acids 28-437 of the receptor protein was inserted into vector pGEX-2T at the EcoRI and BamHJ sites. A stop codon immidiately downstream of the receptor sequence was added. Expression was induced by IPTG and the fusion protein was partially purified by gel chromatography on Sepharyl P S-300 followed by glutathione agarose affinity chromatography. Work is in progress to recover the non-fused receptor after-protease cleavage by thrombin, and to initiate studies on the physico chemical and biological characterization of the recombinant protein; antisera against the receptor will be produced and will be used to purify the natural protein from mammalian cells, and will serve as a probe to study the mechanisms of signal transduction involving interferon-α and its receptor(s).

Thompson, J, Nguyen, NY, Sackett, DL and Donkersloot, JA. Transposon-encoded sucrose metabolism in lactococcus lactis subsp. lactis: Purification of sucrose 6-phosphate hydrolase and genetic linkage to N5-(L-1-carboxyethyl)-L-ornithine synthase in strain K1. J. Biol. Chem., 22:14573, 1991.

Frasch, CE, Rubinstein, Y, Nguyen, N and J Ewell. Structural orientation and antigenic analysis of the class 2 and class 3 outer membrane proteins of neisseria meningitis. Proceedings of the Seventh International Pathogenic Neisseria Conference, Berlin, Federal Republic of Germany, September 9-14, 1990. ed. Achtman, M, Kohl, P, Marshal, C, Morelli, G, Seiler, A, Thiesen, B and W de Gruuter, Berlin, New York, 1991.

Thompson, J, Nguyen, NY and SA Robrish. Sucrose fermentation by fusobacterium mortiferum ATCC: Transport, catabolism and products. J. Bacteriology, 174 (10):3227, 1992.

Zoon, KC, Miller, D, Bekisz, J, zur Nedden, DL, Enterline, J, Nguyen, NY and R-Q Hu. Purification and characterization of multiple components of human lymphoblastoid interferon-α. J. Biol. Chem., 267(21):15210, 1992.

Fujii, N, Minetti, CAS, Nakhasi, H, Chen, SW, Barbahenn, E, Nunes, PH and NY Nguyen. Isolation, cDNA cloning and characterization of an intracellular hemmaglutin and amebocyte aggregation factor from limulus polyphemus. J. Biol. Chem. (in press)

Samudzi, CT, Nguyen, NY and JR Rubin. Crystallization and preliminary X-ray diffraction studies of dogfish-C-reactive protein. Proteins, 1992. (in press)

ABSTRACTS

Li, J, Nguyen, NY and C Lee. Distribution and expression of pneumolysin in streptococcus pneumoniae. FASEB Meeting, Atlanta, Georgia, April 1991.

Nguyen, NY. Limulus C-reactive protein, sigma Xi. The Scientific Research Society, Washington, D.C., April 2-3, 1991.

Nguyen, NY. Electroblotting of proteins at neutral pH: Sigma Xi. The Scientific Society, Washington, D.C., April 2-3, 1991.

Nguyen, NY, de Witte, P, Bekisz, J, Enterline, J and KC Zoon. Performance electrophoresis in non-denaturing neutral and basic buffer systems: Application to the isolation of biologically active interferon alphas. Annual Meeting of the International Society for Interferon Research, Nice, France, November 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 03046-01 LCR

PERIOD COVERED: October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Characterization of a Rec Hemaglutinin and Aggregation Factor from Limulus

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

PI: N. Nguyen, Ph.D., Chemist, LCR, DCB, CBER

COOPERATING UNITS (if any): C. Minetti, Ph.D., H. Nakhasi, PH.D., P. Nunes, M.D., DBB, CBER, FDA; E. Barbehenn, Ph.D., DMEDP, CDER, FDA; N. Fujii, Faculty of Pharm, Scs., Kyoto, Japan.

LAB/BRANCH: Laboratory of Cytokine Research

SECTION: Chemical Biology Section

INSTITUTE AND LOCATION: DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

An 18-kDa hemagglutinin which possesses the property of inducing both aggregation of amebocytes and agglutination of erythrocytes has been isolated from limulus polyphemus amebocytes and purified by ion exchange chromatography. This non-glysolated, single chain polypeptide with an Mr of 18,506 and isoelectric point of 8.3 is stored exclusively in the large secretory granules of amebocytes. Based on the partial N-terminal amino acid sequence of 63 residues, DNA probes have been synthetized for screening a pBR 322 cDNA library constructed from limulus amebocytes. Northern blot analysis indicates the presence of a single mRNA. The primary structure derived from the cDNA sequence reveals an internal homology consisting of two consensus sequences, V-N/D-D/S-W-D and E-D-R-R-W. The formation of 5 disulfide bonds between ten 1/2 cys divides the molecule into three looped domains, each containing the E-D-R-R-W repeat. One of the novel features of this protein is that it shares 37 percent homology with a 22-kDa mammalian extracellular matrix protein isolated from fetal bovine skin. The two proteins exhibit a similar pattern of looped domains, each domain containing a homologous consensus sequence (i.e., E-D-R-R-W). The functional properties of the two proteins are similar in that the limulus 18 kDa protein agglutinates horse erythrocytes and aggregates limulus amebocytes, and the 22-kDa protein is an effective adhesion promoter for dermal fibroblasts.

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

PROJECT NUMBER
Z01 BD 03008-04-LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Interferon Production by Human Monocytes

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

PI: M.P. Hayes, Ph.D., Senior Staff Fellow, LCR, DCB, CBER
J.C. Enterline, Microbiologist, LCR, DCB, CBER
T.L. Gerrard, Ph.D., Chief, LCR, DCB, CBER
K.C. Zoon, PH.D., Director, DCB, CBER
D.J. Lin, COSTEP Student, LCR, DCB, CBER

COOPERATING UNITS (if any)
NIH Blood Bank Apheresis Unit

LAB/BRANCH
Laboratory of Cytokine Research

SECTION

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, 8800 Rockville Pike, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

This study was initiated to assess the role of endogenous interferon on monocyte/macrophage differentiation and activation for host defense functions. Initial observations indicated that bacterial lipopolysaccharide (LPS), a potent monocyte/macrophage activating stimulus, failed to elicit interferon (IFN) production by freshly isolated human monocytes, despite the fact that other monocyte-derived cytokines (tumor necrosis factor, TNF; interleukin-1, IL-1) are produced upon LPS stimulation. However, culture of monocytes in the presence of either interferon-gamma (IFN-γ) or granulocyte-macrophage colony stimulating factor (GM-CSF) induces the capacity for LPS induction of IFN. TNF and IL-1 are differentially regulated under these conditions, suggesting independent regulation of all of these LPS-induced monokines. Neutralization studies indicate that the IFN produced in response to LPS is primarily of the alpha subtype. Northern analysis, using an IFN-α2 cDNA probe, demonstrated that induction by LPS is regulated at the steady-state mRNA level. Further studies have shown that interferon gene expression is quite transient. Down-regulation of expression occurs quickly following induction and is temporally related to the latent induction of the mRNA for interferon regulatory factor 2 (IRF-2), a nuclear factor thought to be involved in repression of IFN gene transcription. A role for NF-κB has not been ruled out, although IFN-α promoters lack the binding site for this factor.

Dr. Hayes is also involved in regulatory duties which include review of investigational new drug applications for cytokines with emphasis on interferons.

PUBLICATIONS

Hayes, MP, Enterline, JC, Gerrard, TL and KC Zoon. Regulation of interferon production by human monocytes:requirements for priming for lipopolysaccharide-induced production. J. Leuk. Biol., 50:176, 1991.

ABSTRACTS

Hayes, MP, Enterline, JC, Gerrard, TL and KC Zoon. Priming of human monocytes for Type I interferon production by GM-CSF and interferon-gamma. J. Leuk, Biol. Suppl., 1:65, 1990.

Hayes, MP and KC Zoon. Regulation of interferon alpha gene expression in human monocytes induced with lipopolysaccharide. Submitted to ISIR meeting for September 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03021-02 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Expression and Characterization of Human Interferon-α Receptor

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

PI: M.P. Hayes, Ph.D., Senior Staff Fellow, LCR, DCB, CBER
K.C. Zoon, Ph.D., Director, DCB, CBER
A.C. Larner, M.D., Ph.D., LCR, DCB, CBER
J.C. Enterline, Microbiologist, LCR, DCB, CBER
N. Nguyen, Ph.D., LCR, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cytokine Research

SECTION

INSTITUTE AND LOCATION

DCB/OBR/CBER/FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

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

The long-term goal of this study, initiated in June, 1991, is to express and characterize the protein encoded by the recently isolated cDNA for the putative Type I interferon receptor. Currently, very little is known about the biochemical and functional nature of the receptor protein. The plasmid containing the cDNA has been obtained from American Type Culture Collection. The full-length insert, coding for the interferon receptor, has been converted, by site-directed mutagenesis, into a cDNA encoding a soluble form of this receptor by changing an amino acid condon into a stop condon just prior to the transmembrane region. This insert was subcloned into a eukaryotic expression vector called pdR (obtained from T. Kishimoto, Osaka University, Japan) for expression in dihydrofolate reductase-negative Chinese hamster ovary cells (DG44, from L. Chasin, Columbia University, NY). The entire cDNA has been sequenced to confirm the changes made. Efforts to generate a soluble form of the receptor protein are underway. The protein products will be purified and used to generate both polyclonal and monoclonal antibodies to the protein. In addition, experiments will be performed to assess the capacity of this protein to bind various species of human interferon-α readily available in our laboratory. These studies should lead to an enhanced understanding of ligand-receptor interactions in the interferon system. Corollary studies are also underway to attempt expression in bacterial systems (primarily in Dr. Nguyen's laboratory) and to make antibodies to these products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03047-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

The Effect of Glutathione on IL-4 Activity

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

PI: Shu-Mei Liang, Ph.D., Research Chemist, LCR, DCB, CBER
Nancy Lee, Chemist, LCR, DCB, CBER

COOPERATING UNITS (if any)

D.S. Finbloom, M.D., Senior Investigator, LCR, DCB, CBER

LAB/BRANCH

Laboratory of Cytokine Research

SECTION

Chemical Biology Section

INSTITUTE AND LOCATION

DCB, CBER, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have previously shown that cellular glutathione (GSH) regulates the T-cell proliferative activity of Interleukin-2 (IL-2). Here, we examined whether and how GSH affects the activity of Interleukin-4 (IL-4) on murine cytotoxic T cells. CT.4R, a T cell line that is responsive to both IL-4 and IL-2, was used as a model. Althoug GSH alone had little effect on the thymidine incorporation of CT.4R cells, it enhanced the response of CT.4R to IL-4 and increased the level of thymidine incorporation up to more than 60 fold in a concentration-dependent manner. GSH affected the binding of IL-4 to cellular receptors. Scatchard plot analysis showed that GSH treatment did not change the dissociation constant significantly, however it increased the receptor number from 1173 ± 126 TO 2112 ± 492 molecules per cell. Internalization and degradation studies of IL-4 showed that the amount of IL-4 internalized and degraded in the GSH treated cells was about 2-fold higher than those in the cells without GSH treatment. These results suggest that GSH regulates the binding, internalizatio, degradation, and T-cell proliferative activity of IL-4; alteration of cellular GSH levels may thus affect the growth and replication of cytotoxic T cells through growth stimulating cytokines such as IL-2 and IL-4.

