NCI/DCBD ANNUAL REPORT

Active Project Numbers - Period 10/1/83 thru 9/30/84

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CB00549	CB04015	CB05117	CB08272	CB08710
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CB00872	CB05035	CB05244	CB08293	CB09002
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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1983 through September 30, 1984

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05596-15 LGN

ERIOD COVERED

ctober 1, 1983 to September 30, 1984

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

athogenesis of plasma cell neoplasia: characterization of antigen-binding proteins

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

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AB/BRANCH

aboratory of Genetics

ECTION

STITUTE AND LOCATION

CI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4	2

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

he major project in this laboratory is to determine the pathogenetic mechanisms n plasmacytoma development in BALB/c mice. The development of this process is ependent upon the genotype of the BALB/c mouse and the participation of spe- ific susceptibility genes. Most conventional strains carry dominant resistance R) genes. DBA/2, for example, has 3 R genes. We have evidence that one of hese is carried on a BALB/c congenic strain carrying the Tol-1^a locus of DBA/2. usceptibility to plasmacytoma genes may be mediated by genes controlling the nflammatory responses to pristane (mineral oil). We have shown that the non- teroidal anti-inflammatory agent indomethacin, a powerful cyclooxygenase inhib- tor, strikingly inhibits pristane and induces plasmacytoma development. These ice, however, do develop oil granulomas and inflammatory exudates. We are ttempting to find the biochemical differences between the pristane and pristane- ndomethacin oil granulomas. One of the important contributions of the oil ranuloma is the provision of growth factors that are required by developing lasmacytoma cells. We have developed a growth dependent transplantable plasma- ytoma line in vitro that reflects a growth factor of macrophage origin. This actor does not appear to be any of the known factors. Accordingly, we are pro- eeding to isolate and characterize this factor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08727-07 LGN

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Organization and control of genetic material in plasmacytomas

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

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LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	2.5	3.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

It is the long range purpose of this project to study the control mechanisms important in regulating cell growth, neoplastic transformation, and protein synthesis in normal and malignant lymphoid cells. To this end we are studying the structure of the genes for cellular oncogenes in normal and tumor tissues from mouse and man and the expression of these oncogenes as mRNAs. In particular we are focusing on the B lymphocytic tumors of mice, plasmacytomas and lymphosarcomas, and we are investigating what role Abelson and Moloney leukemia viruses play in the induction of such tumors and the alteration of cellular oncogenes. We have discovered that increased expression of myc is found in all plasmacytomas, and that altered expression of myb is found in the lymphosarcomas. A morphologically distinct subset of lymphosarcomas has been shown to have altered myb mRNAs owing to the insertion of a deleted form of Moloney leukemia virus in the myb gene. This represents a mammalian example of oncogene activation by promoter/enhancer insertion of virus. We are also studying the expression of oncogenes in mouse and human autoimmune diseases. The study of the organization and expression of mouse and human IgD genes is continuing in mouse and human myelomas, with emphasis on cDNA and genomic cloning of the secreted and membrane forms of this immunoglobulin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05553-15 LGN

RIOD COVERED
tober 1, 1983 through September 30, 1984

LE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
munoglobulin structure and diversity. Characterization of cell membrane proteins

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

: Stuart Rudikoff	Microbiologist	LGN, NCI
E. Jouvin-Marche	Visiting Fellow	LGN, NCI
A. Hartman	Staff Fellow	LGN, NCI

OPERATING UNITS *(if any)*
Osborne, Research Associate, Amherst College, Amherst, Mass.
Hansen, Asoc. Prof., Univ. of Md., College Park, MD

B/BRANCH
boratory of Genetics

CTION

STITUTE AND LOCATION
I, NIH, Bethesda, MD 20205

TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

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

B

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

munoglobulin diversity. 1) Variable region amino acid sequences have been
termined for 3 IgM, κ β(1,6) galactan binding monoclonal antibodies derived
om the same fusion. These sequences in conjunction with Southern blot analysis
dicate that the 3 antibodies derive from a common precursor even though amino
id substitutions are found in the variable regions. These results suggest that
e amino acid substitutions result from somatic point mutations which occur in a
ntinuous manner during ontogeny and are not associated with immunoglobulin
ass switching. To further establish the nature of the observed amino acid
bstitutions, the entire gene family encoding these heavy chains has been cloned
d sequenced. None of the variant protein sequences were found to be encoded in
rm line genes confirming their somatic origin. 2) The question of multigene
olution and mutation is being approached by an analysis of immunoglobulin genes
olated from a variety of mouse species and sub-species representing a spectrum
the evolution of this genus. Genomic libraries have been constructed from
ur different species and appropriate immunoglobulin genes are being isolated
r DNA sequence analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08726-07 LGN

ERIOD COVERED

October 1, 1983 to September 30, 1984

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

Biochemistry and molecular biology of transplantation antigens

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

PI:	Michael J. Rogers	Research Chemist	LGN, NCI
	Richard Swerdlow	Senior Staff Fellow	LGN, NCI
	Giorgio Galetto	Visiting Fellow	LGN, NCI
	David Siwarski	Bio. Lab. Tech.	LGN, NCI
	Dinah Singer	Research Chemist	I, NCI
	Lloyd Law	Chief, Lab of Cell Biology	LCBGY, NCI
	V. J. Hearing	Research Chemist	D, NCI
	G. Jay	Expert	LMV, NCI

OOPERATING UNITS *(if any)*

B/BRANCH

Laboratory of Genetics

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STITUTE AND LOCATION

CI, NIH, Bethesda, MD 20205

TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The purpose of this work is to investigate various biological and chemical roperties of two types of murine cell surface antigens that induce graft ejection: histocompatibility antigens (H-2) and tumor associated transplantation antigens (TATA).

In the case of TATAs, the approach is to purify the molecule bearing these antigens from tumor cells and characterize them. Polyclonal and monoclonal antibodies ay then be prepared against these molecules and used to investigate their iological properties. Ultimately, suitable DNA probes can be prepared and sed to study the genes which encode the molecules. This structural information ill lead to an understanding of the mechanism of induction of these antigens nd their relationship to the oncogenic process. The structure of these molecules ay also provide insights into some of the unique immunogenic properties of tumors, .g., their ability to escape an apparently strong anti-tumor immune response.

In the case of H-2 antigens, the approach is to utilize alloantisera and monolonal antibodies recognizing class I determinants to examine the molecules xpressed on normal and neoplastic cells. Moreover, DNA probes and molecular loning techniques can be used to study the organization and expression of the enes that encode the molecules. Current specific aims are to identify molecules oded for by the many class I genes present in the mouse genome and to obtain nformation about the evolutionary history of this polymorphic multigene family.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05552-15 LGN

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Mammalian cellular genetics and cell culture

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

P.I.:	H. G. Coon	Research Biologist	LGN, NCI
	C. Nelson Sinback	Senior Staff Fellow	LGN, NCI
	S. Yasumoto	Visiting Fellow	LGN, NCI
	E. P. Reddy	Chief, An. Vir. & Field Stud. Sec.	LCMB, NCI
	J. Robbins	Senior Investigator	D, NCI

COOPERATING UNITS *(if any)*

Dr. F. Saverio Ambesi-Impiombato, Istituto di Patologia Generale, Naples, Italy
Dr. Kathy Anderson, Children's Hospital, Wash., D.C.
Dr. Eugene Bell, Dept. of Biology, MIT, Cambridge, MA

LAB/BRANCH

Laboratory of Genetics

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

It is the purpose of this project to analyze and develop new and difficult cell systems in culture. We have developed and are attempting to exploit applications of normal rat thyroid cell cultures. These cells are hormone dependent. They synthesize and secrete a very large protein product, thyroglobulin. They concentrate iodide 100-fold from the medium. They offer a unique opportunity to study secretion, ion uptake and cAMP response. These are being studied in our lab and in other labs, however, our approach is primarily to use electrophysiological techniques. We are attempting to study long term regulation of membrane potential and its relationship to secretion and hormone levels. We are also studying neurons and neuroblasts in cell culture. There are too few mammalian cell systems where "blast" cells can be observed in transition to mature, differentiated cells. We have tried this in nerve cells using cellular hybridization and cellular transformation (with its SV40 viruses) and by using little known cell systems in which blast cells persist throughout life (olfactory epithelium). We are especially interested in the electrophysiology of the cellular response to growth factors and trophic hormones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08950-02 LGN

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunochemistry and genetics of protein-binding immunoglobulins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Sandra Smith-Gill Expert LG, NCI

COOPERATING UNITS *(if any)*
W. Drohan, Molecular Genetics Group, Meloy Laboratories, VA
K. Dorrington, Dept. Biochemistry, University of Toronto, Toronto, Canada
D. Davies, LMB, NIADKD, NIH

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Laboratory of Genetics

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

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

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

Monoclonal antibodies directed against protein antigens are used as probes to study antibody-protein interactions and structure-function relationships, and to study developmentally regulated antigens in normal and neoplastic development. In order to define the complementary structure of an antibody and a protein epitope as precisely as possible, antigenic regions and specific epitopes recognized by monoclonal antibodies to two well characterized proteins, lysozyme c and ovomucoid from avian egg white, are examined. Epitopes are mapped by comparing antibody reactivity with related proteins, and results to date have revealed significant relationships between antigenic and tertiary structure. The antibodies are analyzed structurally by sequencing, chain recombination studies, crystallography and computer modelling, and results to date suggest that properties of the antibody combining site in an anti-protein immunoglobulin may differ significantly from those of anti-hapten immunoglobulins. Structurally and functionally related antibodies are compared to determine genetic mechanisms underlying anti-protein specificity. Experiments are in progress to generate monoclonal antibodies to onc gene protein products; these antibodies will be used to study these proteins in normal and neoplastic B-cell development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08951-02 LGN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Proteins associated with the biological effects of murine leukemia viruses

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

P.I.:	S. K. Ruscetti	Senior Investigator	LGN, NCI
	L. Wolff	Senior Staff Fellow	LGN, NCI

COOPERATING UNITS *(if any)*
Drs. W. Langdon, S. Morse and J. Hartley, Laboratory of Biology of Viruses, NIAID

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

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

B

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

The spleen focus-forming virus (SFFV) and Friend mink cell focus-inducing virus (Fr-MCF) both induce eythroleukemia in susceptible strains of mice. Studies are being carried out to determine which areas of the viral genomes are important in the development of disease and to determine how their products specifically interfere with erythroid cell growth and differentiation. The envelope genes and LTR regions of several strains of SFFV have been sequenced and compared with those of other murine leukemia viruses, and specific, highly conserved changes have been found. Attempts are being made to determine which changes are crucial for pathogenicity and target cell specificity. Additional information about the viral envelope genes and the role of their products in pathogenicity have come from further characterization of the proteins and analysis of their expression in various tissues. In order to determine the mechanisms by which SFFV and Fr-MCF virus alter erythropoiesis, hematopoietic cells from mice infected with these viruses have been analyzed for their ability to proliferate in the presence or absence of the hormone erythropoietin and attempts have been made to determine if their envelope gene products are related to this hormone or its receptor. Finally, attempts to further define the gene in DBA/2 mice responsible for resistance of these mice to F-MuLV-induced erythroleukemia have suggested that it is a single gene on chromosone 5 but is not the $RMCF^r$ gene. Additional studies are being carried out to further define this resistance as well as the resistance that exists in adult mice of susceptible strains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08300-12 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

SAAM, Development and Applications

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

Loren A. Zech, M.D.	Senior Investigator Detail from OD, NIHLB	LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Ray Boston, LaTrobe Univ., Australia; Dr. Naomi Sager, New York Univ., NY; Dr. Trevor Redgrave, Boston Univ.; Dr. Charles Schwartz, Medical College of Virg., Richmond, VA; Dr. Waldo Fisher & Dr. Bruce Patterson, Univ. of Florida, Gainsville;

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Continuing development of a computer system (SAAM) for the simulation, analysis, and modeling of bio-kinetic systems. Further development of a conversational mode of operation increased the versatility, applications and automated the modeling process. The programs which make up SAAM have been revised so that they can run on a DEC-20 computer system and IBM series 370 computer systems, thus making SAAM available to a wider range of users.

Application of the SAAM programs in the development of compartmental models for the metabolism of chylomicrons in rats. Using the model it was determined that chylomicrons of all sizes are taken up as intact lipoprotein particles and that in rats a major portion of the fatty acids taken up by the liver are in the form of triglycerides before hydrolysis.

The development of a compartmental model for the solution species of Bovine lipoprotein lipase resulted in the prediction of an active tetrameric species and an inactive oligomaric species. This provides the first explanation of the loss of activity upon standing at room temperature.

Further analysis of lipoprotein metabolism indicates that the apoB/E receptor plays a major role in apoB-100 metabolism but does not affect the apoB-48 metabolism. In addition the apoE phenotype in humans is a determinant in the kinetics of low density lipoprotein metabolism through the apoB/E receptor.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08303-12 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

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

Robert Blumenthal, Ph.D.,	Chief, Membrane Structure & Function Section	LTB, NCI

Other Professional Personnel:

Ofer Eidelman, Ph.D.,	Visting Fellow	LTB, NCI
Clifford Steer, M.D.,	Expert	LTB, NCI
Peter Greif, M.D.,	Staff Fellow	LTB, NCI
Daniel Margolis	Biological Aid	LTB, NCI

COOPERATING UNITS *(if any)*
Dr. M. Henkart, Dr. P. Henkart, Immunology Branch, DCBD, NCI; Dr. R. Schlegel, LP, DCBD, NCI; Dr. A. Walter, & Dr. J. Handler, LKEM, NHLBI; Dr. S.J. Morris, IRP, NINCDS; Dr. W. Habig, Office of Biologics, FDA; Dr. J. Foulds, LBM, NIAMDD

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Membrane Structure & Function

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We study the organization and changes in organization of membrane components (lipids and proteins), both in the lateral and in the perpendicular direction. (1) We follow the insertion of a protein into a preformed lipid bilayer (either in the form of a planar bilayer or of a lipid vesicle), and study the factors which determine the protein's orientation. We measure electrical properties of Planar Lipid Membranes to study: (a) mechanisms of ion transport; (b) properties of transport systems isolated from natural cell membranes; (c) mechanisms of cytotoxicity; (d) the effect of the membrane potential on the disposition of membrane proteins. (2) We have developed model systems in which fusion of phospholipid vesicles is induced Ca^{2+}, pH, and/or by such proteins as tubulin, clathrin, apocytochrome c and VSV G protein. We study this fusion process using an assay involving resonance energy transfer between two fluorophores incorporated into the vesicle bilayer. (3) We observe lateral organization and movement of fluorescently - labelled molecules on cell surfaces by fluorescence microscopy. We study the mechanism by which asymmetry is maintained between apical and basolateral surfaces in epithelial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08306-12 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Kinetic Modeling of Human Plasma Lipoprotein Metabolism

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

William Beltz, Ph.D., IPA LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Scott Grundy, Center for Human Nutrition & Veterans Administration, Dallas, TX; Dr. Barbara Howard, NIADDK, NIH, Pheonix, AZ

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Kinetic models of plasma apoproteins, cholesterol and triglyceride are being constructed based on data from experiments in man. The models are used to integrate plasma lipoprotein interactions with enzymes and receptors and to provide a better understanding of plasma lipoprotein synthesis and metabolism in health and disease. The models are particularly useful for the rigorous testing of hypotheses, the design of experiments, and the quantification of the effects of various perturbations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08320-09 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

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

Robert Jernigan, Ph.D.	Theoretical Physical Chemist	LTB, NCI

Other Professional Personnel:

Sanzo Miyazawa, Ph.D.	Visiting Associate	LTB, NCI
Percival D. McCormack, M.D., Ph.D.	Senior Staff Fellow	LTB, NCI
Peter Lemkin, Ph.D.	Computer Specialist	IPS, LTB, NCI

COOPERATING UNITS *(if any)*: Dr. J. Ferretti, Laboratory of Chemistry, NIHLB

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Statistically derived Phi-psi maps for each type of residue indicate substantial improvements in X-ray data over previous tabulations.

Position effects in regular secondary regions show strong effects for some types of residues: proline, aromatic and polar groups.

A simple dipolar solvent model indicates an asymmetry to electrostatic interactions. Favorable interactions appear to be enhanced and extended to longer range.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08323-09 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

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

Charles DeLisi, Ph.D., Acting Chief, LTB, NCI

COOPERATING UNITS *(if any)*
Dr. John Inman, Laboratory of Immunology, NIAID; Dr. Irwin Chaiken, Laboratory of Chemical Biology, NIAID; Dr. Jan Cerny, University of Texas; Dr. Herbert Hethcote, Department of Mathematics, University of Iowa.

LAB/BRANCH
Laboratory of Mathematical Biology

SECTION
Theoretical Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A physical chemical analysis of affinity chromatography has led to the design of new techniques for the quantitative study of macromolecular interactions. In particular new methods have been proposed which should allow rapid, accurate determination of thermodynamic and kinetic parameters. These methods are now being tested experimentally.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08331-08 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
An Analysis of Oscillations in the Glucose - Insulin System in Humans

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

David G. Covell, Ph.D. Senior Staff Fellow LTB, NCI

COOPERATING UNITS (if any)
Dr. Rubin Andres, GRC, NIH; Dr. Darish Elhai, SUNY

LAB/BRANCH
Laboratory of Mathematical Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Glucose homeostasis in biological systems if highly regulated. In response to a glucose load, a complex series of hormonal secretions occurs to return plasma glucose concentration to normal. Although these hormonal control mechanisms are poorly understood, experiments suggest they are the result of mutual effects of both glucose and the kinetics of each substance. The complex response dynamics for these hormonal secretions appear to contribute in some organized fashion to glucose homeostasis. To investigate this complex interrelationship we have focused our analysis on the timing of the hormonal secretions during a glucose response. In a normally functioning system such a response exhibits kinetic behaviour characteristic of systems controlled by feedback loops (i.e. damped oscillations to a stable steady state). In certain diseased states or under excessive glucose loads that tax the control mechanisms the dynamic behaviour often appears uncontrolled. The analysis of these states may provide information on the mechanisms involved in glucose homeostasis. Such analysis may also be applicable to a broader class of hormone systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08335-08 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

"Targeting" Liposomes for Selective Interaction with Specific Cells and Tissues

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

John N. Weinstein, M.D., Ph.D.	Senior Investigator	LTB,NCI

Other Professional Personnel:

Robert Blumenthal, Ph.D.	Chief, Mem. Struc. & Func. Sec.	LTB,NCI
Oscar D. Holton, III, Ph.D.	Expert	LTB,NCI
Michael A. Steller	Biologist	LTB,NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have studied three conceptually different ways of "targeting" liposomes:

(1) Antibody-mediated targeting. We find that antibody-bearing liposomes bind in large numbers to cells which bear the appropriate antigen. However, the bound liposomes are internalized only if endocytosis is possible. Upon endocytosis, liposome-entrapped methotrexate (MTX) can escape from the endocytic apparatus and bind to cytoplasmic dihydrofolate reductase, inhibiting growth of the cell. In the course of these studies, we developed the first heterobifunctional method for coupling antibody to liposomes.

(2) Physical targeting. We have designed "temperature-sensitive" liposomes, which break down and selectively release an entrapped drug in vivo at temperatures achievable by local hyperthermia. These liposomes selectively deliver MTX to mouse tumors in vivo and inhibit their growth.

(3) Compartmental targeting. We have demonstrated the delivery of liposomes and entrapped drug to lymph nodes after subcutaneous and intraperitoneal, injection and have determined cellular sites of localization. These studies are being extended to antibody-bearing liposomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08340-06 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physical Chemistry of Antibody Effector Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Charles DeLisi, Ph.D. Acting Chief, LTB, NCI

COOPERATING UNITS *(if any)*
Dr. Ruben Siraganian, Clinical Immunology Section, NIDR; Prof. George Barisas, Biochemistry, Dept., Univ. of St. Louis Med. School; Dr. Alan Perelson, Los Alamos National Lab.; Dr. David Segal & Dr. Steve Dower, Immunology Branch, DCBD, NCI

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Theoretical Immunology Section

INSTITUTE AND LOCATION: NCI, NIH Bethesda, MD 20205

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

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

B

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

Work on the basophil system was continued with the development of a new theory of the kinetics of activation and specific desensitization of cells from allergic and immunized individuals. The theory has been applied to the analysis of a wide variety of data. We also developed new methods, based on measurements of the kinetics of cell activation for determining whether or not descending limb of biphasic dose response curve falls because of insufficient cross-linking. We studied equilibrium and kinetic properties of IgG oligomers of defined size interacting with Fc receptors on a macrophage-like cell line. The results of the equilibrium studies provided the first experimental evidence in support of our predictions that receptor cross-linking can lead to non linear Scatchard plots. In addition, the data suggested two different types of binding sites for dimeric and trimeric oligomers, but only a single type for monomers. The maximum affinity enhancements -- 200 for dimeric relative to monomeric and 2.5 for trimeric relative to dimeric, indicate considerable strain or large differences in the entropic parts of the equilibrium constants for solution phase as opposed to cell surface reactions. The dissociation kinetics of dimer and trimer were biphasic and the rate of dissociation was accelerated by high concentrations of monomer.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08341-06 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physical Chemical Studies of Lipid - Protein Interactions

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

John N. Weinstein, M.D., Ph.D. — Senior Investigator — LTB, NCI

Other Professional Personnel:

Robert Blumenthal, Ph.D. — Chief, Membrane Structure & Function Section — LTB, NCI

COOPERATING UNITS *(if any)*
Dr. T. Innerarity and Dr. R. Pitas, University of Califirnia at San Francisco; Dr. Richard Klausner, LBM, NIAMDD

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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

B

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

We have investigated the interaction of lipoproteins with liposomes to form recombinant particles. A number of lipoprotein fractions (VLDL, IDL, LDL, and HDL) all disrupt liposome stucture by an essentially irreversible and qualistoichometric process. In the case of HDL, the major apoprotein, A-I, recombines with dimyristoyl phsophatidyl choline vesicles 40:1 lipid-protein to form discs approximately 100 Å in diameter and 32 Å in thickness, with proteinon the rim. These structural results were obtained by a combination of neutron scattering, electron microscopy, and column chromatography.

With dipalmitoyl phosphatidylcholine, A-I also forms what we term "vesicular recombinant" particles in a process which may relate to physiological mechanisms by which proteins are assembled into membranes and lipoproteins. To study thie process we have developed a technique called "phase transition release" (PTR) which is also being applied to study incorporation of tubulin into membranes.

Lipoproteins were labelled with the fluorescent lipid 3,3 dioctadecylindocarbocyanine for studies of interaction will cell surface lipoprotein receptors. The lipoproteins are also being labelled with NBD lipids for two-color fluorescence identification of cells in atherosclerotic plaques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08342-05 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Theory of Receptor-ligand Biophysics

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

Charles DeLisi, Ph.D. Acting Chief, LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Alan Perelson, Theor. Div. Los Alamos National Lab., Los Alamos, NM; Prof. Frederik Wiegel, Dept. of of Physics, Twente Univ. of Technology, Enschede, Netherlands; Prof. Federico Marchetti, University of Rome, Italy

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Theoretical Immunology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Rate constants for ligands interacting with cell bound or dispersed receptors have a diffusive part and an intrinsic part. The former depends on geometry, receptor distributions, and diffusion coefficients; the latter on electronic redistributions. We have been focusing on the former and have obtained expressions for diffusion limited association and dissociation rate constants when (1) ligand bind directly and specifically to receptors that are distributed over a spherical surface; (2) ligands bind indirectly by a path that includes non specific association with the cell and diffusion in the surface, toward or away from a specific receptor. We have also developed a formalism that permits calculation of the complete equilibrium and rate constants for cell bound receptors, given the equilibrium or rate constants for dispersed receptors.

Mathematical methods are also being developed to describe aggregation on a two dimensional fluid surface.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08357-03 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

Cell Interactions

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

Charles DeLisi, Ph.D. Acting Chief LTB, NCI

Other Professional Personnel:

Jerome Eisenfeld, Ph.D., IPA LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Richard Asofsky, Laboratory of Microbial Immunity, NIAID

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Theoretical Immunology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews B

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

Helper and suppressor cells form feedback loops which presumably regulate the immune response, and account for the central phenomena of immunology such as tolerance and maturation. We have developed important theoretical criteria involving control loop stability which tells us whether experimentally identified loops can in fact explain phenomena of interest.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08359-03 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Monoclonal Antibodies in the Lymphatics for Diagnosis and Therapy of Tumors

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

John N. Weinstein, M.D., Ph.D.	Senior Investigator	LTB, NCI
Other Professional Personnel:		
David Covell, Ph.D.	Senior Staff Fellow	LTB, NCI
Michael A. Steller	Biologist	LTB, NCI
Oscar D. Holton, III, Ph.D.	Expert	LTB, NCI
Jacques Barbet, Ph.D.	Guest Worker	LTB, NCI
M.J. Talley	Biologist	LTB, NCI
Glenn Spaulding	Biologist	LTB, NCI

COOPERATING UNITS *(if any)* Dr. A. Keenan, Dr. S.M. Larson, LNM, CC: Dr. R. Parker, Dr. S. Sieber, DCCP; Dr. R.K. Oldham, Dr. K.M. Hwang, Dr. M.E. Key, FCRF; Dr. L. Liotta, Dr. G. Bryant, LP, DCBD; Dr. J. Schlom, Dr. D. Colcher, LTIB, DCBD; Dr. M. Lotze, Dr. R. Rosenberg, SB, DCT.

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2	0.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have defined a new approach to the use of monoclonal antibodies for diagnosis and therapy of tumor in lymph nodes: delivery to the nodes via lymphatic vessels after subcutaneous injection. To establish a firm pharmacokinetic basis for this approach, we first studied antibodies to normal cell types in the mouse lymph node. In vitro binding characteristics were combined with in vivo pharmacological parameters to develop a quantitative understanding of the delivery process using the SAAM computer modeling system. Armed with that background information, we then demonstrated and analyzed specific uptake in lymph node metastases of a guinea pig tumor. Imaging studies were followed up with attempts at therapy. For diagnosis of early metastatic tumor in the nodes, the lymphatic route can be expected to provide higher sensitivity, lower background, lower systemic toxicity, and faster localization than the intravenous route. It will also minimize the problem of cross-reactivity with antigen present on normal tissues.

The experimental design of the guinea pig studies is currently being applied to detection of lymph node metastases in clinical stage II malignant melanoma (with S.M. Larson and other collaborators). Similar trials for breast cancer, non-small cell lung cancer and lymphoma have been formulated in conjunction with other investigators.

In vitro and animal studies are being continued both to optimize the clinical procedures and to explore basic functions of the immune system (see project #Z01CB08368-01 Selective Cytotoxicity in the Lymphatics).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08361-02 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Development of a Kinetic Model of GABA Metabolism in Rabbits with Hepatic Coma

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

David Covell, Ph.D. Senior Staff Fellow LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Peter Ferenci, Dr. E. Anthony Jones, Liver Unit, NIAMDD

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.10	.10	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The systemic metabolism of the neurotransmitter GABA was investigated under normal and coma conditions. The plasma levels of GABA are known to be elevated by an order of magnitude over normal in patients with coma resulting from fulminant hepatic failure. To investigate the mechanism(s) for this elevaton, a kinetic model was developed to describe GABA metabolism during various stages of coma in a rabbit model. The major finding of the analysis was that a defect in GABA catabolism could not explain the elevations in plasma levels and additional sources for GABA production must be postulated. Subsequent experimental studies have supported these results by showing that gut bacterial production of GABA is substantially elevated during hepatic coma. The research data were obtained in collaboration with Drs. T. Jones and P. Ferencei of the Liver Unit at NCI.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08362-02 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: The Kinetics of 6-Mercaptopurine in the CSR Following IT & IV Adminstration

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

David Covell, Ph.D., Senior Staff Fellow LTB, NCI

COOPERATING UNITS *(if any)*

Dr. David Poplack, Pediatric Oncology, NCI; P.K. Narang, Clinical Pharmacology, NCI

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The intrathecal administration of the anticancer agent mercaptopurine (i.e. directly into the cerebrospinal fluid, CSF, of the central nervous system) may provide an effective method for the treatment of acute lymphocytic leukemia (ALL). Such treatment requires careful control of drug levels in the CSF. With high speed digital computers it may be possible to use a sophisticated model of mercaptopurine kinetics in conjunction with a mathematical algorithm for dosage selection to rapidly and effectively control the CSF concentration of mercaptopurine. Towards this goal the metabolism of 6-MP have been investigated in monkeys following intrathecal and intravenous administration of mercaptopurine. The major finding of the research has been the development of a physiological-pharmacokinetic model of mercaptopurine kinetics in the CSF. The salient feature of the model is that nearly all of the 6-MP that enters the CSF from the plasma does so via newly formed CSF. As a result of this observation, new experiments are being conducted on the use of the internal cartoid artery as a means of delivering 6-MP to the brain. The methodology is currently being tested on monkeys. This research is being conducted in collaboration with Dr. P. Narang of the Clinical Pharmacology Unit and Dr. D. Poplack of the Pediatric Oncology Unit.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08363-02 LTB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Protein Modeling

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

H. Robert Guy, Ph.D. Expert LTB, NCI

Other Professional Personnel:

Robert Jernigan, Ph.D. Theoretical Physical Chemist LTB, NCI

COOPERATING UNITS *(if any)*

Dr. David Fass, Dr. William Church, Mayo Clinic/Foundation, Rochester, MN

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Methods to predict which portions of α helices are exposed to water, to protein, or to lipid were developed and refined. These methods were tested on proteins of known structure, specifically the globin family, and found to sucessfully classify most residues as buried, partially buried, or exposed. The methods also yield results consistent with experimental findings regarding which residues in bacteriorhodopsin are exposed to water, buried inside the protein, or exposed to lipid. The method was used with other factors to construct new molecular models for colicin A and colicin El membrane channels. This construction process is difficult and is only possible if there are sufficient experimental facts known about the structure. With these methods, it is now possible to screen proteins for probable channel forming properties.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08364-02 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Quantitative Methods for Analyzing Receptor Mediated Binding and Endocytosis

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

Charles DeLisi, Ph.D.	Acting Chief,	LTB, NCI
Other Professional Personnel:		
Marianne Gex-Fabry, M.Sci.,	Visiting Associate,	LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A compartmental model has been developed for the analysis of receptor mediated endocytosis. It considers ligand binding to receptors, diffusion at the cell surface, interaction of ligand-receptor complexes with coated pits, internalization of coated pit contents, lysosomal degradation and recycling to the surface.

The model makes a number of predictions related to the interpretation of binding data. It has been tested against, and applied to the analysis of a large body of data on binding and endocytosis of peptide hormones and modulation of the effects of growth factors by tumor promot rs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08365-02 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Prediction of Protein Function and Cellular Location

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

Charles DeLisi, Ph.D.	Acting Chief	LTB, NCI
Other Professional Personnel:		
Minoru Kanehisa, Ph.D.	Visiting Scientist	LTB, NCI
Petr Klein, Ph.D.	Visting Fellow	LTB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Theoretical Immunology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have developed a method which uses a series of discriminant analyses to allocate a protein sequence of unknown function to one of a number of functional groups (toxins, immunoglobulin variable regions, cytochromes c, etc.). Allocation is based on characteristics of both global and local physical properties (hydrophobicity, charge, etc.) of the amino acid sequence, and also on the appearance in the sequence of some characteristic patterns, such as repeated consecutive appearance of certain residues, or short signature peptides.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08366-01 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Percolation of Monoclonal Antibodies into Tumors

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

John N. Weinstein, M.D., Ph.D.	Senior Investigator	LTB, NCI
Other Professional Personnel:		
David Covell, Ph.D.	Senior Staff Fellow	LTB, NCI
Jacques Barbet, Ph.D.	Guest Worker	LTB, NCI
Oscar Dile Holton, III, Ph.D.	Expert	LTB, NCI

COOPERATING UNITS *(if any)*
Dr. L. Liotta, LP, DCBD; Dr. S.M. Larson, NM, CC
Dr. B. Bunow and Dr. M. Bietermann, LAS, DCRT

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

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

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

B

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

Before a monoclonal antibody (or other biological ligand) can label or kill a tumor cell, it must first reach that cell. For portions of a tumor far from the nearest blood vessel or other source of antibody, access may be limited by the rate at which the molecule can "percolate" through the extracellular space. We are investigating the spatial and temporal profiles of immunoglobulin (Ig) distribution generated by diffusion and convection through tumors, taking into account the possibilities of (a) saturable specific binding to cells, (b) nonsaturable, nonspecific binding, and (c) metabolic degradation.

We first developed theoretical models of the percolation process, using a VAX 11/780 computer and a program package for numerical solution of partial differential equations. Significant predictions thus far include the following: (1) The diffusion coefficient and/or hydraulic conductivity may limit flux of antitumor Ig through tumors. (2) The flux of non-binding control Ig is much less likely to be limited by diffusion or convection. Nonspecific Ig's penetrate more deeply and more quickly into the tumor. (3) Even with saturable binding (but not metabolism), the "C times T" exposure of tumor cells to antibody will be the same throughout the mass. (4) Metabolism will decrease the relative "C times T" exposure of cells farther from the source. This may be a major barrier to effective treatment of solid tumors with ligand molecules (or, for that matter, with standard chemotherapeutic agents).

We are testing predictions of the model using: (1) small cell lung carcinoma spheroids in vitro; (2) human melanoma cells injected i.v. in nude mice to form metastatic nodules. The distribution of antibody will be determined by fluorescence techniques and autoradiography. Concepts arising from this study are being applied to the design of clinical studies with monoclonal antibodies.

In addition to the investigations of immunoglobulin and other ligands as administered agents, we are considering the the physiology of endogenous molecular species including the antibodies, lymphokines, and other growth factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08367-01 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

Selective Cytotoxicity in the Lymphatics

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

Chris D.V. Black, Ph.D. Visiting Fellow LTB, NCI

Other Professional Personnel:

Jacques Barbet, Ph.D. Visiting Fellow LTB, NCI

John N. Weinstein, M.D. Ph.D. Senior Investigator LTB, NCI

COOPERATING UNITS (if any)

Dr. R.J. Parker and Dr. S.M. Sieber, Office of the Chief, DCCP, NCI

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Following subcutaneous injection, radiolabeled monoclonal antibodies bind efficiently to normal and tumor target cells in the lymph nodes (Project # Z01 CB 08359-02 LMB). This finding prompted us to attempt specific therapy using monoclonal antibodies covalently coupled to plant toxins. The first development along these lines has been to synthesize monoclonal antibody-ricin A-chain conjugates using four antibodies of different specificities. We then demonstrated the capacity of these conjugates to bind to their target cells and to inhibit protein synthesis at the ribosomal level in an acellular system. The cytotoxicity of these conjugates for target cells is currently under test. Using the guinea pig hepatocarcinoma cell line (L 10), which expresses large quantities of target antigen, we found only weak cytotoxicity with the monoclonal antibody D3 coupled to ricin A-chain. However, the same toxin coupled to an anti-mouse MHC antibody has proved to be highly toxic for lymphoid cells; similar results are expected with the other conjugates.

Another approach to specific therapy within the lymphatic system is the subcutaneous injection of a monoclonal antibody followed by a similar injection of complement. Such a system attempts to reproduce physiological antibody/complement dependent cytotoxicity. The determination of optimal doses and injection regimes will be facilitated by our current studies on monoclonal antibody pharmacokinetics and by in vitro cytotoxicity assays.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08368-01 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: A Mathematical Model of Subcutaneous Uptake of Monoclonal Antibodies

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

David G. Covell, Ph.D. — Senior Staff Fellow — LTB, NCI

Other Professional Personnel:

John N. Weinstein, M.D., Ph.D. — Senior Investigator — LTB, NCI

COOPERATING UNITS *(if any)*: Dr. Barry Bunow & Dr. Michael Bieterman, DCRT

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Monoclonal antibodies or other ligands are potentially useful for the diagnosis and treatment of tumors in lymph nodes. Their therapeutic and diagnostic potential depends on the ability of the antibody to reach the target cell.

We have developed a theoretical model for the transport of monoclonal antibodies and water into the lymphatic and capillary systems following subcutaneous injection. The model incorporates processes for transcapillary and translymphatic solvent and solute movement that account for a) hydrostatic and osmotic pressure differences between the injected solution and fluid surrounding the injection site, b) differences in the available pore area for transport into the lymphatic and capillary systems and c) specific and nonspecific binding of antibody molecules to tissue cells at the injection site. The partial differential equations describing the model are being solved numerically on a VAX/11-780 computer.

Significant theoretical findings to date include the following: 1) most of the antibody that leaves the injection site to enter the lymphatics does so by convection in the fluid also entering the lymphatics, 2) most of the water leaving the injection site does so by entering the capillary system 3) the repeated administration of smaller doses of antibody over longer times would improve delivery into the lymphatic system and 4) the inclusion of an osmotic agent in the injection solution would tend to reduce water loss into the capillary system and improve antibody entry into the lymphatic system.

The concepts arising from this study are directly applicable to the design of clinical studies with monoclonal antibodies and other ligands.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01CB00885-03 LTB
Formerly
Z01CB00885-02 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Computed Aided Two-dimensional Electrophoretic Gel Analysis (GELLAB)

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

Lewis L. Lipkin, M.D., Chief, Image Processing Section LTB, NCI

Other Professional Personnel:

Peter Lemkin, Ph.D.,	Computer Specialist	IPS, LTB, NCI
Morton Schultz	Senior Engineer	IPS, LTB, NCI
Earl Smith	Expert	IPS, LTB, NCI

COOPERATING UNITS *(if any)*
Dr. Eric Lester, Univ. of Chicago, School of Medicine; Dr. Peter Sondreger, Univ. Univ. of Zurich, Richard Hennebery, Dr, Piotr Grojec, MNS, LMB, NINDS

LAB/BRANCH
Laboratory of Mathematical Biology

SECTION
Image Processing Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

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

B

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

Gellab is a computer based system of analysis of sets of 2D gels It incorporates sophisticated subsystems such as statistical, data base manipulation, image acquisition, etc., It has been applied to a variety of experimental systems in which quantitative changes in one or more proteins among hundreds or thousands of unaltered proteins is the basic analytic problem. During the year numerous extensions to the armamentarium of procedures available to the user have been developed. It has also been applied to several new problems involving both early and late cellular differentiation and or protein synthesis. The objective of defining an exportable version of GELLAB (one that will run on a reasonably powerful microcomputer-affordable by a university department) is being actively pursued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01CB00886-03 LTB
Formerly
Z01CB00886-02 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis and Synthesis of Nucleic Acid Secondary Structure

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

Lewis L. Lipkin, M.D.	Chief, Image Processing Section	LTB, NCI

Other Professional Personnel:

Bruce Shapiro, Ph.D.	Computer Specialist	IPS, LTB, NCI
Morton Schultz	Senior Engineer	IPS, LTB, NCI
Earl Smith	Expert	IPS, LTB, NCI

COOPERATING UNITS *(if any)*
Dr. J.V. Maizel, Dr. K. Currey and Dr. R. Nussinov, Molecular Structure Section, NICHD

LAB/BRANCH
Laboratory of Mathematical Biology

SECTION
Image Processing Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

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

B

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

Research in the structure of nucleic acid molecules has broadened over the past year. We have developed methods for analyzing the effects of perturbations in the standard structure of B-DNA molecules and how these structural alterations may account at least in part for the molecules interaction with its environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08369-01 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
System Software for Protein and Nucleic Acid Structure Analysis

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

Lewis L. Lipkin, M.D.	Chief, Image Processing Section	LTB, NCI

Other Professional Personnel:

Peter Lemkin, Ph.D.	Computer Specialist	IPS, LTB, NCI
Bruce Shapiro, Ph.D.	Computer Specialist	IPS, LTB, NCI
Morton Schultz,	Senior Engineer	IPS, LTB, NCI
Earl Smith	Expert	IPS, LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Image Processing Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective is to functionally unite the wide variety of image processing equipment working in the laboratory so that the results of procedures performed on one processor may be available to the user at any other component. To this end two major software packages have been specified, designed, implemented and largely debugged. These are 1) BMIO, a basic set of input output routines which provide for interprocessor transfer of generalized digitized images, and 2) SPIDER a data independent context free packet switching network in which our DEC System 20 is the permanent master.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08370-01 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

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

Robert Jernigan, Ph.D. Theoretical Physical Chemist LTB, NCI

Other Professional Personnel:

Sanzo Miyazawa, Ph.D. Visiting Associate LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Effective residue-residue interaction energies have been statistically derived from protein X-ray structures. A lattice-like model is used in which each residue type has a coordination number. If a specific residue has an incompletely filled coordination shell, then it is assumed to be filled with equivalent water molecules. Derived contact energies follow intuition: The most favorably interacting pairs are hydrophobic residues. However, those interactions are quite non-specific. More specificity is observed between polar residues.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08371-01 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
B-Z Transitions in DNA

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

Robert L. Jernigan, Ph.D.	Theroretical Physical Chemist	LTB, NCI
Other Professional Personnel:		
Akinoru Sarai, Ph.D.	Visiting Fellow	LTB, NCI
Sanzo Miyazawa, Ph.D.	Visiting Associate	LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

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

B

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

A simple model of the conformational transition between the right handed B double helix and the left handed Z double helix of DNA is proposed. This is a mechanistic model in which the dependence of the energy on twist is represented by two terms, one which depends upon the shape of the potential function through a series in powers of the twist and another inter-unit quadratic potential energy. This second term reflects the resistance of the DNA to deformations. With such a simple model we have studied cases of homogeneous chains with symmetric potential energies, as well as those within homogeneities and asymmetries in which one conformation is preferred over the other, for a portion of the chain. The method yields the location of the B-Z conformational boundaries for different conditions. Comparisons have been made with experiments in which G-C regions have been inserted in circular plasmid DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08372-01 LTB

PERIOD COVERED: October 1, 1983 to September 31, 1984

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

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

Robert Jernigan, Ph.D.,	Theoretical Physical Chemist	LTB, NCI

Other Professional Personnel:

Akinoru Sarai, Ph.D.	Visiting Fellow	LTB, NCI
Gene Barnett, Ph.D.	Detail from ADAMHA	LTB, NCI
Percival D. McCormack, M.D., Ph.D.	Senior Staff Fellow	LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Interactions of DNA with repressor proteins, drugs, carcinogens and free radicals are being studied. Shifts in electronic structures are being calculated with standard molecular orbital methods. The aim is to determine the dependence of the interactions on the DNA sequence and conformation.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08373-01 LTB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure Function Relations in Nucleic Acids

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

Charles DeLisi, Ph.D.	Acting Chief	LTB, NCI
Other Professional Personnel:		
Minoru Kanehisa, Ph.D.	Visiting Scientist	LTB, NCI
Kotoko Nakata, Ph.D.	Visiting Fellow	LTB, NCI
Peter Greif, Ph.D.	Staff Fellow	LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

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

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Methods are being developed to predict whether a given nucleotide sequence is part of an exon, an intron or a non-coding region. We extended and applied the "perceptron" algorithm for distinguishing initiation and termination sites and intron/exon boundaries. Methods were developed that recognize specified patterns in nucleic acid sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00333-21 LB

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Biochemical Basis for Defective Differentiation in Granulocytic Leukemia

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

W. H. Evans	Research Chemist	LB	NCI
E. A. Peterson	Chief, Protein Chemistry Section	LB	NCI
S. Wilson	Biologist	LB	NCI
M. Mage	Immunochemist	LB	NCI
V. Alvarez	Expert	LB	NCI
W. McBride	Chief, Cellular Regulation Section	LB	NCI
R. Balachandran	Visiting Associate	LB	NCI

COOPERATING UNITS *(if any)*

Hematology, Oncology Section, Walter Reed Army Medical Center

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Protein Chemistry Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The main thrust of this work is to develop biochemical methods for the early diagnosis of granulocytic leukemia and methods for inducing leukemic cells to develop some or all of their functional properties as a means of partially or completely restoring host defense mechanisms in leukemia patients. Work is first aimed at establishing which of the many biochemical steps involved in normal granulocyte differentiation are controlled by humoral regulators. The results will be compared with those obtained from similar studies on leukemic cells at corresponding stages of maturity in order to determine the nature and potential reversibility of the arrested differentiation steps. Biochemical analyses are carried out on mature and immature granulocytes isolated from blood and bone marrow and the effects of external cell regulators on granulocyte differentiation, as measured by changes in the synthesis of specific cellular components, are studied in a defined culture system previously developed in this laboratory. Possible relationships between transforming genes in leukemic myeloblasts and factors involved in the regulation of normal granulocyte differentiation are under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00366-14 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Biosynthesis and Assembly of Intracellular Components

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

E. L. Kuff	Chief, Biosynthesis Section	LB NCI	
K. K. Lueders	Chemist	LB	NCI
A. Feenstra	Visiting Fellow	LB	NCI
J. DiPaolo		LB, DCCP	NCI
N. Popescu		LB, DCCP	NCI

COOPERATING UNITS *(if any)*

E. Leiter, Jackson Laboratory, Bar Harbor, ME

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Biosynthesis Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2.5	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have previously characterized intracisternal A-particle (IAP) genes as genetically distinctive retrovirus-like elements that are extensively reiterated in the cellular DNA of Mus musculus and some other rodent species. IAPs are not known to have an infectious extracellular phase. Last year we reported that IAP genes have typical retroviral long terminal repeat units (LTRs) and can act as mobile elements in the mouse genome. Cloned IAP LTRs were shown to promote CAT gene expression when introduced into the appropriate expression vector and transfected into either mouse or monkey cells. We have now found that LTR promoter activity is abolished or greatly reduced by specific methylation of HhaI or HpaII sites on either side of the RNA initiation site, an observation consistent with indirect evidence from this and other laboratories linking DNA methylation with IAP gene expression in intact cells. IAP-specific sequences were found to be 5-10 fold enriched in polyadenylated RNA (polyA-RNA) from BALB/c thumus as compared to the polyA-RNAs from liver, spleen and kidney; IAP sequences were about 1/15th as concentrated in thymus as in several IAP-rich mouse tumors. Major IAP transcripts in BALB/c thymus were 7.2Kb and 5.4Kb in size and corresponded to IAP-associated RNA species previously extracted from mouse neuroblastoma cells. The amounts and relative proportions of these two transcripts varied in the thymuses of different inbred mouse strains, indicating that IAP expression in this tissue is under genetic control. In studies of BALB/c thymus DNA at the genomic level, the number of IAP 5'LTRs demethylated at the HpaII site was below the sensitivity of our detection method; i.e., very few of the 1000 IAP genes were actively transcribed. In situ hybridization of mouse and Syrian hamster (800 IAP elements per haploid genome) chromosomes showed multiple copies distributed over each chromosome. However, in the hamster, 50% of the IAP sequence was concentrated in blocks of constitutive, late replicating, non-centromeric heterochromatin. Clearly defined non-centromeric heterochromatin is not found in the mouse. However, it is possible that in this species also, a large proportion of the IAP elements are sequestered in dispersed genetically silent regions of the chromosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00375-22 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

E. A. Peterson Chief, Protein Chemistry Section LB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Protein Chemistry Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

B

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

Methods for the fractionation and analysis of proteins are developed and applied to the purification of specific proteins for the study of their function and structure. Displacement chromatography is being developed for the fractionation of macromolecules and particles of biological interest, employing polyanions differing in number of charges per molecule as displacers. The procedure is particularly advantageous when large amounts of source material must be used to obtain sufficient amounts of a minor component, since the resolving power of the system can be focused on the narrow range of affinity represented by the protein of interest and its nearest neighbors. However, it is also applicable to ion-exchange HPLC. Recent efforts have been directed toward the simplification of the preparation of narrow-range displacers.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00945-11 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Factors Regulating the Synthesis of Collagen in Normal and Transformed Cells

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

B. Peterkofsky	Research Chemist	LB	NCI
G. Majmudar	Visiting Associate	LB	NCI
R. Spanheimer	Expert	LB	NCI
T. Bird	Visiting Fellow	LB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Biosynthesis Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Two different aspects of regulation of collagen synthesis are being studied. The objective of one project is to determine the mechanism by which vitamin C (ascorbic acid) controls connective tissue metabolism. Previously we showed that decreased collagen synthesis in parietal bone of scorbutic guinea pigs was directly related to the extent of weight loss during the third and fourth week of scurvy, rather than to defective proline hydroxylation. Our current studies show that collagen synthesis in cartilage is similarly affected by scurvy and that synthesis of another major component of cartilage extracellular matrix, proteoglycan, is also decreased. Both effects are directly correlated with weight loss and synthesis of collagen and proteoglycans appears to be coordinately regulated. These, and other results, suggest that ascorbate deficiency indirectly produces these effects by inducing anorexia, which leads to a chronic fasting state. Acute fasting for 96 hr with ascorbate supplementation causes a similar coordinate reduction in collagen and proteoglycan production. Decreased collagen production in both bone and cartilage of acutely fasted animals is not due to an increase in degradation but to decreased synthesis caused by a reduction in the levels of procollagen mRNA.