PUBLICATIONS

Liang, SM, Lee, N, Finbloom, D and CM Liang. Regulation of interleukin-4 activity on cytotoxic T-cells by glutathione. Immunology, 75:435, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 03048-01 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Effect of GSH on IL-2 Activity

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

PI: Shu-Mei Liang, Ph.D., Research Chemist, LCR, DCB, CBER
Nancy Lee, Chemist, LCR, DCB, CBER
Yang Yang Chen, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

Chi-Ming Liang, Ph.D., Laboratory Director, Molecular Oncology, Inc.

LAB/BRANCH
Laboratory of Cytokine Research

SECTION
Chemical Biology Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

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

The relationship between the actions of glutathione (GHS) and Interleukin-2 was examined on murine cytotoxic T-cells. We found that the binding, internalization, and degradation of IL-2 were regulated by the duratin of GSH treatment of CTLL-2 and CT-4R cells. Northern blot analysis of mRNA of IL-2RNA of IL-2Rp55 and IL-2Rp70, the two major components of the high affinity IL-2 receptors, showed that both the IL-2Rp55 mRNA and the IL-2Rp70 mRNA increased 6 h after GSH treatment and then declined to control level. After removing surface IL-2 receptors by trypsin treatment, we found that the reappearance of IL-2 receptors was faster and reached a higher level in GSH treated than untreated cells. GSH also shortened the half-life (from 5 h to 3 h) and thus increased the turnover of surface high affinity IL-2 receptors. These results suggest that GSH may regulate the actions of IL-2 by enhancing the synthesis and turnover of IL-2 receptors.

PUBLICATIONS

Liang, SM, Lee, N, Chen, YY and CM Liang. Effects of glutathione on the synthesis and turnover of interleukin-2 receptors. Cellular Immunology. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03049-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Structure Function Studies of Human Interleukin-2

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

PI: Yang Rong, M.D., Fogarty Fellow, LCR, DCB, CBER
Nancy Lee, M.S., Chemist, LCR, DCB, CBER
Shu-Mei Liang, Ph.D., Research Chemist, LCR, DCB, CBER

COOPERATING UNITS (if any)

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

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

We continue our studies on structure function relationship of human Interleukin-2. We have improved our techniques by developing PCR technique to do site-directed mutagenesis. We have made 11 mutants. Four of them are double substitutions or triple substitutions of cysteine residues to investigate the role of individual cys on IL-2 activity. We found that double substitution of cys at 58 and 125 positions (ala 58/125) has more activity than single substitution at 58 (ala58). This result suggested that the low activity of ala58 is probably due to the formation of wrong disulfide bond. In addition, we made several mutants by moving the cys to different positions to determine the loop size of the disulfide bond. This work is still underway.

PUBLICATIONS

Rong, Y, Chen, YY, Lee, N and SM Liang. The structure-activity study of human interleukin-2: the loop size of the disulfide bond. FASEB J, 6:A1148, 1992.

Liang, SM, Lee, N and Y Rong. The structure-activity of interleukin-2: double and triple substitutions of cysteine residues with alanine. FASEB J, 6:A1148, 1992.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03050-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Protein Engineering of the IL-2 Fusion Protein

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

PI: Shu-Mei Liang, Ph.D., Research Chemist, LCR, DCB, CBER
Yang Yang Chen, Biologist, LCR, DCB, CBER
Yang Rong, M.D., Fogarty Fellow, LCR, DCB, CBER
Nancy Lee, Chemist, LCR, DCB, CBER

COOPERATING UNITS (if any)

Elizabeth Leininger, Ph.D., Scientist LCP, DBP, CBER

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TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 0.7	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In the past 12 months, we have produced 11 mutant IL-2 proteins by site-directed mutagenesis and these mutant proteins have provided useful information as to the functional structure of IL-2. However, none of these mutant proteins can serve as a good agonist or antagonist. We are attempting to reach this goal by engineering IL-2 to fuse with another molecule to generate a multiple function molecule or to generate a more effective molecule. We have constructed an IL-2 mutant protein which has RGDS sequence added to the N terminal of IL-2. The purified mutant protein has both adhesive activity and proliferative activity. On the other hand, mutant protein with RGES added to the N terminal of IL-2 has only proliferative activity but not adhesive activity. The further characterization of these molecules is underway.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03051-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Dichotomy/Glutathione Regulation/Activation of Resting & Preactivated Lymphocytes

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

PI: Shu-Mei Liang, Ph.D., Research Chemist, CBS, LCR, CBER

COOPERATING UNITS (if any)

C.C. Ting, Senior Investigator, DCBD, NCI, NIH
Chi-Ming Liang, Ph.D., Laboratory Director, Molecular Oncology, Inc.

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TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The present study has examined the effect of GSH on two lines of IL-2-dependent activated killer cells, LAK cells and αCD3-activated killer (CD3-AK) cells. We found that GSH added during first 24 hour decreased the generation of LAK and CD3-AK cells from resting lymphocytes, whereas after 48 hours of activation, the addition of GSH increased the killer cell activity. In addition, BSO, an inhibitor of GSH biosynthesis, decreased the proliferation and cytotoxic activities of activated killer cells, and the inhibitory effect was reversed by GSH. These results indicate that GSH downregulates the generation of LAK or CD3-AK cells from resting lymphocytes, but it upregulates the further differentiation of preactivated killer cells. The effect of GSH thus varied with the state of activation of the killer cells. Culturing CD3-AK cells in GSH did not change the distribution of T cell subsets, did not affect the cells' ability to produce lymphokine (IL-2), and did not induce suppressor cells. One striking change as revealed by flow cytometry analysis was that the levels of IL-2 receptor and TCR (α/β)-CD3 were reduced by 80 and 30%, respectively, after 48 hour culturing in GSH. Determination of the mRNA of IL-2 receptor suggests that a post-transcriptional block existed. It appears that the negative effect of GSH on the function of surface IL-2 receptors or T cell receptors on resting lymphocytes severely affected the signal transduction through these receptors and thus abrogated or reduced LAK or CD3-AK cell response. In contrast, for preactivated killer cells, upregulation by intracellular GSH of IL-2 utilization is a dominant effect, thus allowing further differentiation of these killer cells. Our results indicate that the ballance between the activation signal (IL-2 or αCD3) and the immunoregulatory signal (induced by GSH) may determine the outcome of the immune response.

PUBLICATIONS

Ting, CC, Hargrove, ME, Liang, SM, Liang, CM, and SO Sharrow. Dichotomy of glutathione regulation of lymphocyte activation in preactivated and in resting lymphocytes. Cellular Immunol. 142:40-53, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03052-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Effect of Cytokines on the Release of Type IV Collagenase from Human Monocytes

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

PI: Shu-Mei Liang, Ph.D., Research Chemist, LCR, DCB, CBER

COOPERATING UNITS (if any)

Chi-Ming Liang, Ph.D., Laboratory Director, Molecular Oncology, Inc.
Kathleen A. Clouse, Senior Staff Fellow, LCR, DCB, CBER

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

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

The effect of TNF and other cytokines on the release of 72 KD (MMP-2) & 92 KD type IV collagenases (MMP-9) from monocytes was evaluated. The release of MMP-9 from THP-1 cells, a human monocytic cell line, was increased by 0.04 - 25 ng/ml TNF (for 18 hours) in a concentration-dependent manner while that of MMP-2 was only slightly increased in the presence of high concentrations of TNF (≥500 ng/ml). TNF also increased the release of MMP-9 from human primary monocytes. IL-1, like TNF, mainly enhanced the release of MMP-9. The effect of TNF was inhibited by anti-TNF but not anti-IL-1 antibodies, while that of IL-1 was inhibited by anti-IL-1 but not anti-TNF antibodies. IL-6 (25 ng/ml) slightly potentiated the release of MMP-9 but not MMP-2 from THP-1 cells. The potentiating effect of IL-6 was diminished by anti-IL-6 and anti-TNF but not anti-IL-1 antibodies, while the effects of TNF and IL-1 were not altered by anti-IL-6 antibodies. Other cytokines such as IL-4 (up to 25 ng/ml) and IFN-gamma (up to 20 ng/ml) had little effect on the release of MMP-2 or MMP-9. Incubation of the THP-1 cells with TNF in the presence of plasmin or addition of TNF to cells in higher density (1.5 x 10^5 cells/ml) induced the release of an 84 KD gelatinase thought to be the active form of MMP-9. These results indicate that TNF, IL-1, and IL-6 potentiate the collagenolytic activity of monocytes and the potentiating effect of TNF is not mediated via IL-1 or vice versa, while that of IL-6 is mediated at least in part via TNF.