In a second study, we have found that in a nitroquinoline oxide transformant of BALB 3T3 (NQT-3T3), there is almost complete supression of synthesis of type I procollagen, the major product of the parent 3T3 cells. In addition, synthesis of two previously undescribed types of collagen is induced. Both of these molecules appear to have a procollagen type of structure. Each is composed of single subunits with a pepsin-resistant helical region having a typical repeating tripeptide sequence susceptible to bacterial collagenase, plus pepsin-sensitive noncollagenous regions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05202-17 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Isolation, Fractionation, and Characterization of Native Nucleoproteins

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

O. Wesley McBride	Chief, Cellular Regulation Section	LB	NCI
R. Balachandran	Fogarty Associate	LB	NCI
W. Evans	Research Chemist	LB	NCI

COOPERATING UNITS *(if any)*

Drs. David Swan & Stuart Aaronson, LCMB, NCI; Drs. Gerald R. Crabtree & Jeffrey A. Kant, LP, NCI; Drs. E. Hildebrand & D. Nebert, DP, CH; Drs. H. Krokan & C. Harris, NCI; Dr. B. D. Nelkin, Oncology Ctr., Johns Hopkins

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Cellular Regulation Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2.0	3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The purpose of this project is to develop methods for gene transfer to mammalian cells and to use these techniques for gene mapping, analysis of gene expression, and cloning eukaryotic genes. Many independent somatic cell hybrid lines segrating human chromosomes have been isolated and the human chromosome content of each line determined. Analysis of these lines with isotopically labeled cloned DNA probes has previously allowed assignment to specific human chromosomes, and sometimes regional localization, of human cellular onc genes, immunoglobulin genes and pseudogenes, and α, β, and γ fibrinogen genes. Similar procedures have been used to localize the metallothionein multigene family to chromosomes 1, 4, 16, 18, and 20 and the calcitonin gene to chromosome 11p. Chromosomal mapping of cytochrome P-450 genes and the O-methylguanine-DNA methyltransferase gene are in progress. Preliminary studies have failed to detect any rearrangement of cellular proto-oncogenes in guinea pig Leukemia. Transfection assays with this leukemia DNA fail to produce foci on NIH/3T3 monolayers. Other methods are being evaluated to detect transforming genes in the leukemic cells.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05203-16 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immunochemical Purification and Characterization of Immunocytes and Components

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

M.G. Mage	Immunochemist	LB	NCI
L.L. McHugh	Biologist	LB	NCI
L. Romani	Fogarty Visiting Fellow	LB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Protein Chemistry Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Our goal is the development of cell separation methods for the specific isolation of immune cells, particularly for varieties of antigen-reactive cells (ARC) involved in cellular immune reactions, and for their subcellular fractionation in order to study the mechanisms involved in the development of immune reactivities and immune macromolecules. Populations of cells containing ARC are tested for binding to the cell surface antigens of target cells attached to insoluble supports. Separated populations are tested for cytotoxic effector cells (CTL) and their precursors, for activity in allograft rejection and graft-versus host reaction and in the mixed lymphocyte reaction. T cell subpopulations from thymus and spleen are also separated by and characterized with specific reagents such as peanut aggulutinin and antibodies to the Lyt and CTL differentiation antigens. Surface molecules of target cells are isolated to test their binding to ARC. Monoclonal antibodies are prepared against CTL and CTL-derived cell lines in order to characterize their surface antigens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05210-16 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cellular Controls over Growth and Inducible Processes

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

E. B. Thompson	Chief, Biochemistry of Gene Expression Section	LB NCI
P. Earl	Cancer Expert	LB NCI
G. Van Eys	Visiting Fellow	LB NCI
L. Eisen	Chemist	LB NCI
B. Wagner	Animal Physiologist	LB NCI
G. Wasner	Fogarty Internatl. Fellow	LB NCI
J. Remy	CNRS Fellow	LB NCI

COOPERATING UNITS *(if any)* P. Dannies (Yale Univ.); S.S. Simons (NIAMDD); H.J. Eisen (NICHD); L. Zwelling (NCI); M. Costlow (St. Jude's Med. Ctr); T. Antakly (McGill Univ.); J. Harmon (USUHS); J. Schlechte (Iowa Univ.); R. Evans (Salk Inst.); G. Schütz & K. Scherrer (Natl. Can. Inst., Heidelberg, W. Germany); J. Strobl (Univ. of W. Va).

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Biochemistry of Gene Expression Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	11	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Control of transcription of the growth hormone (GH) gene in GH3 cells and GH3 x L cell hybrids has been studied. GH induction by glucocorticoid in GH3 cells is not blocked by cycloheximide. In GH3 x L cell hybrids, and in GH3 subclones, there is correlation between GH expression and methylation at a specific Tha I site 5'-wards of the initiation start site of GH gene transcription. GH gene regions have been joined to the chloramphenicol acetyl transferase (CAT) gene and the hybrid genes are being transfected into cells to test for control by hormones. A new system for studying DNA-steroid receptor interactions on agarose gels is being developed.

In the IM9 and CEM human leukemic cell lines, glucocorticoid effects and glucocorticoid receptors have been studied. Many physical and immunological parameters of the human leukemic cell glucocorticoid receptor have been established, from wild-type steriod-sensitive cells and several classes of steroid-resistant sublines. The phenylpyrazole-substituted steroid cortivazol has been found to have two binding sites in wild-type cells but only one in "receptorless" cells.

A cDNA library from CEM C7 cells is being prepared. An expression library of cDNAs from IM9 cells has been prepared and is being screened with our anti-human glucocorticoid receptor (HGR) antiserum. Immunocytochemical methods for examining HGRs have been developed.

Preliminary examination of tyrosine aminotransferase genes in highly steroid sensitive vs less sensitive rat hepatoma cell lines suggest that the gene may be in different configurations in the two.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05214-13 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

DNA Synthesis in Mammalian Cells

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

S. H. Wilson	Medical Officer	LB NCI
F. Cobianchi	Visiting Associate (3 mo.)	LB NCI
F. Cobianchi	Guest Worker (1 mo.)	LB NCI
P. Kumar	Visiting Fellow (6 mo.)	LB NCI
D. SenGupta	Visiting Fellow (8 mo.)	LB NCI
B. Zmudzka	Visiting Associate (12 mo.)	LB NCI

COOPERATING UNITS *(if any)*

J. Mitchell, NCI; J. Minna, NCI; A. Matsukage, Aichi Cancer Center; E. Baril, Worcester Foundation for Experimental Biology; S. Planck, U. of Arizona; W. Brown, Carnegie-Mellon University

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Biosynthesis Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We continued an investigation of the structure of mammalian DNA polymerase α. Our current results confirm that a 190 KDa polypeptide is an α-polymerase catalytic subunit in growth phase monkey BSC-1 cells and that additional catalytic subunits of ~ 115 KDa and ~ 70 KDa are present also. The 190 KDa polypeptide can be obtained directly from crude soluble extracts of growing cells by immunoprecipitation with antibody to α-polymerase and is enzymatically active after electroelution from an SDS-polyacrylamide gel. Further improvements in our use of immunoblotting techniques have enabled detection of α-polymerase polypeptides in both crude extracts of mammalian cells and extracts of E. coli infected with an expression vector (λgtll) containing mammalian cDNA inserts. Five phage capable of expressing α-polymerase polypeptides have been cloned. A similar approach has been used to obtain other phage clones capable of expressing β-polymerase polypeptide and helix destabilizing protein-1 polypeptide, respectively. Finally, experiments have been conducted toward developing a system for study of polyoma virus DNA replication in vitro. We have obtained evidence that de novo initiation and semiconservative replication occur in reaction mixtures containing plasmid DNA and extract from polyoma virus infected cells. Known requirements of this replication system include 1) the presence in the plasmid DNA of the polyoma virus origin of replication and 2) that the extract comes from infected rather than uninfected cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05231-10 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Role of Subunit Interactions in Enzyme Chemistry and Cellular Regulation

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

C. B. Klee	Chief, Macromolecular Interactions Section,	LB	NCI
A. S. Manalan	Medical Staff Fellow	LB	NCI
D. L. Newton	Research Chemist	LB	NCI
M. H. Krinks	Chemist	LB	NCI
J. R. Miller	Technician	LB	NCI
W. C. Ni	Visiting Fellow	LB	NCI
G. F. Draetta	Visiting Fellow	LB	NCI

COOPERATING UNITS *(if any)*

J. Schiloach, NIAMMD: Mr. Richard Feldman, CR-CCB; Dr. P. Cohen, University of Dundee, Scotland; Dr. L. Heppel, Cornell University, Ithaca, NY; Dr. T. Burke and Dr. K. Rice, NIAMMD; J. Haiech CNRS, Montpellier, France.

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Macromolecular Interactions Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.8	4.3	2.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Calmodulin effects a tight coupling of calcium and cAMP regulation of cellular processes by controlling cAMP levels and cAMP-dependent phosphorylation. It also interacts with the regulatory subunit of cAMP dependent protein kinase and activates a calcium regulated protein phosphatase, calcineurin.

The interaction of calmodulin with calcium and its target proteins is being studied in order to understand the mechanism of the regulation of cellular processes by calcium and cAMP. Using large calmodulin fragments obtained in highly purified states by HPLC, we have identified the two high affinity calcium-binding sites as sites III and IV. Calmodulin fragment 78-148 (sites III and IV) interacts with two different enzymes and with anticalmodulin drugs. The amino-terminal fragment 1-77 also interacts with anticalmodulin drugs and is required for activation of one enzyme studied but not the other. Thus, calmodulin contains at least two drug-interacting domains and different domains are required for activation of different enzymes. A covalent adduct of calmodulin with one mol of norchlorpromazine (CAPP 1-calmodulin) has been prepared. CAPP 1-calmodulin binds to calmodulin-dependent enzymes with high affinity (Ki = 10 nM - 1 nM) but has lost the ability to activate cAMP phosphodiesterase and myosin kinase and is therefore a specific and potent antagonist of calmodulin stimulation of these enzymes. It partially stimulates the phosphatase activity of calcineurin acting as a partial agonist in this case. It fully activates the calmodulin-dependent multifunctional kinase and phosphorylase kinase and does not inhibit protein kinase-C. CAPP 1-calmodulin should be a useful tool to dissect the role of calmodulin and the involvement of distinct calmodulin-regulated enzymes in cellular regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05234-10 LB

PERIOD COVERED: October 1, 1983, to September 30, 1984

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

Interrelations between the Genomes of SV40 and African Green Monkeys

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

Maxine F. Singer	Chief, Nucleic Acid Enzymology Section	LB	NCI
Jeffrey Saffer	Senior Staff Fellow	LB	NCI

COOPERATING UNITS *(if any)*

Professor R. Tjian, Department of Biochemistry, Univ. of California, Berkeley.
S. Adeniyi-Jones and M. Zasloff, Human Genetics Branch, NICHD

LAB/BRANCH: Laboratory of Biochemistry, DCBD

SECTION: Nucleic Acid Enzymology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A cloned segment of the African green monkey (Cercopithecus aethiops) genome that contains DNA sequences homologous to the control region of simian virus 40 is being studied. This sequence, 450 base pairs in length, is embedded in a genomic DNA region that is especially rich in interspersed repeated sequences. The segment homologous to SV40 is flanked by two members of the Alu family. The SV40-like region, which is hypersensitive to DNase I in monkey chromatin, serves as a transcriptional start site in both possible directions for cellular RNA synthesis. Also, the sequence provides information for initiation of transcription from vectors constructed by molecular cloning as measured by expression of an E. coli gene after transfection of the vector into mammalian cells. Expression was measured both by the percent of cells transformed by the E. coli gene and by analysis of messenger RNA transcribed from the vector. Multiple transcriptional start sites were detected in both directions by S1 nuclease analysis. Some of these coincide with the start sites mapped for the genomic transcripts. The SV40-like region also is a bidirectional transcriptional start site in in vitro reactions using fractionated cell free extracts. In vitro transcription depends on the presence of a fraction that is also required for in vitro transcription from SV40 DNA itself but not for other host cell promoters tested. The data suggest that there is a special class of cellular promoters that like SV40 promoters depend on the presence of a short G-rich DNA segment (5'-GGGCGGPuPu) and interact with a specific factor, Sp 1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05244-07 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Organization of Repeated DNA Sequences in African Green Monkeys

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

M. F. Singer	Chief, Nucleic Acid Enzymology Section	LB	NCI
T. Lee	Research Chemist	LB	NCI
R. Thayer	Chemist	LB	NCI
G. Grimaldi	Fogarty Visiting Fellow	LB	NCI
A. Maresca	Fogarty Visiting Fellow/Guest Worker	LB	NCI
S. Contente	Staff Fellow	LB	NCI
G. Humphrey	Guest Worker	LB	NCI
J. Skowronski	Visiting Fellow	LB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry , DCBD

SECTION

Nucleic Acid Enzymology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.3	6.0	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Two types of highly repeated DNA segments in the genome of the African green monkey (Cercopithecus aethiops) are being studied:

(1) Satellite DNAs are characterized by long tandem repetitions and centromeric location. Earlier work indicated that the organization of one monkey satellite, deca-satellite, is highly polymorphic in individual members of the species and undergoes frequent rearrangement in cell culture. Recent data show in addition that the amounts of deca-satellite and α-satellite, the major monkey satellite, vary (independently) in individual genomes. In an effort to understand the maintenance of such extensive but variable DNA sequences, analysis of junctions between satellite and unique genomic sequence has been initiated; several such DNA segments have been cloned and partially characterized.

(2) Previous experiments showed that the KpnI family of long interspersed repeats has members ranging from a few hundred to 6 kbp in length. Cloning and analysis of several new full-length members with surrounding sequences established the sequence at the 2 ends of the element. No terminal repeats occur and while some family members are flanked by target site duplications, others are not. The designated 3'-end varies some but generally includes a polyadenylation site. Assembling data from this and other labs, a sequence for the full 6 kbp was compiled. The sequence contains at least 3.5 kbp of open reading frame, ending 200 bp upstream from the polyadenylation site, at the same position where the previously described homology between the KpnI family and its rodent homologue stops. We conclude that the KpnI family is likely to consist of one or more functional genes as well as pseudogenes. Although transcripts of KpnI family sequences are abundant in the nucleus (and heterogeneous in size), many monkey and human cell lines fail to show significant amounts of homologous polyadenylated cytoplasmic RNA. One cell line revealed such an RNA band about 6 kb long.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05258-05 LB

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Molecular Studies of Eukaryotic Gene Regulation

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

B. M. Paterson	Research Chemist	LB	NCI
J. Hammer	Guest Researcher	LB	NCI
J. Eldridge	Biochemist	LB	NCI
A. Seiler-Tuyns	Fogarty Visiting Fellow	LB	NCI
B. Billeter	Visiting Associate	LB	NCI
A. Levi	Visiting Associate	LB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Using cloned cDNA probes we have isolated the genomic sequences for the following proteins: alpha skeletal actin, alpha cardiac actin, beta cytoplasmic actin, myosin light chains 1-3, vimentin, pyruvate kinase, and glyceraldehyde phosphate dehydrogenase. These have been defined by DNA sequence analysis. The various actin genes have been subcloned into the eukaryotic vector, PSV2-gpt and transfected into L-cells and into the C2 mouse muscle cell line. 5' and 3' probes specific for the various chicken actin genes have been used to monitor expression and regulation in these two mouse cell backgrounds. In vivo transcription of the vimentin gene, a single copy gene, produces two distinct mRNA transcripts, both of which are functional and encode the same polypeptide. The mechanism producing these two functional transcripts has been determined. We have compared the nucleotide sequence of the chicken and hamster vimentin genes to determine intron-exon junctions, codon usage, and sequence conservation. The myosin light chains 1 and 3 are encoded by a single gene. The organization of the gene has been determined by sequence analysis and contains nine exons. Initiation of transcription of LC1 and LC3 starts at different promotors and processing involves differential splicing. The double adenylation sites are used randomly. The expression of the mouse histone H4 gene is cell cycle regulated. The structure of the gene has been modified to localize the cell-cycle dependent regulatory regions in transfection studies with L-cells using the PSV2-gpt vector system. Pyruvate kinase undergoes an isoform shift during myogenesis. The structure and regulation of the gene is under study. The three unique Acanthamoeba myosin polypeptides have been synthesized in vitro and this assay has been used to isolate the corresponding genomic DNA sequences. Structural comparisons of the genes are under way. Nerve growth factor triggers the differentiation of PC12 neuronal cells in vitro. We have isolated cDNA clones representing those genes that are transcriptionally activated in less than 24 hours after exposure to nerve growth factor. Characterization of the genes is under way.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05262-04 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Eukaryotic Gene Regulation: The Metallothionein System

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

Dean H. Hamer	Research Chemist	LB	NCI	C. Seguin	Guest Worker	LB	NCI
				D. Thiele	Staff Fellow	LB	NCI
C. Schmidt	Chemist	LB	NCI				
G.N. Pavlakis	Fogarty Associate	LB	NCI				
A.D. Carter	Staff Fellow	LB	NCI				
B. Felber	Fogarty Fellow	LB	NCI				
A. Leone	Fogarty Associate	LB	NCI				
M.J. Walling	Microbiologist	LB	NCI				

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Cellular Regulation Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.0	7.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The metallothioneins provide a useful model for studying the environmental and developmental regulation of eukaryotic gene expression. The DNA sequences and cellular factors involved in the heavy metal induction of mammalian metallothionein gene transcription have been investigated by in vitro mutagenesis, gene transfer and factor titration experiments. The results indicate that cellular components interact with two distinct regions of the upstream flanking DNA and activate transcription by a positive regulatory mechanism. Cells from patients with Menkes' disease, an inherited disorder of copper metabolism, are defective in some step of the regulatory pathway. Analysis of three human metallothionein genes has shown that regulation also occurs during development, possibly associated with changes in methylation. Genetic strategies have been developed to study the regulation of a metallothionein-like protein in a lower eukaryotic, and metallothionein-based expression vectors have been utilized to overproduce useful proteins such as growth hormone and hepatitis surface antigen.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05263-03 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Eukaryotic Chromatin Structure and Gene Regulation

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

Carl Wu	Visiting Associate	LB	NCI
Thomas Paisley	Biologist	LB	NCI
Zdzislaw Krawczyk	Exchange Scientist	LB	NCI
Barbara Wood	Laboratory Worker	LB	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Developmental Biochemistry Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

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

B

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

The sequential arrangement of nucleosomes along the chromatin fiber is punctuated by highly nuclease-sensitive sites. We previously mapped such sites to the 5' terminus of several heat shock genes in Drosophila by a novel indirect end-labeling technique. Such preferentially accessible sites in chromatin may function as points of entry to the DNA for RNA polymerase and control proteins. We have now developed an exonuclease protection technique for mapping protein binding sites in chromatin, and have found two such sites for both the hsp 82 and hsp 70 genes. Site I is present before and after heat shock gene activation, and covers the TATA box sequence, whilst site II surrounds the upstream heat shock control element and appears only during heat shock. We suggest that heat shock genes are activated by the sequential binding of at least two protein factors, and we are currently developing new methods to assay for these factors in cell-free extracts. To determine the functional relationship of 5' terminal hypersensitive sites in chromatin to gene activity, we have developed an in vitro transcription system from Drosophila nuclei, which is capable of new RNA transcript initiations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05264-03 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of a Mouse Repetitive Gene Family

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

Kira K. Lueders	Chemist	LB	NCI
Joseph Fewell	Microbiologist	LB	NCI
E.L. Kuff	Chief, Biosynthesis Section	LB	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Biosynthesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

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

B

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

Previously we identified and characterized a family of interspersed 400 bp long repetitive sequences (R-sequences) representing 1-2% of mouse genomic DNA. We have now studied the functional role of these sequences in RNA transcription. Plasmid constructs containing R-sequence and the bacterial gene chloramphenicol acetyl transferase have been used in transient expression assays to measure promoter and enhancer functions after transfection into mammalian cells. Several R-sequences increased transcription from the SV40 early promoter in monkey cells, and one R-sequence also increased transcription from an intracisternal A-particle long terminal repeat promoter when present 5' to the promoter.

Polyadenylated RNA transcripts containing R-sequence have been detected in normal (thymus) as well as transformed (neuroblastoma) cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05265-02 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

P. Wagner	Guest Researcher	LB	NCI
J. George	Technician	LB	NCI
Ngoc-Diep Vu	Staff Fellow	LB	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Macromolecular Interactions Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.75	1.0

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

B

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

Cytoskeletal proteins are being studied to understand how these proteins interact to produce the various motile activities of cells. The interaction of cytoplasmic myosin with actin filaments and the hydrolysis of ATP by myosin provide the force that drives these processes. The interactions of cytoplasmic myosins with actin are regulated by specific calcium.calmodulin dependent kinases. Unlike with smooth muscle myosin, there is a linear relationship between the level of cytoplasmic (thymus) myosin phosphorylation and stimulation of the actin-activated ATPase of this myosin. Thus, even low levels of phosphorylation can stimulate motile activity. Turbidity, ultracentrifugation, and electron microscopy were used to examine the equilibrium between myosin filaments and myosin monomers or small oligomers. This equilibrium is dependent on ionic strength, divalent cation concentration, type of anion used, and on whether the myosin is phosphorylated. While phosphorylation promotes filament formation, it appears unlikely that this is the principal mechanism for regulating the participation of cytoplasmic myosins in force development. Fodrin or brain spectrin is a calmodulin binding protein that appears to link the cytoskeleton to the cell membrane. Under approximately physiological conditions, fodrin inhibits the actin-activated ATPase of myosin. More fodrin is required for inhibition in the presence of calcium than in its absence, but calmodulin has no effect on this inhibition. Thus, in the region of the cell where fodrin is localized, the interaction of myosin with actin is inhibited. The calcium sensitivity observed *in vitro* provides a potential mechanism for regulating this inhibition. Detergent treated T-lymphoma cells are being used as a model system for examining the role of cytoskeletal proteins. While these cells are permeable to large proteins, i.e. antibodies and myosin light chain kinase, their cell surface proteins still cap in response to concanavalin A binding. This capping requires calcium and ATP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05266-02 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

Cary Queen	Senior Staff Fellow	LB	NCI
S. Segal	Expert	LB	NCI
J. Stafford	Microbiology Technician	LB	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Macromolecular Interactions Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

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

B

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

We are studying the regulation of expression of the immunoglobulin gene family by attempting to answer two questions: (1) why do only cells of the B-lymphoid lineage synthesize immunoglobulins, (2) how do these cells transcribe only one or a few immunoglobulin genes, while leaving hundreds of other, similar immunoglobulin genes inactive? Our approach to these questions is to insert a cloned, rearranged kappa light chain gene into a plasmid in various configurations, to transfect the plasmid into various types of cells, and to determine whether the transfected gene is transcribed. We have shown that the complete kappa gene is transcribed after transfection into antibody-producing myeloma cells but not in non-lymphoid 3T3 or L cells. Hence the different cell types are able to appropriately regulate the kappa gene even when not in its usual chromosomal environment. By deleting different parts of the cloned gene, we have shown that certain sequence elements actually downstream of the promoter are necessary for its transcription in myeloma cells. Most recently, we have localized the important downstream elements to a 200 base pair region of DNA. We are currently transfecting the kappa gene into a variety of lymphoid cell types (T and B) to study its developmental regulation.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB03663-08 LCO (formerly D)

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tumor virus expression in vitro and in vivo

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

PI: Douglas R. Lowy, Chief, Laboratory of Cellular Oncology, NCI
Other: Sisir K. Chattopadhyay, Visiting Scientist, LCO, NCI
Elliot J. Androphy, Medical Staff Fellow, LCO, NCI
Pierre E. Tambourin, Guest Researcher, LCO, NCI
Timothy F. Kelly, Medical Staff Fellow, LCO, NCI
John T. Schiller, Guest Researcher, LCO, NCI
Marilyn R. Lander, Microbiologist, LCO, NCI
Nancy L. Hubbert, Microbiologist, LCO, NCI

COOPERATING UNITS *(if any)*
Laboratory of Pathology, NCI, Drs. R. Muschel and L. Liotta
Medicine Branch, NCI, Drs. A. Kasid and M. Lippman
Fibiger Institute, Copenhagen, Denmark, Dr. B. Willumsen

LAB/BRANCH
Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.5	6.0	3.5

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

B

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

This project seeks to study mechanisms by which tumor viruses or cellular genes contribute to oncogenesis and to devise approaches to prevent or reverse such changes in cells.

The oncogenicity of p21 ras genes with a single point mutation has not been established for normal cells, although ras genes with single mutations have been found in a variety of human and animal tumors. The ras gene of Harvey murine sarcoma virus (Ha-MuSV), which contains two point mutations (amino acids 12 and 59), is highly oncogenic in vivo. Using recombinants between a normal cellular ras gene and Ha-MuSV ras gene, we have determined that viruses containing either point mutation were oncogenic in vivo. Using Ha-MuSV ras mutants, we have also determined that the carboxy terminus of the p21 protein is required for the transforming activity of the protein, its membrane localization, and its binding of lipid.

Papillomavirus research has been both basic and applied. Using frame shift mutants of cloned viral DNA, we have found that bovine papillomavirus (BPV) contains at least two genes which can independently transform established mouse tissue culture cells. We have also studied the clinical response of patients with epidermodysplasia verruciformis, a disease of chronic widespread wart virus infection, to human leukocyte interferon (IFN). Short term treatment with intralesional or systemic IFN resulted in a marked diminution in the size of warts in each of six patients and to a decrease in the number of virus-positive cells in lesional skin.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05550-15 LCO
(formerly LCBGY)

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Regulation of Retroviral Replication and Cellular Oncogene Expressions

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

PI: Kenneth S. S. Chang, Medical Officer, LCO, NCI

Other: Lai-che Wang, Visiting Fellow, LCO, NCI
Li-Ting Liang, Microbiologist, LCO, NCI

COOPERATING UNITS *(if any)*

V.A. Hospital, Washington, DC
Department of Preventive Medicine, Public Health Service, Washington, DC

LAB/BRANCH

Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

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

B

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

The long range purpose of this project is to investigate the role of type C retroviruses as an etiologic agent and a vector of genetic information for neoplasia and the use of viral and cellular mutants to analyze the mechanism of regulation of gene expression associated with cell differentiation and oncogenesis. The expression of cellular oncogenes are also investigated.

The topics of current interest are: 1) in vitro transmission of the human T cell leukemia virus (HTLV) to nonlymphoid cell lines; 2) HTLV-antibody and virus isolation studies on drug addicts and homosexual patients in D.C. area; 3) oncogene rearrangement, amplification, and expression in human hepatocellular carcinomas, teratocarcinomas, choriocarcinomas, murine reticulum cell neoplasms, and trophoblastic tumors; 4) regulation of retroviral replication in murine trophoblastic tumor cells.

Preliminary results indicate that HTLV (type I) can infect at least some nonlymphoid cells of nonhuman origin, and manifest viral activities through morphological alteration of the cell clones isolated after infection. The unusually high rate of HTLV-I antibody positive serum among the drug addicts in the D.C. area may indicate that exposure to the virus of HTLV-family is not infrequent in this population. Rearrangement and/or amplification of c-myc or c-ras have not been detected in cell culture lines derived from human hepatoma, teratocarcinoma, and choriocarcinoma. Other oncogenes are being tested. Although murine trophoblastic tumor cells are not permissive for type C retrovirus replication, the virus can be activated by treating the infected cells with iododeoxyuridine or azacytidine. Studies on the regulation of integrated viral genome and oncogene of these cells are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04834-08 LCO
(formerly LCBGY)

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Genetic Mechanism of Carcinogenesis and Biological Modifiers as Defense Mechanism

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

PI: S. S. Yang, Chemist, LCO, NCI

Other: J. V. Taub, Biolab Technician, LCO, NCI
R. Modali, Biologist, LCO, NCI

COOPERATING UNITS *(if any)* C. C. Ting, Immunology Branch, NCI
G. C. Yang, OBCB, DCH, CFSAN, FDA, DHHS
P. Yasei, OBCB, DCH, CFSAN, FDA, DHHS
E. Murphy, Jr., Univ. of Texas System, M.D. Anderson Hospital, Houston, TX

LAB/BRANCH

Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

B

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

The major thrust of this study is to elucidate the molecular genetics of neoplastic transformation of normal tissues and the purification and function of two biological modifiers in the cellular defense mechanism. Three experimental systems were used: 1) Two rat retroviruses - a) RHHV, originally isolated in this laboratory and b) WR-RaLV, a wild rat tumor virus; 2) AFB-1 (aflatoxin B-1) interaction with both murine and human DNAs and the subsequent activation of an oncogene; and 3) Interleukin 2 (IL-2), a T-cell product, and CCDF, cytotoxic cell differentiation factor, produced by macrophages for the induction of natural killer (NK)-like cells into cytotoxic cells.

(1) Based on our resolved restriction endonuclease map, we have accomplished the sequence on 3500 nucleotides of various RHHV DNA subgenomic fragment(s) that were found active in recombination with the Kirsten murine sarcoma virus (K-MSV) genome in microinjection studies and critical to the evolution of a transforming virus. We have also resolved the sequence for 650 nucleotides of the WR-RaLV genomic DNA that reflected both the divergent and conservative sequences with a Harvey MSV subgenomic DNA clone in heteroduplex mapping analysis.

(2) The molecular mechanism by which a DNA alkylating agent, AFB-1, activates an oncogene or other cellular genes of both human and murine tissues was investigated. We have identified the human subgenomic DNA fragments of hepatocellular carcinoma that showed preferential binding with AFB-1 at N-7 of deoxyguanine forming DNA-AFB-1 adducts, and, one of which shared extensive homology with the Harvey ras (H-ras) gene.

(3) We succeeded in isolating and purifying murine IL-2 and CCDF by chemical fractionations and column chromatography including HPLC. Using the purified IL-2 and CCDF, the effects of (a) IL-2 on the induction of suppressor T-lymphocytes and (b) CCDF on the differentiation of NK-like cells into cytotoxic lymphocytes, critical to the immune surveillance of tumor growth, have now been better defined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04833-15 LCO
(formerly LCBGY)

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Biological Studies of Various Normal, Virus-infected, and Malignant Cells

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

PI: N. A. Wivel, Senior Investigator, Laboratory of Cellular Oncology, NCI

Other: V. E. Vengris, Visiting Scientist, Div. of Veterinary Drugs, FDA, DHHS
L. W. Redmon, Microbiologist, LCO, NCI

COOPERATING UNITS *(if any)*

P. M. Pitha, Associate Professor, Departments of Microbiology and Oncology,
Johns Hopkins University School of Medicine, Baltimore, MD

LAB/BRANCH

Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.00	1.00	1.00

CHECK APPROPRIATE BOX(ES)

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

B

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

It is the primary purpose of this project to study some of the pertinent factors which influence cell differentiation and malignant transformation, using techniques and approaches which range from the microscopic to the molecular level. Particular emphasis is given to those systems in which murine RNA tumor viruses or chemical carcinogens may be the transforming agent. A variety of mouse model systems are used, including methylcholanthrene-induced sarcomas, plasma cell tumors, mammary tumors, and neuroblastomas. Current projects include: 1) effects of interferon on methylcholanthrene-induced sarcomas of the BALB/c mouse with the aim of defining antitumor activity and relationship to immune response; 2) effects of long term interferon treatment on NIH 3T3 cells transfected with various ras(Ha) related oncogenes; 3) effects of interferon on the assembly and maturation of murine retroviruses with special emphasis on the study of mechanisms whereby virions are rendered non-infectious.

Our results suggest that the major effects of interferon on chemically-induced sarcomas do not appear to be mediated through anticellular activity, but are related to the immune response in the host animal. A number of experiments confirm the necessity of functional T cells in order for interferon to exert its antitumor effect.

A considerable body of our data indicates that interferon affects murine retroviruses during the late stages of virus assembly and release. Even though whole virions are formed there are aberrations in the particle release stage. Those particles which are released have a markedly reduced infectivity which appears to be related to a lack of gp70. Since there is no demonstrable reduction of membrane-associated gp70 in infected interferon-treated cells, it would seem that there is a failure of incorporation of this viral envelope glycoprotein at the virus assembly site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08000-14 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

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

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

PI: Ira Pastan Chief, Laboratory of Molecular Biology NCI

COOPERATING UNITS *(if any)*
University of Oklahoma
Institute of Virology, Tokyo, Japan
Genex Corporation

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.3	.2

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

B

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

cDNA clones coding for the epidermal growth factor receptor were isolated from a cDNA library prepared from A431 cells, a human epidermoid carcinoma. The total sequence of one of these clones which codes for the midportion of the EGF receptor has been determined. Using this clone it has been shown that the EGF receptor is 30-fold amplified in A431 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08001-14 LMB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Role of Cyclic AMP and Transforming Viruses in the Regulation of Cell Behavior

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

PI:	Ira Pastan	Chief, Laboratory of Molecular Biology		NCI
Other:	N. Richert	Senior Investigator	LMB	NCI

COOPERATING UNITS *(if any)*

Dept. of Biochemistry, University of Massachusetts Medical School
Department of Medicine, Duke University Medical School

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Vinculin, a substrate for src kinase has also been shown to be a substrate for protein kinase C. This has been shown using purified protein kinase C and vinculin and in intact cells by stimulating vinculin phosphorylation with phorbol esters.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08006-13 LMB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Control of Gene Expression in Bacteriophage Lambda

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

PI: Max Gottesman Chief, Biochemical Genetics Section LMB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Laboratory of Molecular Biology

SECTION: Biochemical Genetics Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We are continuing our study on the nature of transcription termination in E. coli and of the mechanism of action of the bacteriophage lambda N protein, which suppresses transcription termination.

The phage lambda hin gene product decreases the cyclic AMP levels in E. coli, and has other profound physiological effects. We are attempting to subclone the hin gene and determine the nature of its product.

We are constructing two new vector systems. The first of these is designed to clone DNA fragments bearing promoters active in E. coli. The second, a shuttle vector, is tailored for the cloning of large, and/or unstable DNA fragments, as well as for the reconstitution of genes from overlapping DNA clones.

We are examining the ability of coliphage P1 to stimulate the precise excision of transposons. The responsible gene has been subcloned, and we are attempting to clarify the molecular mechanism of this reaction.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08010-11 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Morphologic Mechanisms of Organelle Function and Transformation in Culture

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

PI: Mark C. Willingham Chief, Ultrastructural Cytochemistry Section LMB,NCI

COOPERATING UNITS *(if any)*

Department of Medicine, Duke University Medical Center, Durham, North Carolina

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Ultrastructural Cytochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Neoplastic transformation produces many changes in cell physiology. Some agents, such as growth-promoting hormones, produce some of these same changes. We have employed morphologic techniques to investigate the mechanism of action of growth-promoting hormones and transforming viruses, as well as the basic cellular mechanisms that regulate the functions commonly altered in neoplastic cells. Endocytosis is a process that regulates the interaction of cells with growth-promoting hormones, such as tumor cell growth factors, and the entry of transforming viruses. In the last year, our study of the pathway of endocytosis in cultured cells has revealed that epidermal growth factor (a growth-promoting hormone)(EGF) and transferrin (a plasma iron-binding protein necessary for cell growth)(TF) are co-internalized in the same pathway into human carcinoma cells through clathrin-coated pits at the cell surface, but diverge from each other in the trans-reticular network of the Golgi system. Cytochemical experiments using electron microscopy have shown that the receptors for EGF and TF are also internalized with the ligands. However, EGF and its receptor have been found to be delivered to lysosomes and degraded, whereas TF and its receptor are recycled intact back to the cell surface. The morphologic divergence of these two ligand-receptor types appears to involve the clathrin-coated pits of the Golgi system. Specialized morphologic studies of the coated pits of the cell surface have shown that images that appeared to be isolated coated vesicles are, in reality, coated pits still connected to the cell surface. Further, the change in shape of these pits has been found to be temperature-dependent. The nucleated erythrocytes of frog and turkey were examined and found to have clathrin-coated pits similar to all other eukaryotic cells, suggesting a possible role for them in the regulation of the surface hormone receptors that have been extensively studied in these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08011-10 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structures and Roles of Transformation-Sensitive Cell Surface Glycoproteins

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

PI: Kenneth M. Yamada Chief, Membrane Biochemistry Section LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Membrane Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	1.7	2.7

CHECK APPROPRIATE BOX(ES)

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

B

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

The glycoprotein fibronectin is usually decreased on tumor cells and is involved in cell adhesion and migration. Fibronectin was present immunologically throughout the animal kingdom, but not in bacteria or plants. Its widespread distribution and conservation suggests its importance, e.g., in cell interactions. The structure of fibronectin was analyzed by a novel surface chemistry approach. Its molecular area was regulated by ionic strength and pH, and calcium ions were essential for maintaining its conformation. These results indicate the importance of ionic and divalent cation-mediated interactions in the structure of this glycoprotein. A dualistic nature of fibronectin in its function as an adhesion protein was discovered. Although it mediated cell adhesion when attached to substrates, fibronectin became an auto-inhibitor of its own function when present at high concentrations in solution. This activity was retained in fragments and even in synthetic peptides. These results suggest that an adhesion protein can be a positive or negative effector depending on its location and concentration, and that the recognition of this glycoprotein may depend on a small peptide sequence. Binding of fibronectin to the cell surface appears to be specific and of moderate affinity. Possible integral membrane protein receptors for fibronectin, especially a 140K protein complex, are being characterized; monoclonal antibodies to the latter antigen inhibited fibronectin-mediated adhesion. In collaborative experiments, the cell surface receptor for insulin was found to be synthesized as a single pro-receptor precursor, then cleaved to form its subunits.

Our future objectives will be to use peptide mapping, immunological criteria, and sequencing of recombinant DNA clones to determine the relatedness of fibronectins, to use synthetic peptides to probe the role of fibronectin in vivo and the function of its putative recognition sequence in vitro, and to identify its receptor using monoclonal antibodies and reconstitution assays.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08700-12 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Collagen Genes in Normal and Transformed Cells

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

Benoit de Crombrugghe, M.D. Chief, Gene Regulation Section, LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)

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

B

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

(1) The Comparison of the DNA sequences of the mouse α2(I) collagen and mouse α1(III) collagen promoter regions reveals only a small number of scattered homologies although these two genes are coordinately transcribed in different tissues and coordinately regulated in several transformed cell lines. In contrast, a segment around the translation initiation site is highly conserved in at least three different collagen genes in two different species, suggesting an additional translational control.

(2) The cloned α2(I) collagen promoter after it has been stably integrated in the genome of mouse cells responds to the same type of down-regulation by oncogenic proteins as the endogenous α2(I) collagen.

(3) Deletions in the mouse α2(I) collagen gene indicate that at least two segments located more that 200 bp and 450 bp upstream of the start of transcription are essential for optimal expression of this gene.

(4) The levels of type III and type I collagen mRNA are coordinately regulated by transformation in some but not all mouse fibroblasts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08702-23 LMB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Endocytosis in the Thyroid Gland

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

Seymour H. Wollman Chief, Cell Organization Section LMB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Cell Organization Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The typical thyroid epithelial cell can take in colloid from the follicular lumen by macropinocytosis. It can also phagocytose red blood cells. We propose to study the mechanism of these processes by electron microscopy, histochemistry and related techniques.

(This project has been suspended during the year, but may be resumed at a later date).

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08704-31 LMB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Thyroid Growth and Involution

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

PI: Seymour H. Wollman Chief, Cell Organization Section LMB NCI

COOPERATING UNITS *(if any)*

Lucio Nitsch, Istituto di Patologia Generale, Universita di Napoli, Naples, Italy

Corrado Garbi, Istituto di Patologia Generale, Universita di Napoli, Naples, Italy

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Cell Organization Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have observed that the lumens of inverted follicles undergo periods of slow dilation followed by rapid shrinkage. The behavior is what would be expected from a closed follicle surrounded by cells that transport fluid into the lumen until a hole is produced through which the transported fluid leaks out rapidly. The hole seals, and the process is repeated.

We have evidence that the microvilli-bearing surface of the inverted follicles has collagen receptors, although the surface never comes into contact with collagen normally.

Separated thyroid follicles are unstable when embedded in a collagen gel. Single cells migrate away from many of these follicles. The migratory cells appear to be of epithelial origin from studies using labeled antibodies.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08705-08 LMB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Genetic and Biochemical Analysis of Cell Behavior

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

Michael M. Gottesman Chief, Molecular Cell Genetics Section LMB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Molecular Cell Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We are utilizing the Chinese hamster ovary (CHO) fibroblast to study the genetics and biochemistry of some aspects of the behavior of cultured cells. Our work has emphasized morphology and its relationship to growth control, and response to cyclic AMP and transforming viruses. We have isolated a variety of different mutants with altered microtubules which express mutated α- or β-tubulin subunits. These mutants are defective in spindle formation because the mutant tubulins are incorporated into spindle microtubules. We have also established two cell systems for examining the ways in which AMP can positively and negatively regulate cell growth. CHO cell growth is inhibited by cAMP; mutants selected for resistance to growth inhibition have defective cAMP dependent protein kinases (cAdepPK). Analysis of these mutants and their revertants indicates that all known cAMP effects are blocked by the kinase mutations. In contrast, drugs such as interferon and tumor promoters which raise cAMP levels, are still able to exert their effects in cAMP-resistant CHO cells, indicating that the major mechanism of action of these drugs is independent of cAdepPK. We have used DNA from cells carrying dominant cAMP-resistant defects to transfer the cAMP-resistance phenotype to sensitive cells as a first step toward the cloning of the genes which make our mutants cAMP-resistant. CHO cells malignantly transformed by RSV are also cAMP-resistant. Formation of tumors by CHO-RSV cells is dependent on prior treatment with cholera toxin which raises cAMP levels within the cells. This increased tumorigenicity is an example of positive regulation of cell growth by cAMP which correlates with phosphorylation of pp60^{src} and activation of its tyrosine kinase activity.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08706-13 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alteration in Gene Expression During Mammary Gland Tumorigenesis

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

Gilbert H. Smith Research Biologist LMB NCI

COOPERATING UNITS *(if any)*

Department of Cell Biology, Baylor College of Medicine, Houston, Texas

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Our laboratory has been studying alterations in MMTV proviral gene expression during mammary gland tumorigenesis in a "clean" inbred mouse strain (C3H/Sm). This strain is of interest because there is "normal" expression of MMTV RNA transcripts in their mammary glands but no viral proteins or virions are produced. We have previously shown that the abundance of these endogenous MMTV transcripts is increased both in mammary tumors induced by chemicals and/or pituitary isografts and in spontaneous mammary tumors from old untreated multiparous C3H/Sm females. By far the most abundant MMTV transcript in these neoplasms was an anomalous 2.2 Kb RNA containing MMTV LTR sequences exclusively. This observation has been extended to include mammary preneoplasias in C3H/Sm mice (hyperplastic alveolar outgrowths) which were induced by hormonal or chemical treatment in virgin female mice. We have demonstrated that these preneoplastic lesions anachronistically possess enhanced activities of their casein and α-lactalbumin genes in the absence of hormone stimulation or pregnancy. Futher study has demonstrated that these preneoplastic mammary lesions release humoral factors which profoundly effect the growth and development of normal mammary tissue in the distal mammary fat pads of their host. The relationship between these altered gene regulatory processes following transformation and the increased expression of MMTV LTR RNA expression is under study.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08709-09 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NAD^+ Metabolism and ADP-ribosylation of Proteins

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

George S. Johnson Research Chemist LMB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Cell Genetics Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

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

B

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

The steroid-receptor protein complex is believed to activate gene transcription by binding to unique DNA sequences within promoter sequences of certain genomes. Research in the present intramural project has uncovered a novel aspect in this activation mechanism--covalent addition to and removal of ADP-ribose from chromosomal proteins are essential control points. During the current time period we have found that certain ADP-ribosylated chromosomal proteins are removed from chromatin by mild digestion with nuclease demonstrating that these modified proteins are not randomly distributed throughout chromatin but rather are probably associated with actively transcribed genes. Glucocorticoids are more effective genomic activators and even glucocorticoid antagonists can function as agonists in cells devoid of ADP-ribosylated proteins suggesting that ADP-ribosylated chromosomal proteins may hinder receptor binding to chromatin and activation of genomes. Nicotinamide and its derivaties also activate a glucocorticoid promoter replicating as an episome.