IMMUNOBIOLOGY SECTION

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

PROJECT NUMBER
Z01 BD 03018-03 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Identification of EBV Proteins that Stimulate Monokine Secretion

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

PI: K. Clouse-Strebel, Ph.D., Senior Staff Fellow, LCR, DCB, CBER
K. (Weih) Faust, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

Don Hochstein, Ph.D., DPQC, FDA; Gary R Pearson, Ph.D., Prof. & Chairman, Dept. of Microbiol. and Susan Pothen, Research Asst., Georgetown University

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INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

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

We have previously shown that certain inactivated viruses, including strains of Epstein-Barr virus (EBV), can stimulate human monocyte/macrophages (MO) to secrete monokines capable of upregulating human immunodeficiency virus (HIV) expression in chronically infected human cells. Our current objective was to focus on EBV proteins to determine whether natural proteins affinity purified using specific monoclonal antibodies, are responsible for the monocyte stimulation leading to this effect. Monokine secretion has been determined in response to the EBV gp350 envelope protein, the gp125 viral capsid protein, the p50 early antigen (diffuse distribution), and the p17 early antigen (restricted distribution). Both gp350 and gp125 are capable of stimulating the secretion of monokines which upregulate HIV expression, while data using p50 and p17 are relatively inconsistent. Monokine secretion caused by the EBV glycoproteins is not attributable to contaminating endotoxin. This suggests that some, but not all, EBV proteins may indirectly affect the expression of HIV during the course of an HIV infection by stimulating monokine production. Whether this is a receptor-mediated phenomenon or whether glycosylation is involved in this response is currently under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03035-02 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Identification of the Receptor(s) on Human MO that Binds to HIV-1 gp120.

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

PI: K.A. Clouse-Strebel, Ph.D., Senior Staff Fellow, LCR, DCB, CBER
D. Finbloom M.D., Senior Investigator, LCR, DCB, CBER

COOPERATING UNITS (if any)

Larry Arthur, Ph.D., PRI/DynCorp, BRMP, NCI, FCRF

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Laboratory of Cytokine Research

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

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

Our previous studies concerning the stimulation of cytokine production from human monocytes/macrophages (MO) by the human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein have demonstrated that natural and recombinant preparations of gp120 differ in their ability to cause monokine secretion. Since those recombinant preparations of gp120 that fail to stimulate monokine production are known to bind to CD4, the major receptor for HIV, this suggests that gp120 binding to CD4 on the MO surface may of itself be insufficient to mediate this effect. Data thus far indicate that the ability of HIV-1 gp120 to cause monokine secretion is determined by primary protein structure, as well as post-translational protein modifications such as glycosylation. Whether this also suggests the existence of an alternative receptor to CD4 or a co-receptor necessary for mediating signal transduction is not clear at present. Thus, studies have been initiated to determine what molecules on the surface of human MO bind/interact with the various preparations of HIV gp120.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03036-02 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Identification of an NK Cell-derived Factor that Suppresses HIV Infection of MO

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

PI: K. A. Clouse-Strebel, Ph.D., Senior Staff Fellow, LCR, DCB, CBER
Co-P.I.: L. Mark Cosentino, D.P.M., Ph.D., DV, CBER
K. (Weih) Faust, Biologist, LCR, DCB, CBER
J. Enterline, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

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Immunobiology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have previously shown that supernatants derived from natural killer (NK) cells of HIV-seronegative individuals suppress HIV replication in human monocytes/macrophages (MO) and monocytic cell lines, but not in human T lymphocytes or T cell lines. Experiments have been undertaken to determine the nature of the factor(s) responsible for this suppression. Our results, thus far, show that the NK cell-derived supernatant contains interferon-γ (IFN-γ) and granulocyte/monocyte colony stimulating factor (GM-CSF), and that each of these cytokines alone can suppress viral replication. Sequential removal of IFN-γ (17 kD) and GM-CSF (23 kD) from the supernatant using immunoaffinity gels did not eliminate the suppressive activity. Size exclusion analysis revealed that the NK cell-derived suppressor factor has an apparent molecular weight greater than 30 kD. An equally suppressive supernatant can be generated using the human NK cell line, NK3.3 When this latter supernatant is subjected to monosulfate column chromatography, HIV suppressive activity is present in both the flow through and the eluted fractions. However, GM-CSF and IFN-γ are only detectable in the flow through, not the eluated, fraction. These data indicate that NK cells synthesize a greater than 30 kD HIV-suppressive factor distinct from IFN-γ and GM-CSF. Work is in progress to identify and characterize this potentially novel factor.

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

PROJECT NUMBER
Z01 BD 03041-01 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Inhibition of HIV Activation by Soluble Tumor Necrosis Factor Receptor

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

PI: K. Clouse-Strebel, Ph.D., Senior Staff Fellow, LCR, DCB, CBER
K. (Weih) Faust, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

W. Farrar, Ph.D., Sr. Scientist, LMI, NCI, NIH; O.M.Z. Howard, PH.D., Scientist, PRI/DynCorp, NCI, FCRF

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DCB, CBR, CBER, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

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

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

The inflammatory cytokine, tumor necrosis factor-α (TNF-α), has been shown to stimulate HIV-1 replication in both chronically and acutely infected T-lymphocytes and monocytes. The activation of the HIV-Long Terminal Repeat (HIV-LTR) and subsequent increase in virus production is linked to TNF activation of the cellular transcription factor, NF-kB. We have tested the ability of two forms of soluble recombinant type 1 (p80) TNF receptor to inhibit TNF-induced HIV activation in vitro. One form of the receptor is a monomer containing the entire 234 residues of the extracellular (ligand-binding) portion of p80. A second form is a homodimer chimeric protein containing these same residues fused to a truncated human IgG(1) immunoglobulin chain, and thus resembles a bivalent antibody without light chains. These recombinant proteins were tested for their ability to inhibit TNF-α-induced expression of HIV-1 in chronically infected human cell lines as determined by viral reverse transcriptase activity. We also examined the ability of the soluble receptors to limit the activation of HIV-LTR transcription. The soluble TNF receptor dimer was most effective at blocking the TNF-α-induced expression in both monocytic and lymphocytic cell lines. The ratio of receptor to TNF-α was critical, with optimal inhibition requiring a 10-fold excess of receptor. Monomeric receptor was less effective at blocking and, at times, was capable of augmenting the activity of TNF-α. Thus, our data suggests that TNF-α-induced HIV-1 expression can be limited in vitro using a dimeric form of the TNF receptor at specific ratios in excess of TNF-α.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BD 03042-01 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Stimulation of Monocytic Endothelin-1 Production by HIV-1 gp120

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

PI: K. Clouse-Strebel, Ph.D., Senior Staff Fellow, LCR, DCB, CBER
K. (Weih) Faust, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

H. Ehrenreich, M.D., DVM, Visiting Scientist, LIR, NIAID, NIH
A.S. Fauci, M.D., Director, LIR, NIAID, NIH

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INSTITUTE AND LOCATION
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TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

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

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

Monocytes/macrophages play a critical role in the pathogenesis of human immunodeficiency virus (HIV)-infection, both as targets for virus replication and sources of production of multifunctional cytokines. Endothelins, peptides with potent vasoconstricting activities, originally isolated from endothelial cells, are also produced and secreted by macrophages in a manner similar to that of other cytokines. In an attempt to explore the potential role of endothelins in HIV-infection, we investigated the effect of the HIV-1 envelope glycoprotein, gp120, on monocytic endothelin-1 production. This glycoprotein has been identified as a potent stimulator of monokines such as TNF-α and interleukin-6, which have been implicated as potential mediators of HIV-encephalopathy. We found that gp120, similar to LPS, stimulates the secretion of endothelin-1 as well as TNF-α from macrophages in a concentration-dependent manner. Using reverse transcriptase polymerase chain reaction (RT PCR), we found that circulating monocytes in HIV-infected individuals show a distinct expression of the endothelin-1 gene which is not detectable in healthy controls, indicating chronic activation of this gene in HIV-infection. In addition, cerebral macrophages in patients with HIV-encephalopathy were strongly positive for endothelin. Thus, monocytic endothelins appear to be stimulated during HIV-infection. Their potent vasoactive properties render them potential candidates for mediating alterations in the cerebral perfusion pattern associated with the AIDS dementia complex.

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

PROJECT NUMBER
Z01 BD 03044-01 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (180 characters or less. Title must fit on one line between the borders.)
Thrombin is a Regulator of Astrocytic Endothelin-1

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

PI: H. Ehrenreich, M.D., D.V.M., Visiting Scientist, LCR, DCB, CBER
K. Clouse-Strebel, Ph.D., Senior Staff Fellow, LCR, DCB, CBER

COOPERATING UNITS (if any)

T. Costa, M.D., Y. Ogino, M.D., LTBP, NICHD, NIH; R. Pluta, M.D., SNB, NINDS, NIH; J.E. Coligan, Ph.D., BRB, NIAID, NIH

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TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Endothelin-1 is produced by cultured primary rat astrocytes and is subject to autostimulatory regulation. In this study, we examine the response of these cells to stimulation by thrombin and report that endothelin-1 is released into the culture fluid in response to thrombin treatment. However, increased production of endothelin-1 is not accompanied by a concomitant increase in steady state levels of endothelin-1 mRNA as assessed by reverse transcriptase PCR, even though thrombin stimulation leads to increased turnover of phospholipid and activation of the nuclear factor AP1. Both endothelin receptor genes (ET-A and ET-B) were found to be transcribed in primary astrocyte cultures and both thrombin and endothelin-1 stimulation result in a distinct temporary decrease in ET-A mRNA. We conclude that astrocytic production of endothelin-1 may be post-transcriptionally regulated in response to thrombin stimulation. These studies suggest a role for thrombin in endothelin-1 mediated neurological processes.

PHS 6040 (Rev. 5/92)

PUBLICATIONS

Clouse, KA, Cosentino, LM, Weih, KA, Pyle, SW, Robbins, PB, Hochstein, HD, Natarajan, V and WL Farrar. The HIV-1 gp120 envelope protein has the intrinsic capacity to stimulate monokine secretion. J. of Immunol., 147(9):2892, 1991.

Farrar, WL, Korner, M and KA Clouse. Cytokine regulation of HIV expression. Review article. Cytokine, 3(6):531, 1991.