The carcinogen N-methyl-N'-nitro-N-nitrosoguanidine rapidly depletes NAD levels in cells by activation of (ADP-ribose)n synthetase and not by causing extrusion of NAD into the culture media. Endogenous ADP-ribosylation of some but not all chromosomal proteins is increased by MNNG treatment.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08710-09 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI: Sue Wickner Research Chemist LMB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

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

B

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

The molecular mechanisms involved in DNA replication are being studied. Proteins involved in both phage lambda and E. coli replication are being purified and characterized in vitro. The present emphasis is to gain insight into the process of initiation of chromosome replication. The initiation of double-stranded λ dv plasmid DNA is being studied in vitro. This reaction requires two phage initiation proteins, O and P gene products, host proteins, RNA transcription in the region of the origin of replication and a specific site, ori, on the λ DNA. By analysis of in vitro constructed deletions of the ori region, I have found the minimal piece of DNA that functions in initiating DNA replication in vitro by an O and P dependent reaction. This piece of DNA is 95 base pairs and contains two O binding sites followed on the right by a region rich in adenine. I have also confirmed the domain structure of λ O suggested by genetic experiments. The DNA binding domain resides in the amino-terminal portion of O as shown by DNA protection experiments with the purified amino-terminal half of O protein. P protein most likely interacts with O protein through the carboxy-terminal portion of O since P protein forms a complex with intact O protein but not with the amino-terminal fragment of O.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08712-09 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Plasma Membrane Proteins in the Regulation of Cell Behavior

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

PI:	Ira Pastan	Chief, Laboratory of Molecular Biology		NCI
Other:	M. Willingham	Chief, UCS	LMB	NCI
	N. Richert	Senior Investigator	LMB	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIH, NCI, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4.7	1.3

CHECK APPROPRIATE BOX(ES)

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

B

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

EGF induces EGF receptor internalization. The ligand and its receptor traverse receptosomes and the transreticular Golgi on their way to lysosomes where they are both destroyed. Each EGF that enters and is destroyed is accompanied by one receptor. Thus there is no recycling of the recptor. Transferrin and its receptor also enter via coated pits and receptosomes and reach the TR Golgi where they are sorted away from EGF and its receptor. Sorting occurs in the TR Golgi. The coated pits of the TR Golgi have an important role in this process.

The α2 macroglobulin receptor has been purified by conventional and affinity chromatography and has been shown to have a subunit M_r of ~ 85K daltons.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08714-07 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Mode of Action of a Bacterial Function Involved in Cell Growth Control

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

PI: Susan Gottesman Research Chemist LMB, NCI

COOPERATING UNITS *(if any)*

Drs. Richard D'Ari and Olivier Huisman, Institut Jacques Monod, Paris, France

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	3.8	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have been studying the role that protein degradation plays in regulating cell growth control, through the study of a mutant defective in protein degradation, the E. coli lon mutant. This strain is defective in cell division regulation after DNA damage; we have demonstrated that this defect is due to accumulation of a highly unstable cell division inhibitor, the product of the SulA gene. We have demonstrated that overproduction of SulA is sufficient to stop formation of septa in E. coli, and have genetically identified the probable target of SulA action. SulA protein has been purified from cells which overproduce SulA, and antibody raised to the purified protein. This will allow future detection of SulA degradation patterns in vivo and development of in vitro assays for SulA degradation. lon mutants also overproduce capsular polysaccharide, and we have developed a system for the simple assay of the regulation of the genes necessary for capsule synthesis (cps) using cps::lac operon fusions. Using these strains, we have isolated and mapped mutations in three genes which regulate capsule synthesis (cpsR, cpsS, and cpsT). From genetic experiments, we have demonstrated the existence of a cascade of regulatory interactions to regulate transcription from the cps structural genes. Future work will allow us to examine this cascade in vitro, and identify the precise role of lon. Using insertional mutagenesis, we have isolated null mutations in lon, demonstrating for the first time the dispensability of this gene for E. coli growth. Our analysis of the proteolysis defect in these mutants will allow us to identify other proteases in the cell with properties similar to lon.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08715-06 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Synthesis of a Transformation-Dependent Secreted Glycoprotein

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

Michael M. Gottesman Chief, Molecular Cell Genetics Section LMB NCI

COOPERATING UNITS *(if any)*
Laboratory of Experimental Pathology, DCCP, NCI

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Cell Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Cultured mouse fibroblasts which are transformed by RNA viruses, a DNA virus or a chemical agent, all secrete a 39,000 Mr-phosphoglycoprotein (major excreted protein, MEP) in large amounts. Nontransformed murine fibroblasts secrete MEP after treatment with tumor promoters such as TPA or growth factors such as PDGF. Human cells also synthesize MEP, and in the case of cultured human melanocytes, its synthesis appears to be increased by TPA treatment. We have purified MEP, prepared monospecific affinity-purified antisera against it and cloned a cDNA which codes for MEP from Chinese hamster and mouse cells. The protein contains mannose 6-phosphate, the lysosomal recognition marker. It is processed intracellularly in both transformed and nontransformed cells to give two specific lower molecular weight forms, the lowest of which has a predominantly lysosomal localization. Transformation, TPA and PDGF stimulate MEP synthesis by increasing levels of MEP specific mRNA. We are studying this system as a model of regulation of lysosomal protein synthesis, processing and secretion as it is affected by transformation and agents which mimic the transformed state, such as tumor promoters and growth factors.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08717-06 LMB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Protease Cleavage Analyses and Carbohydrate Functions of Cell Adhesion Proteins

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

PI: Kenneth M. Yamada Chief, Membrane Biochemistry Section LMB, NCI

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Molecular Biology

SECTION: Membrane Biochemistry Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.3	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Proteases, monoclonal antibodies, and glycosylation inhibitors have provided probes of the organization and activities of adhesive glycoproteins. Protease-resistant and susceptible regions were identified; fibronectin molecules from chicken and human sources were found to retain nearly-identical functional domains for binding to collagen, fibrin, heparin, and other molecules, yet to display variable interdomain regions. These results suggest the evolutionary conservation of functional domains, but not of interconnecting, flexible interdomain polypeptide regions. Retinoic acid increases cell adhesion and may inhibit tumorigenesis. Its molecular mechanism of action was examined in chondrocytes, where it was found to alter glycosylation of fibronectin. Chondrocyte fibronectin was characterized by high-mannose or hybrid N-linked oligosaccharide chains. Treatment with retinoic acid caused a reversion of the structure of these chains to the complex form characteristic of fibroblast fibronectin. These results appear to be the first demonstration of retinoic acid regulation of the glycosylation of a specific glycoprotein. Additional experiments in progress are using the glycosylation inhibitor tunicamycin to help identify critical functional sites on fibronectin for binding to collagen, as well as proteases to generate mitogenic activity from fibronectin. A monoclonal antibody library for membrane proteins identified in immunoblots has been established, and is being applied to identify and characterize new glycoconjugates.

Our objectives will be to continue to use proteases and glycoconjugate probes to identify a small, functionally-essential site in fibronectin for its binding to collagen and to test the role of carbohydrates in protection of function, to determine the origin of the mitogenic activity of proteolytically-cleaved fibronectin, and to use the monoclonal antibody library to characterize novel glyconjugate functions, especially in cell adhesion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08719-05 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development and Uses of Eukaryotic Vectors

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

Bruce H. Howard Chief, Molecular Genetics Section LMB NCI

COOPERATING UNITS *(if any)*

LMV, DCCP, NCI

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We developed techniques for efficient DNA-mediated transfer of genes into primate cells and used these techniques to search for DNA sequences that regulate mammalian cell growth. Studies on growth-stimulatory genes focused on complementation of mutant human c-ras by the adenovirus E1A gene, and involved efforts to detect complementation of c-ras by gene(s) present in preneoplastic or tumor cells. Studies on growth-inhibitory genes focused on sequences present in WI38 human embryo fibroblast DNA that are capable of slowing HeLa S3 cell growth. It was shown that WI38 growth-inhibitory sequences are active in primary and secondary gene transfer experiments; in addition, growth-inhibitory sequences were detected in a cosmid library derived from WI38 DNA. Continuing efforts to improve gene transfer technology included development of improved selection conditions for methotrexate-resistance vectors, further application of a novel vector system based on the bacteriophage lambda lysogenic cycle, and construction of retrovirus vectors carrying dihydrofolate reductase or chloramphenicol acetyltransferase genes.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08750-04 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Genetic Regulatory Mechanisms in Escherichia Coli and Its Bacteriophage

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

PI: Sankar Adhya Chief, Developmental Genetics Section LMB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The cya, crp, rho and nus gene products modulate the expression of a wide variety of bacterial and bacteriophage genes or operons. In order to understand the regulatory processes, we are studying the structure, expression and activity of these genes. We have previously shown that the protein products of the cya, crp and rho genes are autogenously regulated.

We have shown by pulse labeling of RNA and by DNA-RNA hybridization, as well as by operon fusion analysis, that the autogenous regulation of rho is at the level of transcription. We have also found, by similar analysis, that cyclic AMP is a positive effector for Rho gene expression, but also acts as a "repressor" of rho mRNA translation. This opposite control of the rho gene by cyclic AMP would maintain a constant level of Rho in cell. We are currently studying the system in vitro.

We have isolated mutants in the crp gene, which make the CRP protein functional in the absence of cyclic AMP. The mutations are now being sequenced. The amino acid changes in these altered CRPs would allow us to understand the molecular mechanism of gene activation by CRP.

We have found that a NusA amber fragment is still functional for anti-termination, when expressed from a multi-copy plasmid. This suggests that it is the NH_2-terminal portion of NusA that is important for anti-termination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08751-04 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of the gal Operon of Escherichia Coli

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

PI: Sankar Adhya Chief, Developmental Genetics Section LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Developmental Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We are studying the control mechanisms of the expression of the gal operon of E. coli. We have so far shown that the operon is controlled by two promoters, which are modulated by cyclic AMP in opposite ways. Both the promoters are negatively regulated by a gal repressor protein. From our genetic and DNA sequence studies, we had previously proposed that each of the two gal promoters is negatively regulated by two operator elements, one of which (O_E) is located upstream to the promoters and the other (O_I) inside the galE structural gene. Using a new polyacrylamide gel electrophoresis method for studying DNA-protein interactions, we have now demonstrated sequence-specific binding of purified gal repressor to both O_E^+ and O_I^+ DNA but not to mutant O_E^C and O_I^C DNA. By DNase protection experiments we have visualized repressor binding to O_E at -50 to -73 positions and to O_I at +45 to +68 positions of the gal DNA. This confirms the genetically assigned operator role of O_E and O_I. We have also shown that repressor does not compete with cAMP·CRP or RNA Polymerase to bind to gal DNA, suggesting steric hindrance as an unlikely mechanism for gal repression.

We have also determined the complete DNA sequence of the entire gal operon by Dideoxy method of Sanger.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB8752-04

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Mechanism of the Transport of Thyroid Hormones into Animal Cells

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

Sheue-yann Cheng Research Chemist LMB NCI

COOPERATING UNITS *(if any)*

National Biomedical ESR Center; Department of Radiology; Medical College of Wisconsin; Milwaukee, Wisconsin 53226

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	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.)*

We have shown previously the presence of plasma membrane receptors for 3,3',5-triiodo-L-thyronine (T_3) in cultured cells. To explore the possibility of using human placenta or Swarm rat chondrosarcoma as the source for the large scale purification of the plasma membrane T_3 receptors, the binding of T_3 on chondrocytes and the purified plasma membranes of human placenta was characterized. Two classes of specific T_3 binding sites were detected on human placenta and chondrocytes: a high affinity binding site with a Kd of 2.0 nM and 0.5 nM, respectively and a low affinity binding site with a Kd of 18.5 μM and 0.2 μM, respectively. By the affinity labeling, peptide mapping and immunoprecipitation, the plasma membrane T_3 receptors in human placenta and chondrocytes were shown to be similar to those of cultured cells. These results indicate that either tissue can be used as a source for the purification of T_3 receptors.

Polyclonal antibodies against the plasma membrane T_3 receptors were developed using intact GH_3 cells, purified plasma membranes of human placenta and GH_3 cells.

Using electron spin resonance (ESR) and the biologically active spin-labeled T_3 ($SL\text{-}T_3$), the transverse motion (flip-flop) of T_3 in dipalmitoylphosphatidyl choline (DPPC) membranes was examined. The results indicate that $SL\text{-}T_3$ does not flip-flop at any appreciable rate in the membranes. The data suggest that once partitioned into a cell membrane, T_3 would remain in the outer half of the lipid bilayer. This result diminishes the possibility that T_3 enters the cell by passive diffusion and further strengthens the conclusion that the entry of T_3 into cells occurs by a receptor-mediated process.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08753-02 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

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

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

PI:	Ira Pastan	Chief, Laboratory of Molecular Biology,		NCI
Other:	M. Willingham	Chief, UCS	LMB	NCI
	M. Gottesman	Chief, MCGS	LMB	NCI

COOPERATING UNITS *(if any)* Columbia Medical School (College of Physicans & Surgeons)
The Salk Institute, San Diego, California
U.S. Army Medical Research Institute of Infective Diseases, Fort Detrick, MD
Medicine Branch, DT, NCI
Cetus Corporation
Metabolism Branch, DCBD, NCI

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIH, NCI, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.1	4.1	1.0

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

B

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

Pseudomonas toxin has been coupled to monoclonal antibodies to make immunotoxins. When coupled to an antibody to the T cell growth factor receptor to make anti-TAC-PE, it kills leukemia cells that are TAC positive. When coupled to an antibody to the human transferrin receptor (anti-TFR-PE), it kills various tumor cell lines. Cell killing is related to the number of molecules bound and taken into cells.

Adenovirus enters cells in the same vesicle as these immunotoxins. By lysing this vesicle, adenovirus efficiently releases the immunotoxins into the cytosol and selectively increases cell killing. The penton base of adenovirus is important for vesicle lysis.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08754-01 LMB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Genetic Analysis of the Multiple Drug Resistance Phenotype in Tumor Cells

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

PI: Michael M. Gottesman, Chief, Molecular Cell Genetics Section LMB NCI

Other: Ira H. Pastan, Chief, Laboratory of Molecular Biology NCI

COOPERATING UNITS *(if any)*

Division of Cancer Therapy, NCI

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Molecular Cell Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

During the course of chemotherapy of human cancers, variants which are resistant to multiple drugs frequently arise. We have been investigating the genetic and biochemical basis for this pleiotropic resistance of human tumor cells to chemotherapeutic agents. A model system using the cultured KB cell, a human carcinoma cell line, has been developed in which mutant cells selected for resistance to high levels of colchicine have also been found to be resistant to adriamycin, vincristine, vinblastine, puromycin and actinomycin-D. This multiple drug-resistance has been demonstrated to be a co-dominant genetic characteristic in somatic cell hybrids. Resistance to colchicine has been successfully transferred to sensitive human and mouse cells using DNA derived from these drug-resistant human cells. Uptake of several of the drugs involved in the multiple drug-resistance phenotype has been shown to be reduced in the KB cells. Drug-resistance correlates with the appearance of a family of high molecular weight glycoproteins on the cell surface detected by a monoclonal antibody.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05211-12 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Poly(ADP-ribose) and Chromatin structure and Function.

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

PI: W.R. Kidwell — Chief, Cell Cycle Regulation Section, LPP, NCI

Other Professional Personnel:

M.R. Purnell	Visiting Fellow	LPP, NCI
Joel Moss	Sr. Staff Scientist	HIR CM

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Poly(ADP-ribose) synthetase is a chromatin bound enzyme that adds chains of ADP-ribose in tandem to nuclear proteins. This enzyme is activated by DNA damaging agents such as gamma, x-ray and u.v. irradiation and by DNA alkylating agents. We have synthesized and tested 6 compounds which are inhibitors of the synthetase and found that the ability of 4 of 6 of them to block DNA repair is directly correlated with the compound's potency as a synthetase inhibitor. The compounds ranked in order of their ability to block DNA repair are 3-acetl-aminobenzamide> 3-hydroxybenzamide= benzamide>>3-aminobenzamide. 3-nitroben-amide, was found to be much more inhibitory for the repair of DNA chain breaks than was expected based on its potency as a poly(ADP-ribose) synthetase inhibi-tor. Plots of the reciprocal of the repair velocity vs inhibitor concentration normalized against its k_i for synthetase were made. These plots were biphasic indicating that the benzamides had effects on more than one cellular process. At least two processes other than DNA Repair have been implicated as targets. These are RNA synthesis and glutamine synthetase. The latter enzyme was found to be an acceptor protein for mono-ADP-ribose. The enzyme catalyzing this reaction was also found to be inhibited by the benzamides, through their potency as inhibitors for this enzyme was much less than their potency as synthetase inhibitors.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05216-13 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cyclic AMP Binding Proteins in Breast Cancer

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

PI:	Y.S. Cho-Chung	Chief, Cellular Biochemistry Section	LPP, NCI
Other Professional Personnel:	T. Clair	Chemist	LPP, NCI
	M. E. Lippman	Chief, Med. Breast Cancer	M, NCI
	C. L. Kapoor	Lab. Visual Research	LVR, EI

COOPERATING UNITS *(if any)*

Dr. W. R. Miller, U. of Edinburgh, Scotland; and Dr. S. O. Døskeland, U. of Bergen, Norway

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cellular Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Cyclic adenosine 3',5'-monophosphate (cAMP) and its binding proteins are involved in the regulation of the growth of mammary tumors in experimental animals (Cho-Chung, Cancer Res. 38: 4071, 1978). Whilst human breast cancers which possess estrogen receptor (ER) and progesterone receptor (PgR) activities are likely to be hormone responsive, many do not respond to endocrine therapy. In hormone-dependent rat mammary tumors the ratio of steroid receptors to cAMP binding proteins was found to better discriminate hormone dependent from independent tumors than steroid receptor alone.

In this study we will investigate the relationship between cAMP binding proteins, ER and PgR in human breast cancers and clinical parameters including prognosis. Several molecular species as well as proteolytic fragments of cAMP binding proteins have been found in normal and neoplastic tissues. Thus, molecular species of cAMP binding proteins will be determined by utilizing the photo-affinity ligand, 8-azido-[^{32}P]cAMP and immunoprecipitation using affinity purified antibodies to cAMP binding proteins. Utilizing immunocytochemical method intracellular distribution and nuclear compartmentalization of cAMP binding proteins will be also determined. Finally, in a cell-free system, the binding of cAMP binding proteins directly to DNA of normal vs cancer cells will be studied utilizing molecular biology techniques. The goal of this proposal is to provide us a fundamental growth regulatory mechanism of cAMP action in breast cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05219-13 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

In Vitro Simulation of Hormone-dependent Mammary Tumor Regression

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

PI:	R.A. Knazek	Sr. Investigator	LPP, NCI
Other Professional Personnel:	S.C. Liu	Chemist	LPP, NCI
	J.R. Dave	Visiting Fellow	LPP, NCI
	W.B. Rizzo	Clinical Associate	DP, NICHD
	J.D. Schulman	Senior Investigator	DP, NICHD

COOPERATING UNITS *(if any)*

J. Costa, Director (Institut de pathologie, CHUV, Lausanne, Switzerland)

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cell Cycle Regulation

INSTITUTE AND LOCATION

NCI,NIH, Bethesda, Md 20205 & Institut de pathologie,CHUV, Lausanne, Switzerland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Alterations of the hormone-receptors on or within cells will modify the response of target tissues to various hormones thus serving to control cellular growth or function. We have shown that PRL up-regulates its own receptors by modifying target membrane fluidity and that this may occur through modification of prostaglandin synthesis. Extended to the DMBA rat mammary tumor, the regressing tumor membranes are more viscous and bind less PRL than those of the growing tumor. An assay for PG receptor has been developed, which has demonstrated both that these regressing tumors have an increased capacity to bind PG and that copper increases this binding capacity 8-fold. Copper may, thus augment the effect of prostaglandins in vivo and play a role in tumor growth. Patients afflicted with adrenoleukodystrophy or adrenomyeloneuropathy have an inborn propensity to accumulate long chain saturated fatty acids in their cellular membranes. We have demonstrated that this occurs within erythrocytes and thus alters the fluidity of these membranes. Such changes in membrane fluidity may reflect similar changes within the adrenal and gonads and may account for the states of adrenal and gonadal failure observed in these patients. Thus, our studies show quite clearly that membrane-associated receptors are modulated by alteration of membrane fluidity. Metabolism of arachidonic acid by six human tumors is directed toward the lipoxygenase pathway in preference to that of the prostaglandins. The synthesis of prostaglandins by these tumors appears to be determined by relative deficiences in $PGF_{2\alpha}$ isomerase and probable deficiency in phospholipase A_2 and/or cycloxygenase activity. Each tumor type metabolizes arachidonic acid in an unique fashion suggesting that various modes of therapeutic intervention may be employed through the use of agents that inhibit specific enzymes within these pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08215-07 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Angiogenesis and tumor growth

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

PI:	K. Raju	Staff Fellow	LPP, NCI
Other Professional Personnel:	P.M. Gullino	Chief,	LPP, NCI
	S. Ungari	Visiting Fellow	LPP, NCI
	G. Alessandri	Visiting Fellow	LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The mechanism of the angiogenic response has been further studied by extending the search for chemically defined compounds able to mobilize capillary endothelium. The chemotactic activity on endothelium of extract from PGE_1 treated corneas was found to bind to a gelatin-sepharose affinity column. On the assumption that fibronectin could be involved in the chemotactic event we established that (a) mobilization of endothelium by a chemoattractant is most efficient when fibronectin forms the substratum on which the endothelium adheres (b) monoclonal antifibronectin serum blocks the mobilization of capillary endothelium on fibronectin substrate (c) Fibronectin alone or heparin alone do not mobilize capillary endothelium but in combination they substantially enhance mobilization (d) The whole molecule of both fibronectin or heparin is not necessary for the mobilization, fragments of both molecules are sufficient. The characteristics of these fragments are being investigated. The work of the past year has produced evidence to sustain the hypothesis that molecules normally present in the extracellular compartment may act as angiogenesis factors when fragmented by lytic enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08220-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Gene Structure and Sequence of α-Lactalbumin, Whey Phosphoprotein and κ-Protein