Ehrenreich, HE, Costa, T, Clouse, KA, Pluta, RM, Ogino, Y, Coligan, JE and PR Burd. Thrombin is a regulator of astrocytic endothelin-1. Brain Research, 1992. (in press)

Goebel, FD, Rolinski, B, Rieckmann, P, Sinowatz, F, Geier, S, Clouse, KA, Kronawitter, U, Bogner, JR, Klauss, V and H Ehrenreich. In: HIV-1 and the central nervous system - Clinical, pathological and molecular aspects. Satellite Symposium of the European Neuroscience Association Meeting. Weis, S and H. Hippius, eds., Hogrefe and Huber, Publishers. 1992. (in press)

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

PROJECT NUMBER
Z01 BD 03012-03 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of M-CSF Message Expression and M-CSF Secretion by Human Monocytes

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

PI: Marion Gruber, Ph.D, Senior Staff Fellow, LCR, DCB, CBER
Theresa Gerrard, Ph.D., Supervisory Microbiologist, Chief, LCR, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cytokine Research

SECTION
Immunobiology Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

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

We have previously investigated the regulation of M-CSF production from human monocytes induced by various cytokines. The results of these studies have been published. We have expanded this work and have examined the ability of antibodies to the surface antigens: CD45, CD44 and LFA-3 to stimulate M-CSF production. Immobilized antibodies to all three antigens were able to induce M-CSF mRNA while antibodies to other surface proteins did not. F(ab)2 fragments of a CD45 antibody were used to demonstrate that this process did not require Fc binding. Although these same antibodies also induced some measurable M-CSF release, second signals provided by LPS or IL-1β greatly augmented the M-CSF production when added to the cultures. LPS or IL-1β alone were not able to currently investigating the molecular mechanisms involved in the induction of M-CSF message in human monocytes costimulated with CD45 and IL-1β. Preliminary data suggest that for the IL-1β-induced enhancement of M-CSF message of CD45 stimulated monocytes, both transcriptional and posttranscriptional mechanisms are required. M-CSF message induced by these stimuli is not blocked by CHX, indicating that protein synthesis is not required. In addition, we have initiated studies to investigate the role of CD45 in c-fms expression.

PUBLICATIONS

Gruber, MF, Webb, DSA and TL Gerrard. Stimulation of human monocytes via CD45, CD44 and LFA-3 triggers M-CSF production:Synergism with LPS and IL-1. J. Immunol., 148:1113, 1992.

Gruber, MF, Hewlett, IK, Sims, T, Vujcic, L, Manischewitz, J and H. Golding. Study of HIV-1 viral entry and replication in infected subclones of a human CEM T cell line reduced in their ability to form syncytia. Aids Res. Hum. Retrovirus, Vol. 8(6):729, 1992.

PUBLICATIONS IN PRESS

Gruber, MF and TL Gerrard. Production of macrophage colony-stimulating factor (M-CSF) by human monocytes is differentially regulated by GM-CSF, TNFα and IFNγ. Cell. Immunol. (in press).

Gruber, MF, Webb, DSA and TL Gerrard. Engagement of the CD45, CD44 or LFA-3 antigens induces M-CSF production by human monocytes. J. Leuc. Biol. Suppl. 2, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03055-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Regulation of IL-1 and IL-1 Receptor Antagonist Expression by IL-4

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

PI: R.P. Donnelly, Ph.D., Senior Staff Fellow, LCR, DCB, CBER

COOPERATING UNITS (if any)

M.J. Fenton, Ph.D., Asst. Prof., Dept. of Medicine and J.A. Buras, Research Asst., Dept. of Medicine., Boston University School of Medicine.

LAB/BRANCH

Laboratory of Cytokine Research

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DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In this project, we evaluated the effects of the T cell-derived lymphokine, IL-4, on the ability of human monocytes to express IL-1 and the IL-1 receptor antagonist (IL-1ra), a new member of the IL-1 gene family. Activation of human monocytes with lipopolysaccharide (LPS) induces coordinate expression of a number of proinflammatory cytokine genes, including IL-1α, IL-1β, TNF-α, IL-6 and IL-8. IL-4 inhibits expression of these genes in monocytes, suggesting that it may be an important physiologic regulator of cytokine production. We have previously shown that IL-4 reduces steady-state mRNA levels for IL-1β in human monocytes by decreasing both IL-1β transcription and the half-life of newly formed IL-1β mRNA transcripts. We have now extended these findings to show that IL-4 similarly accelerates the turnover of IL-6 mRNA in LPS-stimulated monocytes. However, this inhibition of cytokine expression and dramatic increase in the decay rate of cytokine mRNA does not extend to all LPS-inducible genes because IL-4 treatment did not inhibit expression or accelerate turnover of mRNA for IL-1ra in the same cells. Although IL-1β and IL-1ra are both LPS-inducible genes, they displayed distinct temporal patterns of expression. Peak steady-state mRNA levels for IL-1ra lagged significantly behind that of IL-1β, suggesting a possible endogenous mechanism for limiting IL-1 biologic activity. Furthermore, although IL-4 suppressed expression of both IL-1β and IL-6, it upregulated synthesis of IL-1ra mRNA and protein. Thus, IL-4 inhibits production of the proinflammatory cytokine IL-1β while increasing synthesis of IL-1ra in activated human monocytes. This pathway may have evolved to provide a mechanism by which the host can effectively regulate both the production and activity of this potent proinflammatory mediator. Furthermore, these findings provide additional support for the potential therapeutic use of IL-4 in disease states which are characterized by excessive production of IL-1.

PUBLICATIONS

Donnelly, RP. Regulation of human monocyte activation by interleukin-4. In: Early Decision in DMARD Development II. Biologic Agents in Autoimmune Disease. (Strand, V, Amento, EP and C Scribner, eds.) Arthritis Foundation, Atlanta, pp. 47-54, 1991.

Fenton, MJ, Buras, JA and RP Donnelly. IL-4 reciprocally regulates IL-1 and IL-1 receptor antagonist expression in human monocytes. J. Immunol., 149:1283, 1992.

Fenton, MJ, Buras, JA and RP Donnelly. Posttranscriptional regulation of cytokine and cytokine antagonist expression. Cytokine, 3:452, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03053-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Isolation and Identification of the LPS Binding Proteins on Human Monocytes

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

PI: D.S.A. Webb, Ph.D., Staff Fellow, LCR, DCB, CBER
H.S. Mostowski, Biologist, LCI, DCB, CBER
T.L. Gerrard, Ph.D., Supervisory Microbiologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cytokine Research

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INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

By utilizing two different approaches, we have isolated several proteins which may serve as LPS binding proteins on human monocytes. One method used was to add LPS to 35-S-methionine labeled monocytes, lyse the cells and then, using an anti-LPS antibody, immunoprecipitate the LPS along with any bound monocyte proteins. We have found two proteins, one greater than 200 kd and one approximately a 100 kd in site. The greater than 200 kd protein has been identified by Western blot analysis as CD45. The 110 kd protein has not yet been identified, but we have found that its expression is enhanced by pretreatment with IFN-γ. The second approach used involved covalently linking LPS to CNBr-activated sepharose beads. The beads are then added to a lysate of 35-S-methionine labeled monocytes and washed several times to remove proteins which do not bind the LPS. Using this technique, we have found two additional proteins which are approximately 70 and 90 kd. Work is currently in progress to identify all these proteins and to verify their role in the LPS induced activation of human monocytes.

PUBLICATIONS

Webb, DSA. Stimulation of human monocytes via CD45, CD44 and LFA-3 triggers macrophage-colony-stimulating factor production. Synergism with lipolysaccharide and IL-1p. J. Immunology, 148:1113, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03054-01 LCR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

IL-1 Release by Human Monocytes

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

PI: D.S.A. Webb, Ph.D., Staff Fellow, LCR, DCB, CBER
M. Hayes, Ph.D., Staff Fellow, LCR, DCB, CBER
T.L. Gerrard, Ph.D., Supervisory Microbiologist, Chief, LCR, DCB, CBER

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1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

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

During the course of this work, we have found that pretreatment of human monocytes with M-CSF results in an inhibition of the release of IL-1 from LPS stimulated human monocytes. Northern blot analysis revealed that M-CSF does not alter the LPS induction of IL-1 message. In addition, immunoprecipitations performed using an antibody which binds both the pro and processed forms of IL-1, indicate that the message is translated into intracellular IL-1. However, it appears the intracellular protein is not effectively released from the cells. We plan to use these findings to examine the events involved in IL-1 release from human monocytes and to determine, by use of immunohistochemical staining whether all monocytes stimulated with LPS produce IL-1 or whether only a subpopulation of monocytes is capable of IL-1 production.

BIOCHEMICAL IMMUNOLOGY SECTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 03019-04 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Role of Cytokines and Phagocytic Cells in Inflammation

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

PI: David S. Finbloom, M.D., Senior Investigator, LCR, DCB, CBER
Karen D. Winestock, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

L.M. Wahl, Ph.D., Senior Investigator, LMI, NIDR
G. Garotta, Ph.D., Professor, Hoffmann LaRoche, Basel, Switzerland

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INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

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

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

We have continued to examine the structure of the receptor for Interferon-γ (IFN-γ). We have characterized 2 subunits (Mr of 38 Kd and 200 Kd) by SDS-PAGE analysis of biosynthetically labelled human monocytes. These subunits are in addition to the previously cloned IFN-γ binding protein (Mr, 80-90kD). We are now beginning to preparatively purify these subunits so that eventually antibodies against them can be raised. We have also continued to investigate whether or not somatic cell hybrids expressing combinations of the transfected IFN-γ binding protein and chromosome 21 exhibit the ability to co-immunoprecipitate the 38 and 200 Kd subunits.

Another project pursues an understanding of the role of phosphorylation of the IFN-γ receptor during signal transduction. When exposed to the phorbol ester, PMA, THP1 cells, a monocyte-like cell line, now become capable of responding to IFN-γ as measured by the induction of the gene for IP-10, an IL-8/PF4 family member. The mechanisms underlying the development of competency in these cells for IFN-γ responsiveness is under investigation. Whether or not phosphorylation by various kinases or dephosphorylation by phosphatases are involved in this mechanism is still under study. We are also studying the ability of IFN-γ to induce phosphorylation of the IFN-γ receptor on human monocytes. Using a phosphatase inhibitor, such as okadaic acid, we have been able to measure phosphorylation of the receptor. We are determining if this is necessary for signal transduction as measured by the expression of mRNA for the IP-10 and the FcγRI genes.

We are further examining the mechanisms underlying the ability of IL-4 to inhibit many of the effects of IFN-γ and IFN-α. DNA sequences in the promotor regions of IFN-inducible genes will be identified and tested for their role in the inhibitory effect of IL-4 on IFN-induced gene expression.

PUBLICATIONS

Finbloom, DS. FDA perspective: the ability of preclinical models to either safety or efficacy. In: Early Decisions in DMARD Development II. Biologic Agents in Autoimmune Disease. Amento and Strand, eds. Arthritis Foundation, 1991.

Finbloom, DS, Wahl, LM and KD Winestock. The receptor for interferon-γ on human peripheral blood monocytes consists of multiple distinct subunits. J. Biol. Chem., 266:22545, 1991.

Feldman, GM, Ruhl, S, Bickel, M, Finbloom, DS and DH Pluznik. Regulation of interleukin-4 receptors on promonocytes by the differentiation factor interleukin-6. Blood, 78:1678, 1991.

Liang, S-M, Lee, N, Finbloom, DS and C-M Liang. Regulation by glutathione of interleukin-4 activity on cytotoxic T cells. Immunology, 75:435, 1992.

Montinaro, V, Aventaggiato, L, Esparza, A, Chen, A, Finbloom, DS and Abdalla Rifai. Extrarenal cytokines modulate the glomerular response to IgA immune complexes. Kidney International, 1992. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 BD 03040-01 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analysis of the Mechanism of IL-4R Induction

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

PI: G.M. Feldman, Ph.D., Senior Staff Fellow, LCR, DCB, CBER

COOPERATING UNITS (if any)

David Finbloom, M.D., H. Dov Pluznik, Ph.D., Stefan Ruhl, D.D.S., DCB, CBER; Barry Davidson, Immunex

LAB/BRANCH
Laboratory of Cytokine Research

SECTION
Biochemical Immunology Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

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

This project is focused on the regulation of interleukin-4 receptor (IL-4R) expression on hematopoietic progenitor cells. Using the murine myeloid leukemia M1 line and mature, bone marrow-derived macrophages, neither of which express IL-4R, we have previously characterized the ability of IL-6 and/or interferon-γ (IFN-γ) to induce the expression of IL-4R. We are now continuing with these studies to determine the mechanism of this induction. To this end, we have obtained the putative promoter sequence for the IL-4 receptor gene and are trying to transfect hematopoietic cells with these constructs to localize the promoter region responsible for IL-6 and IFN-γ sensitivity.

In a parallel series of studies, we have determined that although both IFN-γ and IL-6 are individually capable of inducing IL-4R, they have an antagonistic effect on each other when combined. We are currently pursuing studies to characterize and understand the nature of this antagonistic effect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03028-02 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on the Expression and Regulation of Soluble IL-4R

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

PI: G.M. Feldman, Ph.D., Senior Staff Fellow, LCR, DCB, CBER

COOPERATING UNITS (if any)

Patricia Beckmann, Ph.D., Immunex, Seattle; H. Dov Pluznik, Ph.D. and Stefan Ruhl, D.D.S., DCB, CBER

LAB/BRANCH
Laboratory of Cytokine Research

SECTION
Biochemical Immunology Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

In previous studies, we have examined the function and regulation of the soluble form of the IL-4R. Using a cDNA probe that is specific for the soluble IL-4 receptor, we have shown that resting bone marrow-derived macrophages or M1 cells do not express detectable levels of soluble IL-4R unless stimulated with either IL-6 or IFN-γ. When stimulated, they undergo a rapid upregulation of soluble IL-4R expression at both the level of the mRNA and at the level of the protein. Studies are currently underway to determine the mechanism of this induction of soluble IL-4R, and to determine the physiologic role that soluble IL-4R may play in the immune response.

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

PROJECT NUMBER
Z01 BD 03020-04 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanisms of IFN-regulated Gene Expression

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

PI: A. Larner, M.D., Ph.D., Senior Investigator, LCR, DCB, CBER
E. Petricoin, Ph.D., Ph.D., NRC Fellow
R. Hackett, Ph.D., Staff Fellow, LCR, DCB, CBER
M. David, Ph.D., Visiting Fellow, LCR, DCB, CBER

COOPERATING UNITS (if any)

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DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

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

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

Treatment of a variety of cultured human cells with Type I interferon (IFNs) induces a rapid increase in the rate of transcription of several cellular genes. This lab is interested in the mechanism by which IFNs are capable of activating and then subsequently inhibiting the expression of such genes.

Phorbol ester-mediated inhibition of IFN-induced ISG54K expression: The following facts concerning the inhibitory effects of phorbol esters on IFN-activated ISG54K expression have been established. The actions of phorbol esters are reversed by inhibitors of protein synthesis. The mechanisms by which long term IFN treatment of cells down regulates ISG54 gene expression are distinct from those by which phorbol esters inhibit ISG54 expression. We have mapped two regions within the ISG54 gene that allow phorbol esters to repress IFN-activated gene expression. One is region at the cap site and one is the interferon-stimulated response element (ISRE). IFN-induced proteins that bind the ISRE are also inhibited by phorbol esters. We are presently purifying a protein which destroys the ISGF3 transcription complex from all PMA treated cells. We have recently developed a cell free system where IFNα can activate the formation of ISGF3 in vitro. This system has enabled us to demonstrate that the component of the ISGF3 transcription complex which is modified by IFNα (ISGF3α) is associated with the plasma membrane, and its activation involves a protein kinase. Using a combination of specific tyrosine kinase and phosphatase inhibitors, and monoclonal antiphosphotyrosine antibodies, we now are able to demonstrate that IFNα activated transcription involves at least a two step process where a tyrosine phosphatase and a tyrosine kinase lead to modification of ISGF3α and subsequent formation of the complete complex. The ISGF3 complex is specifically disrupted by protein tyrosine phosphatase and can be reversibly dissociated by the phosphotyrosine analogue phenylphosphate. This suggested that SH2 and/or SH3 domains may be required for the stable formation of this transcription complex.

PUBLICATIONS

Nakagawa, Y, Rivera, V and AC Larner. A role for the Na/K ATPase in the control of human c-fos and c-jun transcription. J. Biol. Chem., 267:8785, 1992.

Nakagawa, Y, Akai, H, Rupp, B, Grimley, P and AC Larner. Interferon induced gene expression: Evidence for a selective effect of ouabain on activation of the ISGF3 transcription complex. Virology. (in press)

Petricoin, E, Hackett, R, Akai, H, Igarashi, K, Finbloom, DS and AC Larner. Coordinated mechanisms by which phorbol esters modulate interferon signaling in human fibroblasts. Mol. Cell. Biol. (in press)

David, M and AC Larner. Activation of transcription factors by interferon α in a cell free system. Science. (in press)

HEMATOPOIETIC GROWTH FACTORS SECTION

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

PROJECT NUMBER
Z01 BD 03038-01 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biosynthesis of Hematopoietic Growth Factor

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

PI: D.H. Pluznik, Ph.D., Research Microbiologist, LCR, DCB, CBER
R.B. Cohen, M.D., Senior Staff Fellow, LCB, DCR, CBER
Y. Iwai, M.D., Ph.D., Fogarty Fellow, LCR, DCB, CBER
K. Akahane, Ph.D., Guest Worker, LCR, DCB, CBER
S.F. Dougherty, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

M. Bickel, D.D.S., University of Bern, Bern, Switzerland

LAB/BRANCH
Laboratory of Cytokine Research

SECTION
Hematopoietic Growth Factors Section

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	2.8	0.3

CHECK APPROPRIATE BOX(ES)

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

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

Stimulation of T and B cells by various agents is followed by expression of the GM-CSF gene in T cells. In T cells, we have shown that the expression of this gene in these cells is post-transcriptionally controlled mainly by stabilization of the mRNA. The mechanism of mRNA degradation and stabilization is being examined using murine EL-4 thymoma cells as a model system for T cells, and murine M12.4.1 lymphoma cells as a model system for B cells.

Induction of GM-CSF mRNA in EL-4 cells by the Ca++-ionoiphore A23187 occurs by stabilization of the mature transcript in the cytoplasm. Using CAT constructs in which portions of the GM-CSF 3 prime UTR have been substituted for the SV40 poly adenilation signals and transfection of EL-4 cells, we have determined which sequences in the GM-CSF mRNA regulate degradation and stabilization. The AU-rich elements in the 3 prime UTR are principally responsible for mRNA degradation. mRNA stabilization is mediated by two regions within the GM-CSF mRNA half-life. Using PCR, we have produced linker-substitution mutations of the GM-CSF 3 prime UTR and have mapped the A23187-responsive elements of the 3 prime UTR to the AU-rich boxes and to a 60 bp region upstream from the AU-rich area. The 60 bp region is identical to the phorbol ester TPA-responsive element (see 1990-91 Annual Report).

Using PCR analysis of nuclear RNA with probes specific for GM-CSF intron sequences, we found that induction of GM-CSF mRNA in M12.4.1 cells by IL-1 occurs by stabilization of the nuclear precursor RNA followed by stabilization of the mature transcript in the cytoplasm. IL-4 inhibits the IL-1 induced production of GM-CSF in M12.4.1 cells. This inhibition is not regulated by changes in the transcription rate of GM-CSF gene. IL-4 causes only a slight decrease in the half-life of the mature transcript in the cytoplasm. The inhibitory effect of IL-4 is mainly due to intranuclear destabilization of the primary transcripts of GM-CSF.

PHS 6040 (Rev. 5/92)

PUBLICATIONS

Iwai, Y, Bickel, M, Cohen, RB and DH Pluznik. Concanavalin A induced granulocyte macrophage colony stimulating factor production in a murine T cell line is post-transcriptionally controlled. Exp. Hematol., 20:271, 1992.

Akahane, K, and DH Pluznik. Interleukin-4 inhibits interleukin-1α induced granulocyte-macrophage colony stimulating factor gene expression in a murine B lymphocyte cell line via downregulation of RNA precursor. Blood, 79:3188, 1992.

Bickel, M, Iwai, Y and DH Pluznik. Binding of sequence-specific proteins to the adenosine-uridine (AU)-rich sequences of the murine granulocyte macrophage colony stimulating factor (GM-CSF) mRNA. Proc. National Acad. Sci. (in press)

Akahane, K and DH Pluznik. Regulation of IL-1-induced GM-CSF mRNA expression in a murine B cell line. The 1992 FASEB J., part II, p. A2053.