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

PI: P. K. Qasba LPP, NCI

Other Professional Personnel: S. Matarazzo Staff Fellow LPP, NCI

P. Hutzell Microbiologist LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.75	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Using the cDNA clones for α-lactalbumin, whey phosphoprotein and κ-protein, we have screened a bacteriophage Charon 4A/rat partial EcoRI genomic library and isolated phages containing genomic DNA corresponding to these cDNA's. The genomic maps for the three individual genes have been established from the phages carrying overlapping DNA fragments. Entire α-lactalbumin gene sequence has been determined and compared with the chicken lysozyme gene, since it was proposed that the two genes have arisen from a common ancestral gene. These results show that: a) the 5'-flanking sequence of α-lactalbumin gene contains almost identical short repeat sequences; b) a nanonucleotide sequence ATCCCTTTC is repeated 3 times which resembles with the part of 19 nucleotide concensus sequence ATC^{CC} ATTT^{A}TCTG^{G}TTGTA thought to be involved in the progesterone receptor recognition site in ovalbumin gene; c) both, α-lactalbumin and lysozyme genes contain 3 introns at similar positions; d) the first three exons of the two genes show high nucleotide homologies and are of comparable lengths and e) the fourth exon of α-lactalbumin, which codes for the amino acid residues essential for its interaction with galactosyltransferase, is markedly different from the corresponding exon of lysozyme and is preceded by two $(TG)_n$ repeats, $(TG)_{25}$ and $(TG)_{22}$. It is suggested that the 4th exon of α-LA coding for a new functional unit, might have replaced the DNA region of a primordial lysozyme gene and led to a protein with a new function.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08226-08 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Hormones and Growth Factors in Development of Mammary Glands & Tumorigenesis

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

PI:	B.K. Vonderhaar	Research Chemist	LPP, NCI
Other Professional Personnel:	E. Ginsburg	Biologist	LPP, NCI
	W. Kidwell	Biologist	LPP, NCI
	H. Nakhasi	Staff Fellow	LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.75	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

This project is designed to understand the role of hormones and growth factors in normal mammary gland development and differentiation. We wish to understand how milk-protein production is controlled by various hormones. Studies include: 1) examination of the role of thyroid hormones, adrenal steroids and Vitamin D in synthesis and secretion of milk proteins in organ culture, 2) examination of the role of epidermal growth factor and mammary gland-derived growth factors in lobulo-alveolar development of the immature mouse mammary gland, 3) defining the roles of estrogen and progesterone in priming the mammary tissue prior to whole organ culture to determine their effects on induction of EGF receptors, mammary gland-derived growth factor receptors and the production of growth factors by the animals.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08229-08 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Role of Dietary Lipids in Mammary Cancer

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

PI: W. R. Kidwell Chief, Cell Cycle Regulation Section LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cell Cycle Regulation Branch

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Growth regulation of the mammary epithelium of both normal and neoplastic states is a complex process involving the interaction between the epithelium and stromal cell populations, including the adipocytes. These interactions may be important in the process of preneoplastic to neoplastic conversion of mammary epithelium and the role of dietary lipids in this process. Experiments to date indicate that the epithelium is dependent on essential fatty acids for proliferation and that prolactin stimulated epithelium recruits these fatty acids from mammary adipocytes. Prolactin's role in this process appears to be mediated by signals from the epithelium directed at mast cells in the gland. The activated mast cells release histamine and this compound then triggers the release of fatty acids from the proximal adipocytes. The prolactin activated epithelial cells then selectively take up the unsaturated fatty acids. Part of these are inserted into membrane phospholipids with the consequent stimulation of cell growth. Some of the essential fatty acids are converted to prostaglandins. Of these, prostaglandin E_1 is a potent growth stimulator of the epithelium. The essential fatty acids thus appear to be important for mammary cell growth by serving as membrane structural components and as substrates for prostaglandin synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08249-05 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Hormonal Control of Growth of Normal and Neoplastic Mammary Cells

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

PI: William R. Kidwell, Chief,Cell Cycle Regulation Section LPP, NCI

Other Professional Personnel:

M. Bano	Visiting Fellow	LPP, NCI
D. Salomon	Expert	LTB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A growth factor has been purified to apparent homogeneity from human milk and primary human mammary tumors. The factor appears to be a new, as yet underscribed, protein. It has a molecular weight of 62,000 and pI of 4.8. Because of the tissue of origin, we have named it mammary derived growth factor 1, MDGF1. MDGF-1 stimulates the growth of normal mammary cells and mammary tumor cells such as MCF-7. Optimal response is seen at about 10 ng/ml. The factor appears to act synergistically with estrogen since mammary epithelium from estrogen primed animals is responsive while that from unprimed animals is not. 125I-MDGF1 binds specifically to high affinity receptors on cell membranes. In addition to stimulating growth, the factor differentially amplifys collagen synthesis as much as 7 fold. In NRK cells the differential stimulation of collagen synthesis is produced via a MDGF-1 stimulation of collagen mRNA production. Mammary cell responsiveness is substratum dependent, being manifest on stromal collagen or tissue culture plastic but not on a basement membrane collagen substratum. These findings suggest that responsiveness to MDGF-1 might be facilitated as proliferating mammary cells penetrate through the basement membrane and contact the stroma. Such a mechanism might explain how a new basement membrane is synthesized by proliferating epithelium.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08250-04 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

'Label-fracture'--A method for high resolution labelling of cell surfaces

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

PI: P. Pinto da Silva Chief, Membrane Biology LPP, NCI

Other Professional Personnel: F. Kan Visiting Fellow LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, MD 21701

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have in the past year developed a new technique "label-fracture" which allows the observation of the distribution of a cytochemical label of antigens and receptors on cell surfaces. Cells are fixed in glutaraldehyde and labeled with an electron dense marker (colloidal gold). They are then frozen, freeze-fractured and replicated by platinum/carbon evaporation. The exoplasmic halves of the membrane, stabilized by the deposition of the Pt/C replica, are washed in distilled water. Mounted on formvar coated grids and then examined on an electron microscope. This new technique reveals the surface distribution of the label coincident with the Pt/C replica of the exoplasmic fractured face. We are now applying this technique to study the cell surface microdomains of sperm and insulin binding sites on the surface of animal fat cells. "Label-fracture" has extraordinary resolution (avg. <5nm) and will allow mapping at the supramolecular level of receptors and antigens on cell surfaces while relating directly to the freeze-fracture morphology of the plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08251-05 LPP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Growth Factor Production by Neoplastic Rat Mammary Epithelial Cells

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

PI:	W. R. Kidwell, Chief Cell Cycle Regulation Section		LPP NCI
Other Professional Personnel:	J. Zwiebel	PHS Fellow	LPP, NCI
	M. Bano	Visiting Fellow	LPP, NCI
	D. Salomon	Expert	LTB, NCI
	Graeme Bell	Scientist	Chiron Corp.

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Pathophysiology

SECTION
Cell Cycle Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

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

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

B

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

The ability of cells to proliferate independently of a surface substratum is a property that distinguishes transformed cells from normal cells. Current thinking is that this ability of tumor cells is brought about by the production in tumor cells of anchorage-indepent growth conferring factors, or transforming growth factors (TGF). New observations that we and others have made indicate that normal tissues in addition to tumor tissues can make TGF. For example, we have found that TGF activities are made by or accumulate in proliferating bovine mammary gland, in rat, mouse and human adenocarcinomas and in fact are present in large amounts in human milk. The TGF activities in human milk and human mammary tumors have been partially purified. The major species from the two sources have identical PI's and are probably the same protein. Milk TGF has been purified about 2000 fold using isoelectric focusing, HPLC gel permeation and reverse phase chromatography. The activity is similar to human EGF in its size, its insensitivity to heat and in its inactiviation by proteases and disulfide reducing agent. If differs from human EGF in its pI and in its potency in promoting anchorage independent growth of NRK cells.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08268-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Structure, Topology, and Dynamics of Tight Junctions

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

PI: P. Pinto da Silva, Chief, Membrane Biology Sec. LPP, NCI

Other Professional Personnel: None

COOPERATING UNITS *(if any)*

Dr. J. Chevalier, Laboratoire d'Hemostase et de Thrombose, Institut de Recherches sur les Maladies du Sang, Paris, France

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The experimental portions of this project has been temporarily interrupted because of lack of personnel and of access to freeze-fracture equipment (placed in storage since March 1983). Work will pursue in 1984 to the extent that laboratory and personnel conditions are made available.

HHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08269-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Membrane differentiation: Role of Integral Components in Membrane Domains

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

PI: P. Pinto da Silva, Chief, Membrane Biology Sec. LPP, NCI

Other Professional Personnel: A.P. Aguas Visiting Fellow LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, MD 21701

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We investigated the participation of transmembrane proteins on the expression of chemically-homologous domains on the surface of mammalian cells. Conjugates of lectins and colloidal gold were employed to label intact, hypotonic-disrupted, and freeze-fractured membranes. The precise location of the lectin-gold label was analysed in situ by electron microscopy. To study the a regionalization of surface components, a highly polarized cell (the spermatazoon) was choosen as the first experimental model. Our results showed that: a) the large intramembrane particles seen on freeze-fracture faces of cells are the morphological counterpart of integral membrane sialo-proteins; b) the surface of flagella has a high density of transmembrane glycoproteins that may be involved in the transduction of movement from the cytoskeleton to surface exposed elements; c) lysosomal and plasma membrane may express unequal complements of fully glycosilated components.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08270-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Fracture-label: A comparative study of membrane glycocomponents in duodenum

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

PI: P. Pinto da Silva Chief, Membrane Biology Section LPP, NCI

Other Professional Personnel: F. Kan Visiting Fellow LPP, NCI

COOPERATING UNITS *(if any)*

Dr. M.R. Torrisi, Institute of General Pathology, University of Rome

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, MD 21701

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Thin section fracture-label was used to determine the distribution of wheat germ agglutinin (WGA) binding sites in intracellular membranes of secretory and non-secretory rat tissues as well as in human leukocytes. In all cases, analysis of the distribution of WGA led to the definition of two endomembrane compartments: one, characterized by absence of the label, includes the membranes of mitochondria and peroxisomes as well as those of the endoplasmic reticulum and nuclear envelope; the other, strongly labeled, comprises the membrane of lysosomes, phagocytic vacuoles, and secretory granules, as well as the plasma membrane. The Golgi apparatus was weakly labelled in all studied tissues. This appears to reflect the short lived presence of fully glycosylated membrane proteins in this organelle.

The fracture-label technique was also used to determine the distribution of Concanavalin A (Con A), wheat germ agglutinin (WGA) and Ulex Europaeus 1 (UEA 1) binding sites in the plasma membranes, intracellular membranes as well as secretory products of duodenal columnar and goblet cells. Emphasis was placed on the comparison of labeling density of various lectin binding sites over the plasma and intracellular membranes.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08271-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

T Lymphocyte Heterogeneity: Characterization of T cell leukemia

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

PI: P. Pinto da Silva Chief, Membrane Biology LPP, NCI

COOPERATING UNITS *(if any)*

Dr. M.R. Torrisi and A. Pavan, Institute of General Pathology, First University of Rome, Rome, Italy

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIH, FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Fracture-label cytochemistry revealed that T cells are heterogeneous with respect to the expression of transmembrane proteins. It is now important to study lymphocyte populations in T cell leukemias. Preliminary findings indicate that with patients suffering from Micosis fungoids the T cells are homogeneous. The results of this study can therefore lead to the classification, diagnostic and therapeutic assessment of T cell leukemias. The work should also be expanded LS B cells and B-cell leukemia. At present this project is suspended to lack of personnel and laboratory conditions.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08272-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Membrane glycoproteins and glycolipids of normal and transformed human cells

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

PI: P. Pinto da Silva Membrane Biology Section LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, Maryland 21701

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

This project has been temporarily interrupted because of lack of personnel. Should resources be allocated it will be continued using both fracture-label (see project #Z01CB08270-03 LPP and Z01CB08269-03 LPP and the new "label-fracture" (see project #Z01CB08250-04 LPP) techniques.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08274-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Regulation of Lactogenic Hormone Receptors in Mammary Tissue

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

PI: B.K. Vonderhaar	Research Chemist	LPP,	NCI
Others Professional Personnel: Maria Nascimento	Guest Researcher	LPP,	NCI
Ratna Biswas	Visiting Fellow	LTIB,	NCI
Erika Ginsburg	Biologist	LPP,	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.65	0.9	.75

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

This project is designed to evaluate the nature of lactogenic hormone receptors and the factors (including other hormones) which affect binding of the hormone to this molecule. Studies include 1) purification of the receptor from human tissue and preparation and characterization of an antibody against it; 2) examination of the nature of the interaction of prolactin and human growth hormone with native as well as cryptic forms of the receptor 3) characterize the selectivity of the effect of changes in the membrane lipid environment on binding of lactogenic hormones to their receptors vs other peptide hormones (such as EGF) binding to their receptors and 4) examination of the effects of various lectins on binding of lactogenic hormones to their receptors and the subsequent biological activity of the lactogens in cell culture.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08279-03 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Effect of proline analogs on normal and neoplastic breast epithelium

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

PI:	W. R. Kidwell	Chief, Cell Cycle Regulation Section	LPP, NCI
Other Professional Personnel:	S. J. Taylor	Medical Staff Fellow	LPP, NCI
	M. Bano	Visiting Fellow	LPP, NCI
	F. Grantham	Bio. Lab. Tech.	LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

D

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

A series of proline analogs have been analyzed for their effects on collagen synthesis inhibition in cultures of primary DMBA-induced rat mammary tumors and for their effects on mammary tumor growth in tumor bearing animals. Azetidine carboxylate, thioproline and cis-hydroxyproline were found to be potent, selective inhibitors of collagen synthesis, blocking amino acid incorporation into collagen by 7 to 27 fold more than incorporation into total tumor cell protein. In vivo all 3 of these compounds at doses of 50-200 mg/kg S.C., caused tumor growth arrest or regression. The conditions favoring proline analog sensitivity of mammary tumors have been assessed. A positive correlation exists between the ability of a tumor to synthesize basement membrane and its analog sensitivity. The analogs do not produce any discernable, general toxicity at concentrations which affect tumor growth. Sensitivity is approximately proportional to the efficacy of the analog in blocking collagen synthesis in cultured tumor epithelium. The epithelium of normal mammary glands and mammary adenocarcinomas is dependent on proline for optimal growth, especially when cells are plated on stromal collagen substrata. Blocking basement membrane deposition and thereby favoring tumor cell contact with stroma may, therefore, promote proline analog uptake and tumor cell kill.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08280-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Oncogene Expression in Mammary Cancer

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

PI: Y.S. Cho-Chung Chief, Cellular Biochemistry Section LPP, NCI

Other Professional Personnel: M. DeBortori Visiting Associate LPP, NCI
T. Clair Chemist LPP, NCI
F. L. Huang Expert LCCTP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cellular Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.5	0.7

CHECK APPROPRIATE BOX(ES)

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

B

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

Nineteen distinct transforming genes have been identified in the genomes of oncogenic retroviruses. Each of these oncogenes has a homologue in the chromosomal DNA of all vertebrate species. Current evidence indicates that this highly conserved set of genes may play a vital role in cell proliferation and/or differentiation. In addition, inappropriate expression of some of these genes has been implicated in the genesis of cancer.

Our efforts have been concentrated on the cellular homologue of the ras gene, the oncogene carried by Harvey and Kirsten Sarcoma viruses. An amplified expression of the cellular ras gene has been correlated with the oncogenesis of human bladder, lung and colon carcinomas.

In this study we are investigating the role of ras gene expression in the induction of rat and human mammary carcinomas. It is our hypothesis that mammary cancer can be triggered by activation of ras gene expression. Enhanced expression might result from genetic changes at the ras locus itself such as a rearrangement involving new promoter sequences, or from changes at other, so-called "regulatory" loci.

The expression of the ras gene will be determined in growing vs regressing mammary tumors, hormone-dependent vs hormone-independent tumors, and the mammary gland of rodents during normal development or chemical or viral carcinogenesis. The ras gene expression will be also determined in hyperplastic alveolar nodules, benign and malignant breast diseases of humans. The goal of this proposal is to provide us a fundamental basis for better understanding the mechanisms by which oncogenes involved in neoplastic growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08281-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Mechanism of Reverse Transformation

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

PI.: Y.S. Cho-Chung Chief, Cellular Biochemistry Section LPP, NCI

Other Professional Personnel:

T. Clair	Chemist	LPP, NCI
P. Tagliaferri	Biochem. Oncogenes Sec.	LTIB, NCI
B. Bassin	Chief, Biochem. Oncogenes	LTIB, NCI
C.L. Kapoor	Retina Foundation Fellow	LVR, EI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Cellular Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Occasionally, tumor cells differentiate spontaneously and then regress completely. It has been suggested that cAMP may be linked with the morphological differentiation of neoplastic cells since treatment of some tumor cells with dibutyryl cAMP, prostaglandin E_1 and inhibitors of cAMP-phosphodiesterase induces irreversible morphological differentiation. That this differentiation may be a reversion of malignancy is supported by the observation that no tumor is produced when these treated cells are inoculated into animals.

Avian sarcoma virus-transformed mammalian cells also occasionally revert to a normal phenotype. Current information suggests four major categories of mechanisms by which transformed cells may revert to a normal phenotype: (1) loss of the viral genome; (2) mutation in the transforming gene(s) (by deletion, insertion or base change); (3) reduction in transforming-gene expression at either transcriptional, translational, or posttranslational levels; and (4) the appearance of host-cell resistance to the effects of viral transforming genes.

To investigate factors that affect phenotypic reversion of transformed cells, we have chosen a cell line 433 of NIH 3T3 cells containing the transforming ras-gene of Harvey sarcoma virus flanked by LTR of MMTV; the expression of ras-gene in 433 cells is therefore controlled by mouse mammary tumor virus promoter (MMTV-LTR) which is under control of glucocorticoid. Thus, the phenotypically normal 433 cells become transformed and produce the ras-gene product, p21 only upon addition of glucocorticoid. The goal of this study is to investigate the effect of intracellular regulatory factors, such as cyclic nucleotides, hormones, and growth factors on the controlling element, MMTV-LTR to gain knowledge on the mechanism of reverse transformation.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08282-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

In Vitro Assembly of Gap Junctions

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

PI: Pedro Pinto da Silva Chief, Membrane Biology Section NCI, LPP

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have discovered the first experimental system for in vitro assembly of gap junctions. Assembly of gap junctions is pre-conditioned by disruption of cytoskeletal elements and proceeds even in the presence of inhibitors of protein synthesis. We now want to investigate the role of temperature in the assembly process, the ontogenetic and structural relationships between tight and gap junctions and the role of lipid molecules in the structure of the connexon (its building unit). This project has been interrupted for lack of personnel and access to freeze-fraccture equipment.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08283-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Study of Cytoplasm Compaction by Permeation of Probes into Freeze-Fractured Cells

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

PI: Pedro Pinto da Silva Chief, Membrane Biology Section LPP, NCI

Other Professional Personnel: Maria Luiza F. Barbosa Visiting Fellow LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We developed a new method -- "fracture-permeation" -- to assess the compactness of the cytoplasmic matrix. Cells fixed in glutaraldehyde were frozen, cross-fractured in liquid nitrogen and thawed. Cell fragments were immersed in concentrated solutions of native ferritin (30% w/v). Permeation by ferritin, an electron-dense probe, tested the existence and distribution of intermolecular spaces within the cytoplasmic matrix of glutaraldehyde-fixed cells. Ferritin molecules were unable to permeate the cross-linked cytoplasm of human neutrophils, fungal zoospores and cysts, used here as examples of non-dividing cells with low levels of protein synthesis. In resting lymphocytes from human peripheral blood permeation of ferritin was limited or absent, but it became massive in cells activated by phytohaemagglutin. Massive permeation of ferritin was also observed within the cytoplasmic matrix of active cells (sarcoplasm of skeletal muscle, fungal sporangia, germinating cysts). We show that compactness of the cytoplasmic matrix depends on the physiological state of the cell: in cross-fractured skeletal muscle ferritin permeation of the sarcomere readily differentiates rigor from relaxed states. Our results accord with the existence in the native cytoplasm of interactive soluble and insoluble protein phases.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08284-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Differential regulation of a rare mRNA in rat mammary gland and liver

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

PI: Hira L. Nakhasi LPP, NCI

Other Professional Personnel: K. Daruwalla Guest Researcher LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

In most eukaryotic cells mRNA levels vary widely depending upon their turnover and differential rates of trancription of their genes. Considerable information is available on the role of abundant mRNAs. Comparatively little is known about rare mRNAs in eukaryotic cells. To understand the importance of the rare mRNAs in cell function, we have isolated a cDNA clone for rare mRNA from a cDNA library generated from lactating rat mammary gland. This cDNA clone codes for a protein of M_r24,000 in an in vitro system. The mRNA corresponding for this cDNA clone is present both in mammary gland and in liver and is of the same size in both organs. However, there is an altered expression of this mRNA in some mammary tumors as well as in some hepatomas. Expression of this mRNA in the mammary gland is under the control of prolactin, where as the expression in liver is under the control of androgens, glucocorticoids and thyroid hormones.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08285-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Purification and Modulation of N-acetylglucosaminide β1→4 Galactosyltransferase

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

PI: Hira L. Nakhasi		Staff Fellow	LPP, NCI
Other Professional Personnel:	K. R. Daruwalla	Guest Researcher	LPP, NCI
	P. K. Qasba	Research Chemist	LPP, NCI
	L. Nagarajan	Visiting Fellow	LTIB, NCI
	W. B. Anderson	Research Chemist	LTIB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Glycosyl transferases are a group of enzymes which are associated with the cell membrane and have been implicated to play a role for intercellular adhesion and tumor invasion. We are studying one of these enzymes namely N-acetylglucosaminide-β1→4 galactosyl transferase (GT). This enzyme besides transferring galactose from UDP-galactose to nonreducing terminal residue N-acetyl glucosamine in glycoproteins, is also modified by milk specific protein α-lactalbumin to transfer galactose to glucose, for the synthesize of lactose in mammary gland. GT was purified from rat milk by affinity chromatography on N-acetylglucosamine-sepharose and α-lactalbumin-sepharose columns. The purified enzyme from rat milk showed three polypeptides of M_r 59k, 54k and 27k. GT purified from human milk under similar conditions, was electrophoretically homogenous showing one polypeptide of M_r 54k. Rat and human milk GT differed in its substrate constants besïdes being antigenically different.

Since glycosyl transferases are involved in transferring the carbohydrate moieties onto the cell surface glycogconjugates and cell surface carbohydrates are known to alter during embryogenesis, we studied the changes in cell surface GT in mouse embryonal carcinoma cells (F_9) upon differentiation with retinoic acid and cyclic AMP. There was an increase in activity with the treatment and this increase could be blocked by either actinomycin D or cycloheximide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08286-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Regulated expression of cloned milk protein gene transfected into mammalian cells

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

PI:	P. K. Qasba	Research Chemist	LPP, NCI
Other Professional Personnel:	S. Matarazzo	Staff Fellow	LPP, NCI
	P. Hutzell	Microbiologist	LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.75	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Milk protein gene expresssion is multihormonally regulated. Insulin and and hydrocortisone are required for the transcription of these genes, whereas prolactin is essential for the stability of these messages. We have inserted various regions of these genes in the vectors carrying, either a) Chloramphenicol acetyl transferase genes (CAT vectors) or b) neomycin resistant (pSV2 neo) genes, to characterize the DNA sequences through which these hormones induce α-lactalbumin and WP-genes. Primary mammary epithelial cell cultures grown on collagen substratum and several cell lines carrying insulin and hydrocortisone receptors are transfected with the plasmid constructs and the DNA sequences through which these hormones induce these genes are presently being characterised.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08287-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Characterization and cDNA cloning of α-LA-like activity from epididymis

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

PI: P. K. Qasba — Research Chemist — LPP, NCI

Other Professional Personnel: I. K. Hewlett — Visiting Fellow — LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

α-lactalbumin, a modifier protein that changes the substrate specificity of galactosyltransferase, to promote the synthesis of lactose, is found in the mammary glands of lactating mammals and in milk. Molecules similar to mammary gland α-lactalbumin but distinct in their modifier activity have been found in the epididymal fluid. This activity differs from mammary gland α-LA activity in that it transfers galactose from UDP-galactose to either glucose or myo-inositol with equal efficiency. The products of these reactions, lactose and galactinol were characterized by paper chromatography. Using rat mammary gland α-lactalbumin cDNA clone as a hybridization probe, RNA sequences homologous to α-lactalbumin mRNA were also detected in total RNA from rat epididymis. This finding suggests that α-lactalbumin or similar molecules, in addition to regulating lactose synthesis in the mammary gland, may have other important functions, e.g., synthesizing specific oligosaccharide sequence on the cell surface glycoproteins which are recognized as new antigenic determinants. Specifically in the male reproductive tract, where lactose is absent and free glucose levels are barely detectable, α-LA-like activity may modulate sperm surface glycoproteins which may play an important role in sperm-egg surface interactions during fertilization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08288-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

cDNA cloning of galactosyltransferase

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

PI:	P. K. Qasba	Research Chemist	LPP, NCI
Other Professional Personnel:	S. Matarazzo	Staff Fellow	LPP, NCI
	P. Hutzell	Microbiologist	LPP, NCI
	H. Okayama		LMG, NICHD

COOPERATING UNITS *(if any)*

Dr. K. Brew and Dr. S. Sinha, Dept. of Biochemistry, Miami Medical School, Miami, Florida

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.25	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Galactosyltransferases are the family of enzymes which transfer galactose from UDP-gal to the non-reducing residues of oligosaccharides of various glycoconjugates as well as to monosaccharides. N-acetylglucosaminide β1→4 galactosyltransferase is a specific transferase, secreted in milk as part of lactose synthetase complex which transfers galactose through β1→4 linkage to terminal N-acetylglucosamine residues in glycoproteins. α-Lactalbumin modifies the activity of this galactosyltransferase in such a way that it inhibits the transfer of galactose from UDP-galactose to N-acetylglucosamine either free or linked as a terminal sugar of a glycoprotein, but facilitates the transfer to glucose or myo-inositol. To understand the modulation of galactosyltransferase activity essential for generating specific cell surface antigenic determinants, we have first isolated and characterized cDNA clones corresponding to α-lactalbumin. Protein sequence, isolation and the sequence of the cDNA clones corresponding to the galactosyltransferases, which is essential in understanding the molecular mechanism of the modulation of the transferases and the control of their gene expression, is being investigated.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08289-01 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Expression of κ-casein gene in normal and neoplastic rat mammary gland

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

PI: Hira L. Nakhasi		Staff Fellow	LPP, NCI
Other Professional Personnel:	P.M. Gullino	Medical Officer	LPP, NCI
	K. Daruwalla	Guest Researcher	LPP, NCI
	F.H. Grantham	Bio. Lab. Tech.	LPP, NCI
	M.D. Thompson	Biologist	LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Caseins are a major group of secretory phosphoproteins synthesized during lactation in mammals and are stored and secreted as stable calcium phosphate complexes called micelles. The micelles are composed of αS_1, αS_2 and β caseins which interact with calcium and κ-casein. κ-casein has two important functions in the lactational process of mammals, one to stabilize milk micelles which can be assimilated slowly and second it has a labile band in its primary structure which is important for milk clotting. Therefore, production of κ-casein represents an important step in the functional differentiation of the mammary epithelium and an alteration of this production may be a marker of neoplastic transformation.

A full length cDNA clone for the rat κ-casein was isolated and its nucleotide sequence was determined. The deduced amino acid sequence from the nucleotide sequence revealed a signal peptide of 21 amino acids and a mature protein of 203 amino acids long. The mature protein is 33 amino acids long at the carboxyterminal end as compared to the known κ-caseins. κ-casein mRNA content of the mammary tissue was found to increase during its functional differentiation. Prolactin appears to modulate the production of κ-casein mRNA both in normal mammary cell and some carcinogen induced mammary tumors.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08290-01 LPP

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Growth of breast cancer metastases

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

PI:	P. M. Gullino	Medical Officer, Chief	LPP, NCI
Other Professional Personnel:	F. H. Grantham	Bio. Lab. Tech.	LPP, NCI
	D. M. Hill	Bio. Lab. Tech.	LPP, NCI
	H. M. Pettigrew		

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Pathophysiology

SECTION: Office of the Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

An experimental model of mammary carcinoma was developed in the rat and characterized by the ability to produce diffuse metastasis in 95% of subjects within 72 hr from transplant. This model permitted preparation of animals with clinically silent metastases as observed in women undergoing a mastectomy for mammary carcinoma. The model was utilized to study the influence of pregnancy and lactation on the growth rate of clinically silent metastases at the time of conception. The results showed that pregnancy prolongs the survival time of rats bearing metastases of this mammary carcinoma. For the first time, to our knowledge, support has been obtained under experimentally controlled conditions of sporadic clinical observations suggesting that pregnancy in women of child bearing age operated on for breast cancer need not necessarily be avoided, providing it occurs after the treatment for the primary breast cancer has been completed and lactation following delivery is not permitted.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08291-01 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Fracture-label:cytochemical localization of glycocomponents in crossfractured nuclei

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

PI: P. Pinto da Silva Chief, Membrane Biology Sec. LPP, NCI

Other Professional Personnel: Frederick W.K. Kan Visiting Fellow LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have shown that localization of glycocomponents in chromatin can be visualized at the ultrastructural level by the fracture-label technique. Concanavalin A and Ulex Europaeus 1 were used to localize glycocomponent in the chromatin in the nucleus of duodenal columnar and exocrine pancreatic cells. We have found that both Con A and UEA I bind to the chromatin in the nucleus of the above two cell types. Furthermore, the binding sites are confined to the euchromatin region of the nucleus. Our finding are the first to assign to exchromatin the location of glycocomponents within the nuclear matrix. The importance of glycoconjugates in gene expression is, thereby, anticipated.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08292-01 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

"Fracture-permeation":compactness of the sarcomere during muscle contraction

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

PI: Pedro Pinto da Silva Chief, Membrane Biology LPP, NCI

Other Professional Personnel: Maria L.F. Barbosa Sr. Staff Fellow LPP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.40	0.20

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We use "Fracture-permeation" to study the compactness of myofilaments within the sarcomere during muscle contraction. As models of skeletal muscle, we utilize sartorius muscle from toad (Bufo marinus) and papillary muscle from the left vantriale of Sprague-Dawley rats. Tissue fixed in glutaraldehyde was frozen, cross-fractured in liquid nitrogen and thawed. Tissue fragments were immersed in concentrated solutions of native ferritin (30% w/v). Permeation by ferritin, an electron-dense probe, tested the existence and distribution of intermolecular spaces within the sarcomere of glutaraldehyde-fixed muscle cells. Our results lead to the first unequivocal ultrastructural characterization of the contracted stage in cardiac muscle cells. Qualitatively distinct patterns of ferritin permeation into the sarcomere lead to immediate identification of all stages of muscle contraction. Macromolecular permeation of freeze-fractured skeletal muscle characterizes and distinguishes rigor, contracted and relaxed states. Morphological identification of contracted muscle and the pattern of permeation by ferritin into sarcomere at this state raises questions concerning the molecular mechanisms of muscle contraction. These new results have reinforced our expectations on "Fracture-permeation" as a new approach to study intracellular matrices.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08293-01 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Essentiality of insulin for the accumulation of rat milk protein mRNA's

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

PI: P. K. Qasba	Research Chemist	LPP, NCI
Other professional personnel:	Y. Topper, Chief,	LBM, NIADDK
	P. Chomczynski, Visiting Scientist	LBM, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The control of overt differentiation of mammary gland in vitro involves an interplay between polypeptide and steroid hormones. 1) There is absolute requirement of hydrocortisone for accumulation of these messages, specifically the accumulation of 42K casein mRNA in mammary tissue from adrenalectomized, virgin rat is almost 20x higher in the presence of exogenous hydrocortisone than in its absence. Accumulation of 25K casein mRNA is also totally dependent on the steroid. 2) Insulin is absolutely required for the expression of these milk protein genes and can be considered as a developmental hormone in the mammary system. Neither fetal calf serum nor Multiplication stimulating activity (MSA) or epidermal growth factor (EGF) can substitute insulin effect on differentiation, though these hormones can sustain mammary cell viability in culture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04022-02 MET

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Molecular and Biochemical Characterization of the Human Interleukin-2 Receptor

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

PI:	Warner C. Greene	Senior Investigator	MET,NCI
	Warren J. Leonard	Senior Staff Fellow	MET,NCI
	Joel M. Depper	Expert	MET,NCI
	Martin Kronke	Guest Researcher	MET,NCI
	Thomas A. Waldmann	Branch Chief	MET,NCI
	Gerald Crabtree	Senior Investigator	LP,NCI
	Stuart J. Rudikoff	Senior Investigator	LG,NCI

COOPERATING UNITS *(if any)*

Richard J. Robb, Principal Investigator, E.I. duPont de Nemours, Glenolden, PA

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The human receptor for interleukin-2 (IL-2, T-cell growth factor) has been molecularly cloned and biochemically characterized. The human genome normally contains a single copy of this receptor gene, however, when transcribed, two mRNAs are produced which vary in length due to use of different polyadenylation signals. Sequence analysis of cloned cDNAs also indicates the presence of alternate pathyways of mRNA splicing. Splicing of a 216 base pair intron contained within the coding region of this gene results in an altered protein unable to bind IL-2. In contrast, cDNAs corresponding to unspliced mRNA when ligated to SV-40 regulatory elements and transfected into COS-1 cells result in the expression of membrane receptors capable of binding IL-2 and anti-Tac. The receptor is composed of 272 amino acids including a signal peptide of 21 amino acids. The protein backbone (33,000) daltons) is modified cotranslationally by addition of N-linked carbohydrate then exported to the Golgi and membrane where O-linked sugar, sialic acid, phosphate and sulfate are added. 30,000-60,000 IL-2 receptors are displayed on the surface of PHA activated lymphoblasts while leukemic T cells infected with human T cell leukemia-lymphoma virus express 5-10 fold more receptors. The number of receptors in normal activated T cells, but not HTLV transformed T cells, markedly declines during long term culture suggesting that T cell responsiveness is regulated not only by the availability of IL-2 but also by the expression of IL-2 receptors. Reexpression of receptors occurs following stimulation with antigen or lectin or with agents that activate protein kinase C (phorbol diesters and phospholipase C). The presence of large humbers of IL-2 receptors on the surface of HTLV infected leukemic T cells has been exploited to selectively kill these cells using anti-IL-2 receptor antibody (anti-Tac) coupled to the toxic A chain of ricin.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB04021-02 MET

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetic Mechanisms in Human Lymphoid Neoplasms

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

PI:	Stanley J. Korsmeyer	Senior Investigator	MET,NCI
	Ajay Bakhshi	Senior Staff Fellow	MET,NCI
	Andrew Arnold	Medical Staff Fellow	MET,NCI
	Katherine A. Siminovitch	Guest Researcher	MET,NCI
	Paul Guglielmi	Guest Researcher	MET,NCI
	Thomas A. Waldmann	Branch Chief	MET,NCI
	Warner C. Greene	Senior Investigator	MET,NCI
	David G. Poplack	Senior Investigator	PB,NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

B

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

The examination of immunoglobulin (Ig) genes has revealed an ordered hierarchy in which heavy chain genes rearrange before light and κ generally rearranges before λ. Part of this ordered process is a deletional loss of κ gene segment within λ producing B cells, and we have identified a new recombinatorial element that uniformly mediates this κ loss. The "non-T, non-B" acute lymphoblastic leukemias were shown to be a developmental series of B-cell precursors with a coordinate sequence of cell surface antigen expression and Ig gene rearrangements. These leukemias represent landmarks enabling the identification of genes that are transcriptionally activated during the early stages of B cells. As cells of non-B lineage retain germline light and usually heavy chain genes, the configuration of Ig genes provides a molecular lineage marker. Ig gene analysis definitively established hairy cell leukemia as a genotypic B-cell, but one which expressed receptors for interleukin-2. Furthermore, Ig gene rearrangments have served as sensitive and specific markers capable of identifying minority populations of clonal B cells in tissues of mixed cellularity; these tumor-specific molecular markers have been of great use in early detection, classification, and following the natural history of lymphoid noeplasms. B-cell differentiation was also inducible with phorbol esters and allowed elucidation of the role of c-Myc in maturational arrest. Frequently Ig gene rearrangements are intermediate or aberrant preventing Ig production while other molecular errors account for the truncated proteins of heavy chain disease (HCD). A case of μ HCD proved to have an RNA splicing error responsible for its small heavy chain without light chain; whereas, in contrast a γ HCD had a DNA deletional rearrangement. Chromosomal translocations can also rearrange in Ig gene loci, and we are utilizing such a heavy chain rearrangement to identify a new cancer related gene being introduced from chromosome 18 in certain lymphomas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04020-08 MET

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Genetic Control of the Immune Response to Natural Antigens

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

PI:	Jay A. Berzofsky	Senior Investigator	MET,NCI
	Hajime Kawamura	Visiting Fellow	MET,NCI
	Howard Streicher	Medical Staff Fellow	MET,NCI
	Ira Berkower	Investigator	BB,NCDB
	John Minna	Branch Chief	NMOB,NCI
	Frank Cuttitta	Investigator	NMOB,NCI

COOPERATING UNITS *(if any)*

Frank R.M. Gurd, Department of Chemistry, Indiana University
Mark Busch, Department of Chemistry, Indiana University

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The mechanisms of determinant-specific Ir gene control and of antigen recognition by T and B lymphocytes were explored at several levels in the response to myoglobin. An immunodominant epitope of myoglobin centering on Glu 109 was identified for high responder T cells restricted to I-A^d, under Ir gene control. Cloned T cell lines specific for this epitope were produced, along with clones specific for a minor epitope at Lys 140. All 109-specific clones were I-A restricted, whereas all I-E restricted clones were specific for Lys 140. Monoclonal antibodies to the Lys 140 site could block the latter clones, a novel result presumably possible because of T cell-antibody shares specificity. The site recognized by Lys 140-specific T cell clones, as well as any site which must react with I-E, has been narrowed to the 11-residue sequence 136-146 using natural and synthetic peptides. Inhibitors of proteolysis inhibited presentation of native myoglobin but not of a small fragment or an intact but unfolded form of myoglobin to the same T cell clone, implying a requirement for proteolytic processing of native myoglobin, but probably in order to unfold the molecule, not just to reduce size. A major antimyoglobin idiotype was discovered using rabbit antibodies to a monoclonal antimyoglobin. The idiotype was expressed by all strains tested, representing 5 Igh allotypes, but the relative proportion of antibodies bearing the idiotype was influenced by H-2-linked Ir genes - an important link between these 2 major regulatory systems. Syngeneic monoclonal antiidiotypes were prepared and used to delineate several idiotopes. A novel mutual enhancement between two antiidiotopes was observed.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04018-08 MET

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Study of Human Immune Defense Mechanisms and Its Control

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

PI:	Andrew V. Muchmore	Senior Investigator	MET,NCI
	R. Michael Blaese	Section Head	MET,NCI
	Basil Golding	Medical Staff Fellow	MET,NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

A

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

These studies are designed to explore the role of cell surface lectin-carbohydrate interactions, in cellular recognition, cooperation and regulation. Special emphasis is placed on the role of complex carbohydrates and glycoproteins in the regulation of immune response during human pregnancy. A mannose 1-6 dimer of mannose and a more complex glycoprotein have been purified from human pregnancy urine. Both compounds are being extensively characterized for their immunoregulatory properties. A second set of studies is examining a T independent antigen specific model of human antibody production in vitro. These studies are concentrating on 1) cellular requirements, 2) B cell subset diversity, and 3) fine specificity of V region products. A third line of research has explored spontaneous monocyte mediated cytotoxicity with the development of cytotoxic cell lines. Finally, we have used intact and F(ab)2 anti Dr antibodies to disect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04017-08 MET

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Biology of the Immune Response

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

PI:	David L. Nelson	Senior Investigator	MET, NCI
	Robert Yarchoan	Investigator	MET,NCI
	Claudia Quijano	Guest Researcher	MET,NCI
	Lawrence Rubin	Guest Researcher	MET,NCI
	William Biddison	Senior Staff Fellow	NI,NINCDS
	Brian Murphy	Senior Investigator	LID,NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch

SECTION

Immunophysiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

A

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

Studies were undertaken to study the maturation and immunoregulation of in vitro antigen-specific cytotoxic T-cell responses and humoral antibody responses by B-cells in normal individuals and patients with immune deficiency states. Patients were heterogenous with regard to their ability to generate influenza virus specific ctotoxic T-cells in vitro. Most patients with hypogammaglobulinemia produced cytotoxic T-cells normally, while patients with the Wiskott-Aldrich syndrome and ataxia-telangiectasia produced alomost no virus specific cyotoxic T-cells. The latter two patient groups were also deficient in their ability to generate alloimmune cytotoxic T-cells in vitro. Normals produce specific antibody which are macrophage and T-cell dependent. Co-cultures of T-cells with allogeneic B-cells and macrophages with antigen demonstrated allogeneic T-cell help and radiosensitive T-cell suppression. Cord blood cells: 1) produced no specific antibody, 2) had B cells which made no antibody in the presence of mature T-helper cells, and 3) had T-cells capable of providing allogeneic T-helper effects. Normal cells produced mostly IgG antibody and small amounts of IgM and IgA, due to a variation in precursor isotype frequency. No evidence of "isotype switching" was found. Among immunodeficient individuals, cells from 5 of 11 patients with hypogammaglobulinemia who made no antibody in vivo produced specific antibody in vitro. Cells from patients with the Wiskott-Aldrich syndrome and ataxia-telangiectasia produced less antibody than controls. Among the latter patients, defects in both T-cells and B-cells but not monocytes contributed to the poor response. Defects in the production of cytotoxic T-cells and specific antibodies may contribute to the increased incidence of neoplasia observed in immunodeficient patients.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB04016-12 MET

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Action of Insulin-Like Growth Factors

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

PI:	S. Peter Nissley	Senior Investigator	MET,NCI
	Lynne Gaynes	Medical Staff Fellow	MET,NCI
	Joyce Haskell	Guest Researcher	MET,NCI
	Matthew M. Rechler	Senior Investigator	LBP,NIADDK
	Wayne Anderson	Senior Investigator	LTIB,NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolism Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	3	2.5

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

B

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

We have demonstrated that the Type II IGF receptor is phosphorylated in intact cells. The phosphorylation is not dependent upon IGF-II and could not be demonstrated with solubilized receptor preparations. We have developed two assays to screen mouse sera and hybridoma supernatant for antibodies to the type II IGF receptor. One assay measures the ability of the serum or supernatant to block the binding of radiolabeled IGF-II to type II receptor bearing cells (blocking antibody). The second assay is an immunoprecipitation assay which measures the ability of the serum or hybridoma supernatant to bind to a preforemed radioligant-solubilized receptor complex and be immunoprecipitated with a goat antimouse serum. Using these assays we have measured receptor antibodies in sera of mice whose spleen cells are being fused to plasmacytoma cells (NS-1) to form hybridomas. We have shown that mouse embryonal carcinoma cell lines produce IGF-II but little or not IGF-I. We are characterizing IGFs and IGF binding proteins produced by human fetal fibroblasts and postnatal fibroblasts.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB04015-14 MET

PERIOD COVERED: October 1, 1983 through September 30, 1984

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

Development and Function of Humoral and Cellular Immune Mechanisms

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

PI:	R. Michael Blaese	Section Head	MET,NCI
	Andrew V. Muchmore	Senior Investigator	MET,NCI
	Giovanna Tosato	Expert	MET,NCI
	Frank M. Orson	Medical Staff Fellow	MET,NCI
	Alfred D. Steinberg	Senior Investigator	A&R,NIAMDD
	Robert Yarchoan	Investigator	MET,NCI
	Steven E. Staus	Senior Investigator	LCI,NIAID
	Fred Wang	Medical Staff Fellow	MET,NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Metabolism Branch

SECTION: Cellular Immunology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

The Cellular Immunology Section has continued and extended its studies of the immunobiology of the Epstein-Bar virus. EBV is a unique viral pathogen for man in that its target cell for infection is the immune system itself. Not only are B lymphocytes infected by the virus, but once infected they become functionally activated to proliferate and secrete immunoglobulin and significantly, they acquire the property of autonomous growth as well. In addition, like other herpes viruses, EBV persists in B cells for life after primary infection and retains its capacity to activate these cells at any time. These properties present a considerably more complex problem of host defense than encountered with other pathogens and the body has evolved a broad array of humoral and cellular mechanisms to control this pathogen and regulate its effects on its lymphoid cell target. This research project has identified both suppressor and cytotoxic mechanisms of control which evolve from EBV nonspecific mechanisms in acute primary infection to EBV antigen specific mechanisms which appear during convalescence. Each of these direct control processes are in turn regulated by another series of immunoregulatory cells with contrasuppressor activity. Arrangements in this complex system of regulation are associated with several diseases and we have described such defects in patients with agammaglobulinemia, rheumatoid arthritis and chronic mononucleosis.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB04004-23 MET

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulatory Functions of Amino Acids on Ribonucleotides

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

PI:	James M. Phang	Section Head	MET,NCI
	G. Alexander Fleming	Medical Staff Fellow	MET,NCI
	Marshal Merrill	Guest Researcher	MET,NCI
	Quinton R. Rogers	Visiting Scientist	MET,NCI
	Grace C. Yeh	Expert	CPB,NCI

COOPERATING UNITS *(if any)*

David Valle, M.D., Johns Hopkins Hospital School of Medicine, Baltimore, Maryland