Bickel, M, Iwai, Y, Cohen, RB and DH Pluznik. Binding of proteins to adenosine-uridine (AU)-rich sequences regulates the stability of granulocyte macrophage colony stimulating factor (GM-CSF) mRNA. 8th Intl. Congress of Immunol., Budapest, 1992.

Iwai, Y, Cohen, RB and DH Pluznik. Two distinct pathways, dependent and independent of Ca++ are involved in granulocyte macrophage-colony stimulating factor mRNA stabilization in murine T cells. Exp. Hematol., 20:800, 1992.

Akahane, K and DH Pluznik. Interferon-γ destabilizes granulocyte macrophage colony-stimulating factor mRNA induced by interleukin-1 in murine endothelial cells. Exp. Hematol., 20:756, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 03039-01 LCR

PERIOD COVERED
October 1, 1991 through September 30, 1992

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

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

PI: D.H. Pluznik, Ph.D., Supervisory Microbiologist, LCR, DCB, CBER
K. Akahane, Ph.D., Guest Worker, LCR, DCB, CBER
S. B. Midura, Microbiologist, LCR, DCB, CBER
S.F. Dougherty, Biologist, LCR, DCB, CBER

COOPERATING UNITS (if any)

Stefan Ruhl, D.D.S., NIDR, NIH; Stefan Karlsson, M.D., Ph.D., NINDS, NIH

LAB/BRANCH
Laboratory of Cytokine Research

SECTION
Hematopoietic Growth Factors

INSTITUTE AND LOCATION
DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.6	PROFESSIONAL: 1.3	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

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

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

Leukocyte integrins which share the $\beta 2$ subunit CD18 are involved in cell adhesion to extracellular matrix and also mediate cell-cell interactions. To examine changes in integrin expression on differentiating myeloid cells the M1, an immature murine myeloid leukemic cell line, was used. Upon stimulation with interleukin-6 (IL-6), M1 cells express surface markers, enzymes and functions of normal, mature macrophages. By FACS analysis, the α subunits CD11a (LFA-1) and CD11b (Mac-1) plus CD18 expression on IL-6 stimulated M1 cells were found to increase in a time dependent fashion reaching levels expressed on normal mature macrophages. The increase in integrin expression was correlated with an increase in the adhesion of IL-6 stimulated M1 cells to culture dishes. Our study shows that the expression of both the α and β subunits of the leukocyte integrin family are increased during differentiation due to de-novo synthesis, similar to other markers of myeloid differentiation. Furthermore, it also shows that CD11b and CD18 are transcriptionally controlled while the expression of CD11a is post-transcriptionally regulated.

Gaucher's disease is a lipid storage disorder caused by a deficiency in the enzyme glucocerebrosidase (GC). The human GC gene has been successfully transfected into murine BM stem cells which have been transplanted into lethally irradiated mice. Macrophages from these reconstituted mice, evaluated 6 months after transplantation, express the human GC protein. To develop a quick screening method for testing the retroviral vector expression of the GC gene in macrophages and to study the effect of differentiation on this gene expression, we used the M1 cell line. Transduced M1 clones with the Molony virus containing the GC cDNA were analyzed for the expression of the vector generated human GC RNA and protein before and after induction of differentiation with IL-6. Our data show that the viral LTR from the Molony virus efficiently promotes the expression of human GC RNA before and after induction of differentiation of the transducted M1 clones.

DIVISION OF CYTOKINE BIOLOGY

LABORATORY OF IMMUNOLOGY

IMMUNOBIOLOGY SECTION

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

PROJECT NUMBER
Z01 BD 04002-04 LMI

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of B Cell Growth by Epstein Barr Virus

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

PI: G. Tosato, M.D., Laboratory Chief, LI, DCB, CBER
S.E. Pike, Microbiologist, LI, DCB, CBER
K. Jones, Microbiologist, LI, DCB CBER

COOPERATING UNITS (if any)
S. Markey, NIMH, NIH
C. Ijames, NIMH, NIH

LAB/BRANCH
Laboratory of Immunology

SECTION
-

INSTITUTE AND LOCATION
DCB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 3	PROFESSIONAL: 2.9	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

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

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

Epstein-Barr virus (EBV) infects human B cells and induces their immortalization. The molecular basis for EBV immortalization is largely unknown. Recently, it was observed that when EBV immortalized cells are cultured without serum at critically low densities, proliferation ceases and the cells die. However, addition of cell-free supernatants from exponentially growing EBV-immortalized cell lines can rescue these cells from death and promote their proliferation. Recently, we have identified IL-6 as being one of the autocrine growth factors for EBV-immortalized B cells (Tosato, G. et al, J. Virol., 1990) However, only approximately 25% of autocrine growth factor activity is attributable to IL-6 while the remaining autocrine growth factor activity resides in a low molecular weight component.

We sought to identify this low molecular weight activity. The purification was accomplished, sequentially, by gel filtration chromatography, exchange chromatography, adsorption and gel permeation chromatography, reverse phase HPLC and hydrophylic-interaction HPLC. The material purified in this manner was analyzed by mass spectrometry. Two major components were identified as lactic acid (LA) and oxalic acid. Synthetic LA stimulated growth in EBV-immortalized B cells at 1 to 10 mM, a concentration of LA achieved in the culture supernatant of EBV-immortalized cell lines. This molecule alone was found to account for all the low molecular weight autocrine growth factor activity in supernatants of EBV-immortalized B cells. Thus, LA is an autocrine stimulatory molecule that induces the proliferation of EBV-immortalized B cells.

PUBLICATIONS

Pike, SE, Markey, SP, Ijames, C, Jones, KD and G Tosato. The role of lactic acid in autocrine B-cell growth stimulation. Proc Natl Acad Sci, USA, 88L:11081, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04003-04 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Immunoregulation of EBV-infected B Lymphocytes

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

PI: G. Tosato, M.D., Laboratory Chief, LI, DCB, CBER
S. Pike, Microbiologist, LI, DCB, CBER

COOPERATING UNITS *(if any)*

S. Straus, M.D. and J. Cohen, M.D., NAID, NIH
M. Blaese, M.D., NCI, NIH

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

DCB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.3	PROFESSIONAL: 1.8	OTHER: .5

CHECK APPROPRIATE BOX(ES)

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

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

Epstein-Barr virus latently infects most normal adult individuals worldwide. The mechanisms by which this occurs are incompletely understood. A critical role for T cell immunity is suggested by the observation that severe T cell immunodeficiency is associated in vivo with the occurrence of polyclonal and oligoclonal proliferations of B lymphocytes naturally infected with EBV. In vitro, removal or inactivation of T lymphocytes results in the generation of EBV-immortalized cell lines. T cell specific cytotoxicity, natural cytotoxicity, suppression and secretion of inhibitory molecules have all been shown to inhibit EBV-infected B cells in vitro and are believed to contribute to their control in vivo. In the present study, T-lymphocytes were found to proliferate in response to cell-free supernatants of EBV-immortalized B cells and to deplete EBV-immortalized cells of the growth factors they require for autocrine growth. Interleukin-6 was found to be one of the autocrine growth factors produced by EBV-immortalized B cells that T cells utilize. As a consequence of T cell utilization of growth factors produced by EBV-immortalized B cells and required by the virally infected cells for continuous growth, B cell suppression occurs. Thus, T cell competition for growth factors may represent an important and novel regulatory mechanism for maintenance of EBV latency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04014-02 LMI

PERIOD COVERED

October 1991 through September 30, 1992

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

Interleukin-6: A Transcription Activating Cytokine.

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

PI: J. Tanner, Ph.D., LI DCB, CBER
G. Tosato, M.D., Laboratory Chief, LI, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

-

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Interleukin-6 (IL-6), a 20-26 kDa phospho-glycoprotein whose gene has been cloned and sequenced in a variety of heterologous systems, has been reported to induce Ig secretion in activated B cells, acting as a growth and differentiation factor for these cells.

Because both the Ig genes and their regulatory elements have been characterized in detail, study of the molecular mechanisms governing Ig secretion induced by IL-6 provides an ideal model system for investigation. Recently, IL-6 was reported to transcriptionally activate γ_1 heavy chain and λ light chain genes in a subclone of the lymphoblastoid cell line CESS that was selected on the basis of high density IgG1 surface expression.

The purpose of the present study was to investigate further the molecular basis for Ig production induced by IL-6. Using a panel of B cell lines obtained by EBV immortalization of normal human B lymphocytes, we confirmed that IL-6 stimulates Ig gene transcription, causing significant increases in the amounts of mRNA per cell. We further showed that IL-6 induces expression of the growth responsive genes c-myc and actin, followed by increases in DNA, RNA and protein synthesis as well as cell numbers. Thus, IL-6 does not selectively activate Ig transcription but acts as a B cell stimulatory factor transcriptionally activating several growth responsive genes.

PUBLICATIONS

Tanner, J and G. Tosato. Regulation of B cell growth and immunoglobulin gene transcription by Interleukin-6. Blood, 79:452, 1992.

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

PROJECT NUMBER
Z01 BD 04020-01 LMI

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Interleukin 6 Serum Levels in Solid Organ Transplant Recipients

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

PI: G. Tosato, M.D., Laboratory Chief, LI, DCB, CBER
K. Jones, Microbiologist, LI, DCB, CBER
Y. Taga, M.D., Visiting Scientist, LI, DCB, CBER

COOPERATING UNITS (if any)

J. McKnight, Ph.D., Univ. of Pittsburg; M. Ho, M.D., Univ. of Pittsburg, M.K. Breining, R.N., Univ. of Pittsburg

LAB/BRANCH
Laboratory of Immunology

SECTION
-

INSTITUTE AND LOCATION
DCB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

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

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

Recipients of solid organ transplants develop lymphoproliferative disorders of Epstein-Barr virus (EBV)-infected B cells. In addition, interleukin-6 (IL-6) promotes growth of EBV-infected B cells in vitro and in vivo. To study a potential role of IL-6 in the development of post-transplant lymphoproliferative disorders (PTLD), we have measured IL-6 levels in serum samples of 14 solid organ transplant recipients. Six of these patients who did not develop PTLD had normal IL-6 levels (5-15 U/ml) for 45 to 215 days post transplant. In contrast, 6 of 7 patients who developed PTLD had abnormally elevated serum IL-6 levels (85-1040 U/ml)at various timepoints post transplant (5-310 days). In all but one of these 7 cases, serum IL-6 levels were abnormally elevated before (3-45 days) PTLD was diagnosed. One patient with microscopic PTLD of the transplanted kidney had relatively normal serum IL-6 levels (18 U/ml). Finally, one of the 14 patients who died of sepsis 17 days post transplant had abnormally high IL-6 levels (50-103 U/ml) prior to death. Mean IL-6 levels in the culture supernatants of 5 single cell suspended PTLD lesions were greater than 1500 U/ml, while mean IL-6 levels in culture supernatants of peripheral blood of patients with acute infectious mononucleosis were less than 15 U/ml. Together, these findings demonstrate that, generally, IL-6 levels are abnormally elevated in sera of PTLD patients, and suggest that IL-6 may have a role in the pathogenesis of PTLD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04021-01 LMI

PERIOD COVERED

October 1, 1991 through Septenber 30, 1992

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

Regulation of Death in EBV Immortalized Cells

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

PI: B. Cherney, Ph.D., Staff Fellow, LI, DCB, CBER
G. Tosato, M.D., Laboratory Chief, LI, DCB. CBER

COOPERATING UNITS (if any)

K. Bathia, Ph.D., POB, NCI, NIH
I.T. Magrath, M.D., POB, NCI, NIH

LAB/BRANCH

Laboratory of Immunology

SECTION

-

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Despite the fact that EBV-immortalized B cells are capable of continuous growth when cultured under optimal culture conditions, they die when incubated at sufficiently low cell densities. We have examined the process of death in EBV-immortalized cells cultured at critically low cell densities. We asked whether death occurs by necrosis or by apoptosis. Two lines of evidence suggest that death occurs through apoptosis. First, the cellular DNA was found to separate in discrete fragments on agarose gels. Second, the protein synthesis inhibitor cycloheximide inhibited cell death, suggesting that the process involves protein synthesis. A number of gene products have been implicated in apoptic death processes, including BCL-2, p53 and C-myc. So far, we have examined the role of two of these genes in our system, BCL-2 and p53. Levels of expression of BCL-2 did not change significantly in EBV-immortalized cells destined to die by apoptosis. In contrast, levels of p53 in RNA were increased by 2-3 fold 6 to 12 hours before death occurred. Thus, EBV-immortalized cells die by apoptosis when deprived of autocrine growth factors and this process is associated with increased expression of the p53 tumor suppressor gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 BD 04016-02 LMI

PERIOD COVERED: October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): IL-6 is Tumorigenic for EBV Immortalized B Cells

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

PI: G. Tosato, M.D., Laboratory Chief, LI, DCB, CBER
J. Tanner, Staff Fellow, LI, DCB, CBER
S. Pike, Microbiologist, LMI, DCB, CBER
K. Jones, Microbiologist, LMI, DCB, CBER

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Immunology

SECTION: -

INSTITUTE AND LOCATION: DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	3.0	0

CHECK APPROPRIATE BOX(ES)

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

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

Expression of the human IL-6 gene in EBV-immortalized normal human B lymphocytes following retroviral-mediated transduction rendered these cells highly tumorigenic in athymic mice. The tumors were lymphomas composed of the originally inoculated human lymphoblastoid cells. Coinjection of IL-6 expressing EBV-immortalized cells with IL-6 non-expressing control cells resulted in increased tumorigenicity of the IL-6 non-expressing cells. The lymphoblastoid cells expressing IL-6 were indistinguishable from parental cell lines in morphology and in a variety of cell surface characteristics and did not exhibit growth advantage over parental cell lines in vitro, such that increased tumorigenicity is unlikely to depend upon a direct oncogenic effect of IL-6 on the B cells. Rather, at high concentrations, IL-6 markedly inhibits human lymphoblastoid cell killing by IL-2-activated murine splenocytes in vitro, suggesting that IL-6 related tumorigenicity might depend upon IL-6 inhibiting cytotoxicity at the tumor site. Thus, production of IL-6 by tumor cells which results in natural killer cell dysfunctions illustrates a novel mechansism of tumor cell escape from immune surveillance.

PUBLICATIONS

Tanner, JE and G Tosato. Impairment of natural killer functions by IL-6 increases lymphoblastoid cell tumorigenicity in athymic mice. J Clin Invest. 88:239, 1991

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04017-02 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Interleukin-10 Inhibits T Cell Proliferation and Interferon γ Production

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

PI: Y. Taga, M.D., Visiting Scientist, LI, DCB, CBER
G. Tosato, M.D., Laboratory Chief, LI, DCB, CBER

COOPERATING UNITS (if any)

H. Mostowski, Microbiologist, DCB, CBER; F. Wang, M.D., Harvard Medical School; Dr. K. Moore and Dr. P. Vieira, DNAX Research Institute, Palo Alto, CA

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Interleukin-10 (IL-10) was originally identified as a cytokine produced by mouse TH2 cells that inhibits interferon γ production by TH1 cells. Recently, human IL-10 has been sequenced and found to have approximately 70 % homology with the BCRF-1 open reading frame of Epstein-Barr virus. Similar to mouse IL-10, human IL-10 has been found to inhibit interferon γ production by human T cells, a property shared by the protein encoded by BCRF-1. The purpose of our investigation was to assess a possible role for IL-10 and BCRF-1 in the control of EBV infection.

A number of experiments have demonstrated that both human IL-10 as well as BCRF-1 protein are potent inhibitors of mitogen-induced T cell proliferation. This inhibitory effect is associated with inhibited IL-2 production and is reversed by addition of exogenous IL-2 to the suppressed cultures. Confirming previous observations, we also found that IL-10 and BCRF-1 protein inhibit Interferon γ production. This effect, however, appears to be independent of the growth inhibitory functions of IL-10 and BCRF-1.

In a subsequent set of experiments, we have examined whether IL-10 inhibition of T cell growth occurs through a direct effect on T cells or rather occurs indirectly acting upon a different cell type. Using purified T cells activated by immobilized OKT3, we found that IL-10 acts directly on the T cell.

PHS 6040 (Rev. 5/92)

PUBLICATIONS

Taga, K and G Tosato. IL-10 inhibits human T cell proliferation and IL-2 production. J. Immunol. 148:1143, 1992.

RETROVIROLOGY SECTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04011-04 LMI

PERIOD COVERED

October 1,1991 through September 30, 1992

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

Role of Proteoglycans and Polyanionic Polysaccharides in HIV Infection

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

PI: M.A. Norcross, M.D. Senior Investigator, LI, DCB, CBER
G. Rodriquez, M.S., Microbiologist, LI, DCB, CBER
D.C. Bou-Habib, M.D.,Ph.D., LI, DCB, CBER
T. Orevecz, PhD., LI, DCB, CBER

COOPERATING UNITS (if any)

M. Patel, Hoechst, India; M. Yanagashita, NIDR; V. Hascall, NIDR

LAB/BRANCH

Laboratory of Immunology

SECTION

Retrovirology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

Our laboratory has continued investigating the molecular mechanism of the antiviral effects of polyanionic compounds and the role of cell surface proteoglycans in mediating HIV-1 infection. Our results have revealed that the inhibitory effects of polyanionic polysaccharides on viral binding, viral replication and syncytia formation are mediated by interactions with the V3 principle neutralizing domain of gp120 and with envelope regions near the CD4 binding site. We have also identified a cell surface sulfated polysaccharide, heparan sulfate proteoglycan, as a novel HIV binding site on the T-cell surface which functions together with CD4 to mediate HIV entry. We have biochemically identified proteoglycan synthesis in HIV susceptible T-cell lines and have demonstrated by enzymatic treatment of T-cell lines with heparitinase and by inhibition of glycosaminoglycan sulfation that heparan sulfate is required for HIV-1 infection. We have quantitated direct virus binding to cells and have found that treatment of cells with heparitinase inhibits HIV-1 binding to the T-cell surface. Exogenous HS added to cultures inhibited virus infection in a manner analogous to dextran sulfate, further supporting a functional role for HS in HIV-1 binding. These results provide direct evidence for participation of cell surface heparan sulfate proteoglycans in HIV-cell attachment and virus entry. The description of this new cell entry site potentially should allow for additional modes of antiviral therapies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04013-04 LMI

PERIOD COVERED

October 1,1991 through September 30, 1992

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

Anti-HIV Antibodies Which Interfere with CD4-HIV Envelope Interactions.

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

PI: M.A. Norcross, M.D. Senior Investigator, LI, DCB, CBER
G. Roderiquez, M.S., Microbiologist, LI, DCB, CBER
T. Orevecz, Ph.D., LI, DCB, CBER
D.C. Bou-Habib, M.D.,Ph.D., LI, DCB, CBER

COOPERATING UNITS (if any)

E. Bonvini, CBER,DTS

LAB/BRANCH

Laboratory of Immunology

SECTION

Retrovirology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

We have continued this study by 1) measuring autologous serum for CD4 blocking activity on autologous virus, by 2) analyzing serum from chimps immunized and infected with HIV, and by 3) studying the effect of CD4-blocking antibodies on monotropic HIV strains.

To analyse autologous serum-virus interactions, we have tested serum from the labworker infected with IIIB and have found that, on cell-associated CD4 binding assays, this individual has the highest blocking activity against IIIB of any serum yet tested, but has only weak cross reactivity on the other laboratory strains used in the blocking assays in contrast to most other sera. Surprisingly, this serum although containing high concentrations of antibody to gp120 in the ELISA assay, has no ELISA CD4-gp120 blocking activity. Analysis of immunized and HIV infected chimp sera, in contrast to the human sera, has found that HIV envelope immunized chimp sera contain high levels of blocking antibodies to rgp120 in the ELISA format but have no cell-associated CD4 blocking activity. These results demonstrate that dramatic differences exist between the conformation of gp120 when in solution (or on the ELISA plate) and when it is associated with gp41 on the cell surface or virus membrane. The results also show that the immune response to envelope differs greatly depending on the form in which envelope is presented to the immune system.

These studies have been extended into an analysis of primary, monotropic and chimeric virus isolates. These isolates have shown a dramatic resistance to neutralizing antibodies which appears to involve resistance both to antibodies directed to the CD4 binding site as well as to the V3 neutralizing domain. Our studies provide new insight into role of antibodies directed towards the CD4 site on envelope in protective immunity and provide the foundation for vaccine and therapeutic approaches directed at this epitope.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04022-01 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Signalling Function of CD4 and its Role in Modifying T-cell Activation.

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

PI: M.A. Norcross, M.D., Senior Investigator, LI, DCB, CBER
G. Roderiquez, M.S., Microbiologist, LI, DCB, CBER
T. Orevecz, Ph.D., LI, DCB, CBER
D.C. Bou-Habib, M.D., Ph.D., LI, DCB, CBER

COOPERATING UNITS (if any)

E. Bonvini, CBER, DTS

LAB/BRANCH

Laboratory of Immunology

SECTION

Retrovirology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

To investigate the biological and immunological consequences of HIV-CD4 interactions, we have initiated studies to analyze the functional changes which occur secondary to the binding of either virus, virus envelope, or antibodies to CD4 on the surface of normal T-cells. The initial studies are to evaluate a variety of monoclonal antibodies to CD4 and other cell surface molecules, along with envelope proteins and purified HIV to determine whether these reagents either co-stimulate or inhibit T-cell receptor initiated T-cell activation. We have found that different epitopes on the CD4 molecule, as defined by monoclonal antibodies, are able to transmit different signals to the T-cell. Some sites are very potent at inhibiting T-cell activation when presented in solution, where as others evoke strong stimulatory activity in the context of a crosslinked simultaneous signal from the T-cell receptor. These functional differences may represent differences in the type or quality of signal generated in response to distinct epitopes on the CD4 molecule. When the effects of HIV are analyzed in the same systems, activities similar to those of monoclonal antibodies to CD4 are apparent. However, the functional effects of virus and envelope are much broader and extend beyond interactions that can be assigned to an exclusive effect on the CD4 molecule. These stimulatory and inhibitory activities may depend on envelope sites outside of the virus CD4 binding region or even be secondary to other proteins present on the viral surface. We are also currently studying the biochemical signaling generated by different antibodies to CD4. Recent observations suggest that crosslinking of CD4 can potentiate increases in intracellular calcium concentrations possibly secondary to protein kinase activation. Our goal is to define and understand the molecular and biochemical differences between CD4 generated negative and positive signalling. These pathways presumably are routes by which HIV can modify the function of the host T-cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04018-02 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Analysis of HIV-induced Autoantibodies to Cryptic Epitopes on Human CD4.

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

PI: M.A. Norcross, M.D., Senior Investigator, LI, DCB, CBER
G. Roderiquez, M.S., Microbiologist, LI, DCB, CBER
T. Orevecz, Ph.D., LI, DCB, CBER
D.C. Bou-Habib, M.D., Ph.D., LI, DCB, CBER

COOPERATING UNITS (if any)

E. Bonvini, CBER, DTS

LAB/BRANCH

Laboratory of Immunology

SECTION

Retrovirology Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

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

We have continued to define the mechanism of induction and to characterize human anti-CD4 auto-antibodies. This study describes a model for virus induced autoimmunity which supports the hypothesis that, as a consequence of HIV infection, abnormal processing of the CD4 receptor occcurs. This process uncovers cryptic epitopes near the membrane anchoring region on CD4, which are then recognized as foreign antigen leading to autoimmune antibody production. Using the CD4 antigen as a model, we are studying how and where in the cell or on the cell surface, HIV and presumably the envelope protein complex interacts with CD4 to induce cleavage of the CD4 molecule at the membrane-external protein junction. These studies are performed using live HIV interacting with CD4 postive cells and, in addition, using protein expression systems with vaccinia vectors expressing high levels of mCD4 together with vectors expressing the envelope proteins. Degradation of CD4 has been followed kinetically using radioisotope labelling and immunoprecipitation and western blotting. To determine whether cleavage of CD4 occurs on the membrane surface as a consequence of virus binding, cells expressing high levels of CD4 have been incubated with free virus or purified envelope and then degradation of CD4 monitored. We are also planning to determine whether this autoimmune process extends into the cellular immune compartment by testing patients for cellular response to sCD4. If these are positive we will then attempt to map these reactivities using peptide reagents generated to analyze autoantibodies. This project has led to the hypothesis that abnormal antigen processing can generate autoimmune reactivities and presents a theoretical paradigm which may provide insight into molecular mechanisms involved in other autoimmune disorders.

PUBLICATIONS

Callahan, L, Roderiquez, G, Mallinson, M and MA Norcross. Analysis of HIV-induced auto-antibodies to cryptic epitopes on human CD4. J. Immunology. (in press)

GENE REGULATION SECTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04008-04 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Regulation of the Expression of Human Immunoglobulin Genes

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

PI: E.E. Max, M.D., Ph.D., Senior Investigator, LI, DCB, CBER
J.G. Judde, Ph.D., Fogarty Fellow, LI, DCB, CBER
M. Malarkey-Briggs, Biologist, LI, DCB, CBER
M. Mitchell, Biologist, LI, DCB, CBER

COOPERATING UNITS (if any)

Drs. Vinay Jain and Ian Magrath, NCI, NIH

LAB/BRANCH

Laboratory of Immunology

SECTION

Gene Regulation Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 1.50	OTHER: .50

CHECK APPROPRIATE BOX(ES)

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

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

We previously identified three functional elements in the enhancer located downstream of the human immunoglobulin κ gene. By extending our analysis of site-directed mutations, we were able to characterize the sequence requirements for functional activity of the three motifs. The motif that we designate HM resembles a binding site for Helix-Loop-Helix transcription factors in its sequence requirements within the symmetric consensus element (CANNTG) but appears unique in that it also contains additional sequence requirements 8 bp downstream. Although the second motif (which we designate PU) exactly matched the previously reported μB element of the heavy chain enhancer, the sequence requirements for enhancer function were not that of μB, but rather resembled the consensus binding site for the ETS family of transcription factors including PU.1. Our analysis of the third element (which we designate DR) has demonstrated important functional nucleotides at the 5′ and 3′ ends of the element. We hope to purify and characterize the protein(s) binding to this motif.

Previously, our transfection studies employed primarily the Burkitt lymphoma line CA46, but we have begun a collaboration with Drs. Jain and Magrath to examine a panel of other Burkitt lines to determine how well the function of several immunoglobulin enhancers are supported in these lines. These studies are designed in part to explore the hypothesis that the unregulated expression of the c-myc oncogene in these cells stems from translocation of this gene into the vicinity of the immunoglobulin enhancers. Current results suggest that, despite the derivation of all of these lines lines from B lymphocytes expressing immunoglobulin genes, there is a wide variation in the ability of these cells to support function of the various immunoglobulin gene enhancers. We are currently attempting to determine how well the sequence requirements for the human 3′ κ enhancer, as previously studied in CA46, generalize to other Burkitt lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BD 04019-02 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Lymphokine Regulation of Immunoglobulin Isotype Switching

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

PI:E.E. Max, M.D., Ph.D., Senior Investigator, LI, DCB, CBER
F.C. Mills, Ph.D., Staff Fellow, LI, DCB, CBER
M. Malarkey-Briggs, Biologist, LI, DCB, CBER
M. Mitchell, Biologist, LI, DCB, CBER

COOPERATING UNITS (if any)

Drs. George Thyphronitis and Fred Finkelman, USUHS; Drs. Charles Chu and William Paul, NIAID; Drs. Andrew Saxon and Ke Zhang, UCLA School of Medicine

LAB/BRANCH

Laboratory of Immunology

SECTION

Gene Regulation Section

INSTITUTE AND LOCATION

DCB, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.50

CHECK APPROPRIATE BOX(ES)

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

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

To study the mechanism of the IL-4 induced switch from synthesis of immunoglobulin μ to ϵ in human lymphocytes, we have continued our analysis of PCR-amplified composite μ-ϵ switch regions formed by the switch recombination. From the sequence of the composite switch region in the cell line 2C10, we identified an insertion at the μ-ϵ junction that apparently derived from the γ1 gene switch region. This insertion implies that the 2C10 line switched initially to γ1 and subsequently to ϵ. Our finding that similar insertions occur with some frequency in fresh human lymphocytes switching to ϵ in culture led us to ask whether such sequential switching was obligatory and whether it always involved γ1. To approach these questions, we have developed PCR assays for the reciprocal products of the isotype switch recombination. Preliminary evidence suggests that (1) the sequential switch can involve other γ genes besides γ1, and (2) sequential switching is not obligatory, as some reciprocal products indicative of a direct μ-to-ϵ switch are observed. Our collaborative study of ϵ mRNA transcripts in cells switching to ϵ has led to the identification of two alternative mRNA splice forms. One of these contains a putative transmembrane (TM) region but is unusual in having a very large extracellular segment between the last immunoglobulin domain and the TM region; this segment could be important for the signal transduction machinery of ϵ-expressing lymphocytes. The second form includes the second of two "membrane" exons but this is read in an alternative reading frame; this form lacks a hydrophobic segment and would presumably be translated into a secreted protein that would be 134 amino acids larger than the classical secreted form. We have obtained Western blot evidence for an ϵ-related protein of the size expected for this larger secreted form and are currently attempting to generate antibodies specific for the C-terminal end predicted for this larger form in order to verify the existence of the protein and study its function.

PUBLICATIONS

Saxon, A, Kurbe-Laemer, M, Behle, K, Max, EE and K. Zhang. Inhibition of human IgE production via FcεR-II stimulation results from a decrease in the mRNA for secreted but not membrane ε heavy chains. J. Immunology, 147:4000, 1991.

Saxon, A, Zhang, K and EE Max. Regulation of human B cell production of IgE proteins. Proceedings of the 9th Immuno-Pharmacology Symposium, 63-89, 1991.

PUBLICATIONS IN PRESS

Mills, FC, Thyphronitis, G, Finkelman, FD and EE Max. Immunoglobulin μ-ϵ isotope switch in IL-4 treated human B lymphoblastoid cells; evidence for a sequential switch. J. Immunology. (in press)

Zhang, K, Saxon, A and EE Max. Two unusual forms of human IgE encoded by alternative RNA splicing of ϵ heavy chain membrane exons. J. Exp. Med. (in press)

Chu, CC, Paul, WE and EE Max. Quantitation of immunoglobulin heavy chain switch recombination Sμ-Sγ1) by novel digestion-circularization polymerase chain reaction method. Proc. Natl. Sci. USA. (in press)