LAB/BRANCH
Metabolism Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

B

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

The metabolism of proline and pyrroline-5-carboxylate provides a mechanism for the intercompartmental, intercellular and inter-organ transfer of redox potential. Mediated by the transfer of redox potential, pyrroline-5-carboxylate stimulates the pentose phosphate pathway, PP-ribose-P synthesis and nucleotide production. This mechanism links amino acid and nucleotide metabolism. This effect of pyrroline-5-carboxylate has been shown to be synergistic to the effect of growth factors on ribonucleotide synthesis. This effect suggests that pyrroline-5-carboxylate may mediate hormonal effects and, indeed, may act as a "primitive hormone." The concentration of pyrroline-5-carboxylate is especially high in aqueous humor (7-10X plasma) suggesting that the regulatory effects of pyrroline-5-carboxylate may be especially important for ocular tissues. It has also been shown that the synthesis of pyrroline-5-carboxylate from glutamate and its subsequent conversion to ornithine may play a role in maintaining ornithine as a critical intermediate in the urea cycle.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04003-28 MET

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Studies of Porphyrin Metabolism in the Tumor-Bearing Host and Porphyria

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

PI: Donald P. Tschudy Senior Investigator MET,NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Work on the inhibitory effects of succinylacetone on heme biosynthesis led to the discovery of its immunosuppressive activity. This compound is now being studied in animal model systems for possible application to organ transplantation.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB04002-15 MET

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Defects in Immunoregulatory Cell Interactions in Patients with Immune Dysfunction

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

PI:	Thomas A. Waldmann	Branch Chief	MET,NCI
	Andrew Arnold	Medical Staff Fellow	MET,NCI
	Stanley J. Korsmeyer	Senior Investigator	MET,NCI
	Ajay Bakhshi	Medical Staff Fellow	MET,NCI
	Warner C. Greene	Senior Investigator	MET,NCI
	Warren Leonard	Senior Staff Fellow	MET,NCI
	Joel M. Depper	Expert	MET,NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

A

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

Studies were directed toward the defining the role of disorders of lymphocyte maturation and of immunoregulatory cell interactions in the pathogenesis of immune dysfunctions. Recombinant DNA technology has been applied to the study of the arrangement and rearrangement of immunoglobulin genes and antigen specific T cell receptor genes in lymphocytic leukemia. Such rearrangement of these genes were used to define the lineage and clonality of T and B cell malignancies as well as to define the causes for the failure of maturation of lymphoid cells in patients with non-T and non-B lymphocytic leukemia. Using a monoclonal antibody, anti-Tac, the human receptor for T cell growth factor (interleukin-2) has been purified to homogeneity. The receptor is a 55,000 dalton glycoprotein composed of a 33,000 dalton peptide backbone that is post-translationally modified by introduction of N and O linked carbohydrates, sialic acid, as well as phosphate and sulfate yielding mature receptors. The gene for this receptor has been cloned and expressed and shown to encode a 251 amino acid polypeptide. The anti-Tac monoclonal inhibits in vitro T cell proliferation induced by antigens, the development of cytotoxic and suppressor cells and as well as B cell immunoglobulin production. Activated B cells were shown to bear the IL-2 receptors. Leukemias of helper T cells (Sezary leukemic cells) are Tac antigen negative. In contrast, the adult T cell leukemia which is associated with the type C retrovirus (human T cell leukemia/lymphoma virus, HTLV) universally displays large numbers of IL-2 receptors on the cell surface. The consistent display of IL-2 receptors which may be aberrant in size on adult T cell leukemic cells may play a role in the uncontrolled growth of these cells. Anti-Tac is evaluated for the therapy of patients with adult T cell leukemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB03657-10 D

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immunopathologic Mechanisms Involved in Inflammatory Skin Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

S.I. Katz, Branch Chief, Dermatology Branch, NCI

COOPERATING UNITS *(if any)*

Dermatology Department, USUHS, Bethesda

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	5	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.)*

My clinical and laboratory endeavors involve three major areas of immunodermatology. The first deals with studies of patients with various forms of vesiculobullous diseases. We have not only provided detailed clinicoimmunopathological correlations of several heretofore poorly defined diseases, i.e. dermatitis herpetiformis, acquired epidermolysis bullosa and herpes gestationis but we have characterized the antigens to which the antibodies in some of these diseases bind, i.e., pemphigus and pemphigoid antigens. These studies are closely linked to my second major area of interest which is to provide an understanding of and to chemically characterize ultrastructurally-defined components of the epidermal basement membrane and to determine the function of each of these. We have demonstrated that epidermal cells synthesize both the skin specific pemphigoid antigen and the ubiquitous laminin, both of which are localized to the lamina lucida of the basement membrane zone. We have also described another stratified squamous epithelial specific basement membrane protein which is defined by the KF-1 monoclonal antibody. This basement membrane zone antigen is a noncollagenous component of the lamina densa and is specifically absent or markedly diminished in the dystrophic forms of epidermolysis bullosa which is a severely mutilating disease characterized by marked skin fragility and blisters. The antigen appears when the fetus is approximately 16 weeks of age. My third and major area of interest is the role of the epidermis as an immunological tissue. We have demonstrated that within normal epidermis Langerhans cells are the only cells which 1) synthesize and express Ia antigens, 2) can present both soluble antigens and haptens to sensitized T cells, 3) are capable of allogeneic T cell stimulation in a mixed epidermal-lymphocyte proliferation system, 4) can induce hapten and allogeneic cytotoxic T lymphocytes in vitro, and 5) are of a mesenchymal origin. We have also demonstrated that keratinocytes produce an Interleukin 1-like cytokine which may serve as a second signal in generating T cell responses.

GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB03666-06 D

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Chemical Mediators of Inflammation

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

(Name, title, laboratory, and institute affiliation)

Thomas J. Lawley, M.D., Dermatology Branch, DCBD, NCI

COOPERATING UNITS *(if any)*

LCI, NIAID, Metabolism Branch, NCI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.2	.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.)*

Antigen-antibody complexes play important roles in a variety of human systemic and cutaneous diseases. This laboratory studies how immune complexes are formed, how they cause tissue damage, and how they are cleared from the circulation. We have identified and partially characterized the immune complexes which exist in a variety of human diseases utilizing highly sensitive radioimmunoassays. We have developed a sensitive radioimmunoassay for the detection of IgA containing immune complexes. We have determined the antibody classes present in the immune complexes and examined the physiochemical characteristics of these complexes, as well as the reaction of these complexes with mediators of inflammation such as the complement system. We have examined the correlation between absolute levels of circulating immune complexes, the extent and severity of clinical disease, and reticuloendothelial system function. We have also examined the influence that certain genes of the major histocompatibility complex exert on immune function in vivo and in vitro in humans. Since immune complexes may activate the complement system and since the complement fragments C5a and C3a are thought to be important in the pathogenesis of the inflammatory response in cutaneous and systemic diseases, we have purified C5a and studied its in vivo and in vitro reactivity. Its in vivo role was assessed by the first in-depth analysis of the cutaneous reactivity of this complement fragment in man. We have also studied the ability of C5a and C3a to modulate cell surface receptors for immunoglobulin and complement on the surface of leukocytes. Increasing evidence indicates that human endothelial cells, under certain circumstances, can be induced to become immunologically competent. In order to evaluate the role endothelial cells in immune complex mediated vasculitis we have isolated human umbilical vein endothelial cells, grown them in cell culture, examined them for the presence of immunologically relevant cell surface antigens and receptors before and after stimulation with soluble mediators of immunoregulation.

PHS 6040 (Rev. 1/83)

GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB03659-10 D

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Therapy of Skin Cancer, Disorders of Keratinization, and Cystic Acne

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

(Name, title, laboratory, and institute affiliation)

G.L. Peck, Senior Investigator, Dermatology Branch, NCI

COOPERATING UNITS *(if any)*

1) Clinical Chemistry Service, NIH, Bethesda, Maryland 20205
2) Molecular Disease Branch, NHLBI, NIH, Bethesda, Maryland 20205
3) Cancer Prevention Studies Branch, DCPC, NCI, NIH, Bethesda, Maryland 20205

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

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.)*

Oral 13-cis-retinoic acid was effective in the treatment of skin cancer, and a variety of disorders of keratinization (lamellar ichthyosis, Darier's disease, pityriasis rubra pilaris), and cystic acne. An oral synthetic aromatic derivative of retinoic acid (RO-10-9359) was more effective and less toxic than 13-cis-retinoic acid in the treatment of the disorders of keratinization. A high initial followed by a low maintenance dosage of 13-cis-retinoic acid was comparably effective but less toxic than previously used continuous high-dosage schedules in the treatment of cystic acne. The high-low dosage schedule was superior to the high initial dose schedule used alone and to a continuous low dose schedule. 13-cis-retinoic acid led to small but significant elevations in plasma lipids and changes in lipoproteins during therapy. RO-10-9359 produced similar changes which were dose dependent and responsive to dietary management. Absorption of RO-10-9359 is greater with milk as a source of long-chain fatty acids, than with water. Etretinate is bound in plasma to beta-lipr - oproteins. Administration of etretinate with milk vs. water yielded different ratios of drug to metabolite in the serum. Etretinate persists in the serum after discontinuation of therapy and trace amounts have been detected after more than 2 years. Etretinate is stored in fat and serum etretinate concentration correlates with percent of ideal body weight. One chronic toxicity, "retinoid hyperostosis," has been observed with long-term, high-dose isotretinoin characterized by anterior spinal ligament calcification and osteophyte formation of vertebrae.

PHS 6040 (Rev. 1/83)

GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB03630-14 D

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Effects of Vitamin A and Analogs on Chick, Mouse and Human Skin

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

G.L. Peck, Senior Investigator, Dermatology Branch, NCI

COOPERATING UNITS *(if any)*

Dept. Dermatology, UCSF
Lab of Vision Research, NEI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

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

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

This project proposed to morphologically and biochemically define the mechanism of action of vitamin A and its derivatives (retinoids) in altering epidermal differentiation in normal skin, and in benign and malignant lesions of skin. Topical all-trans retinoic acid, but not systemic 13-cis-retinoic acid, increased gap junction density and decreased desmosome density in treated basal cell carcinomas. This indicates that topical and systemic retinoids may exert their antineoplastic activity by different cellular mechanisms.

A specific cytosol retinol binding protein (CRBP) has been identified in mouse, normal human skin and skin and human skin from patients with Darier's disease, psoriasis and basal cell carcinomas. A specific cytosol retinoic acid binding protein (CRABP) has also been identified in newborn mouse and normal human adult skin and newborn foreskin. The qualitative and quantitative distribution between the epidermis and dermis of both CRBP and CRABP has been determined in adult human lower limbskin.

PHS 6040 (Rev. 1/83)

GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB03638-14 D

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Studies of DNA Repair in Human Degenerative Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

J.H. Robbins Senior Investigator Derm NCI

COOPERATING UNITS *(if any)*

Biostatistics Branch, DCCP, NCI.

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.9	2.9	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.)*

Studies in this laboratory are designed to elucidate the role of DNA repair processes in human diseases and in carcinogenesis and in normal and abnormal aging. Most studies have been conducted with cells from patients with xeroderma pigmentosum (XP) who have defective DNA repair plus multiple cutaneous malignancies, and premature aging of sun-exposed skin and of the nervous system. Cells from patients with ataxia telangiectasia, diseases with abnormal cell growth and differentiation, Alzheimer disease, Parkinson disease, Huntington disease, Duchenne muscular dystrophy, retinitis pigmentosa, and Cockayne syndrome and from patients with the following primary neuronal, muscular and retinal degenerations are also being studied. These studies are designed to elucidate the pathogenesis of these disorders. We assess the biological effectiveness of DNA repair primarily by in vitro assays of cell survival after treatment of the cells with the DNA damaging agents.

PHS 6040 (Rev. 1/83)

GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB03656-11 D

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Chemistry, Structure and Biosynthesis of Mammalian Epidermal Keratin Filaments

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

P.M. Steinert, Visiting Scientist, Dermatology Branch, NCI

COOPERATING UNITS *(if any)*

Experimental Pathology Branch, DCCP, NCI; Laboratory of Molecular Biology, DCBD, NCI; Laboratory of Physical Biology, NIADDKD

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.75	5.25	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The biosynthesis, structure and function of the polypeptide chains which comprise the subunits of the keratin filaments of normal human, murine and bovine epidermis are being investigated. The subunits polymerize in vitro into native-type filaments. The details of filament ultrastructure are being investigated using image analysis procedures of filaments examined by transmission electron microscopic and scanning transmission electron microscopic techniques. Model structures generated from these methods will be computationally tested for compatibility with other physico-chemical data and amino acid sequence studies of individual filament subunits. cDNA cloned probes that encode human and mouse epidermal keratins have been isolated and are being used to determine the amino acid sequences of the proteins, and to study the structure and expression of keratin genes. The 10nm filaments of fibroblasts, muscle cells and neuronal tissues have been shown to be structurally similar to, but immunologically different from keratin filaments. A histidine-rich basic protein isolated from human epidermis and the slightly different protein of mouse epidermis specifically aggregate keratin filaments and other 10nm filaments in a manner suggestive of an interfilamentous matrix component. cDNA cloned probes will be isolated to study their structure, expression and amino acid sequence.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00853-31 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Surgical Pathology

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

PI: E.E. Lack Chief, Surgical Pathology & Postmortem Section LP, NCI

OTHER: (see next page)

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Surgical Pathology & Postmortem Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
20	20	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

The Surgical Pathology and Postmortem Section, together with the Cytopathology Section, Ultrastructural Pathology Section and Hematopathology Section provide complete service in anatomic pathology for the Clinical Center patients and collaborate with the research staff of all institutes in those investigations which involve the use and study of human pathological material. A new frozen section and surgical pathology processing area has been constructed adjacent to the new operating rooms and became operational on April 18, 1983. This new facility has greatly enhanced processing of specimens and communication of diagnostic findings with attending physicians.

The staff is engaged in a variety of projects involving clinicopathological correlation and pathologic characterization of disease studied at the Clinical Center. Immunocytochemical techniques have been applied to the characterization and study of tumors and other non-neoplastic diseases. The use of immunohistochemical staining has greatly facilitated more precise diagnosis in selected difficult cases and with the increasing number of monoclonal antibodies available this technique should have even greater value in diagnostic and research pathology.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00872-02 LP

ERIOD COVERED

ctober 1, 1983 to September 30, 1984

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

utopsy Service

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

I: C.M. Reichert Chief, Autopsy Service LP, NCI

THER: (see next page)

OOPERATING UNITS *(if any)*

AB/BRANCH

aboratory of Pathology

ECTION

urgical Pathology and Postmortem Section

ISTITUTE AND LOCATION

CI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3 1/2	1	2 1/2

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

he Autopsy Service of the Laboratory of Pathology provides complete service in utopsy pathology for the Clinical Center patients and collaborates with the esearch staff of all institutes in those investigations which involve the use nd study of human pathological material.

he staff is engaged in several projects involving clinicopathological correlation nd pathologic characterization of disease studied at the Clinical Center. mmunocytochemical techniques have been applied to the characterization and tudy of tumors and other non-neoplastic diseases.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00852-31 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Exfoliative cytology applied to human diagnostic problems and research problems

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

PI:	E.W. Chu	Chief, Cytopathology Section	LP, NCI
OTHER:	S.E. Martin	Staff Pathologist	LP, NCI
	S. Kan	Visiting Fellow	LP, NCI
	E. Magyarosy	Visiting Fellow	LP, NCI
	T.A. Wood	Biologist	LP, NCI
	L. Galito	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Cytopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	4	4

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

A

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

The Cytopathology Section provides complete diagnostic service in exfoliative cytology, medical cytogenetics, and fine needle aspiration cytology. The section has also initiated applying new immunocytochemistry techniques to improve and enhance cytological diagnostic efficacy. In addition, the section collaborates in various clinical research projects utilizing special techniques including special staining, tissue culture techniques, as well as investigating chromosomal and/or somatic cell hybridization techniques in mapping genes.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00897-01 LP

ERIOD COVERED

)ctober 1, 1983 to September 30, 1984

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

Cytological diagnosis of lymphomas by immunocytochemistry

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

PI:	S.E. Martin	Surgeon	LP, NCI
OTHER:	E. Magyarosy	Visiting Fellow	LP, NCI
	H.-Z. Zhang	Visiting Fellow	LP, NCI
	E.S. Jaffe	Chief, Hematopathology Section	LP, NCI
	S.-M. Hsu	Medical Staff Fellow	LP, NCI
	E.W. Chu	Chief, Cytopathology Section	LP, NCI

OOPERATING UNITS *(if any)*

AB/BRANCH

aboratory of Pathology

ECTION

ytopathology Section

STITUTE AND LOCATION

CI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

he cytological diagnosis of malignant lymphoma can be extremely difficult because he cytological features of the malignant cells in small cell and mixed small and arge cell lymphomas may be indistinguishable from those of reactive lymphoid ells. We are studying the usefulness of the avidin biotin immunoperoxidase tech-ique and a battery of antibodies to T and B cell markers to the diagnosis of ymphoma in cytological specimens.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00518-06 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fate of IgE bound to mast cells

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

PI:	C. Isersky	Senior Investigator	LP, NCI
OTHER:	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI
	S.J. Mims	Biologist	LP, NCI
	J. Rivera	Biologist	A&R, NIAMDD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pathology

SECTION
Ultrastructural Pathology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	6	6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

IgE bound to the surface of mast cells and/or basophils is responsible for the immediate hypersensitivity reaction. This response once established, persists for prolonged periods of time. We have shown that the mechanism is not due to internalization (J. Immunol. 122: 1926-1936, 1979). Cross linking of the IgE by allergen (or other means) is normally necessary to elicit cell degranulation, which results in histamine release. We wished to determine if analogous, chemically induced cross-linking affects the fate of IgE compared to monomeric IgE. The possible effect of oligomerized IgE binding to the recently described IgG Fc of basophils was also being investigated. We found that IgE binds exclusively to its own (Fcε) receptor and is internalized but not reexpressed upon cross-linking by "allergen" (ie, DNP-albumin + DNP-binding IgE). This is virtually unprecedented for known receptors.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00520-06 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Surface disposition and fate upon ligand binding of IgE and its receptor

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

PI:	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI
OTHER:	C. Isersky	Senior Investigator	A&R, A
	S.J. Mims	Biologist	LP, NCI
	J. Rivera	Biologist	A&R, A

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Antigenic responsiveness to allergens is imparted to mast cells and basophils by specific membrane binding of allergen binding IgE. Other cells have been shown to bind ligands non-randomly, especially to microvilli (dePetris, Nature 272: 66-68, 1978). Further, cell bound IgE has been shown to survive for prolonged periods of time on the cell surface (Isersky, Rivera, Mims, and Triche, J. Immunol. 122: 1926-1936, 1979). Finally, binding of cell-bound IgE with multi-valent ligand results in rapid internalization without re-expression of both IgE ligand and its receptor. We are studying the native distribution of IgE receptors on the cell surface by two techniques and comparing their fate following ligand binding. Of special interest is the fate of planar cell surface receptors compared to those on microvilli. In addition, the role of a pre-lysosomal compartment ("CURL") in ligand-IgE-receptor uncoupling and subsequent degradation is being investigated by double label techniques (colloidal gold-ligand and IgE-ferritin or α-receptor ferritin).

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00545-06 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Extracellular matrix synthesis by human tumors in vitro

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

PI:	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI
OTHER:	A. Modesti	Visiting Fellow	LP, NCI
	S. Scarpa	Visiting Fellow	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

LCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The type and amount of matrix proteins synthesized by human tumor cells in vitro appears to parallel that of cultured normal cell counterparts to some extent. We have broadened these observations to a variety of human tumors to determine whether these patterns might allow more precise categorization of the tumor's origins. In addition, we are characterizing a new matrix protein synthesized by some of these tumors. The identity, function, and molecular organization within the extracellular matrix of this component is currently unknown.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00874-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Neurone-specific enolase in childhood tumors

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

PI:	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI
OTHER:	M. Tsokos	Visiting Scientist	LP, NCI
	R.I. Linnoila	Medical Staff Fellow	LP, NCI
	R. Chandra	Children's Hospital, Washington, D.C.	

COOPERATING UNITS *(if any)*

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Laboratory of Pathology

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NCI, NIH, Bethesda, MD 20205

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☐ (a1) Minors

☐ (a2) Interviews

A

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

The diagnosis, and thus therapy, of solid tumors of childhood is often difficult due to lack of distinguishing characteristics. This is especially true of Ewing's sarcoma, neuroblastoma, primitive soft tissue sarcomas, and (occasionally) lymphoma. We have evaluated the presence of a specific neural enzyme, neurone-specific enolase (NSE), in paraffin-embedded sections of a diverse group of solid childhood tumors, including previously unrecognized variants of neural tumors, employing immunocytochemistry with antisera to NSE. We find uniform reactivity of all neural tumors with this antibody. No cross-reactivity with non-neural tumors, save a rare example of differentiated rhabdomyosarcoma, was found. We conclude that NSE is a reliable, readily detected marker in even primitive childhood tumors of neural origin. Also, we have defined the neural histogenesis of a newly described, "round cell" tumor of chest wall resembling Ewing's sarcoma. Finally, we have recently confirmed the unique character of so-called peripheral neuroepithelioma, which is NSE-positive but which displays hybrid neural and Schwannian morphologic characteristics.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00875-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Differentiation, matrix proteins, & in vitro invasiveness of human neuroblastoma

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

PI:	M. Tsokos	Visiting Scientist	LP, NCI
OTHER:	S. Scarpa	Visiting Fellow	LP, NCI
	U.P. Thorgeirsson	Visiting Scientist	LP, NCI
	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI
	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI

COOPERATING UNITS *(if any)*

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3	3	0

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☐ (a1) Minors

☐ (a2) Interviews

B

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

Neuroblastoma is a neoplasm known to show spontaneous histologic maturation in vivo which correlates with a better biologic behavior and prognosis. Extracellular matrix (ECM) proteins, on the other hand, have been shown to influence tumor invasion and metastasis. Production of ECM proteins has been previously reported only for the C1300 murine neuroblastoma cell line. We have studied ECM synthesis (ie, fibronectin (FN), laminin (LM), and collagen type IV) in relation to differentiation of human neuroblastoma in vitro. The qualitative and quantitative differences in ECM protein synthesis by neuroblastomas in vitro before and after differentiation have been assessed by immunofluorescence, polyacrylamide gel electrophoresis and quantitative scanning densitometry of autoradiograms of these gels. Differentiation has been induced by dibutyryl-cyclic AMP and retinoic acid and studied by light and electron microscopy as well as biochemical expression of neurotransmitter enzymes. Finally, the biologic behavior of the neuroblastoma cells before and after differentiation with the above agents was tested in vitro, employing a human amnion invasion assay. Our results indicate that neuroblastoma shows tripartite differentiation in vitro into three main cell types: neuronal, Schwannian and melanocytic. Each cell type has different light and electron microscopic characteristics and exhibits a specific pattern in terms of ECM protein expression. Quantitative studies of the synthesized ECM proteins showed no definite changes in any of the three studied proteins with differentiation. Morphologic differentiation, however, was accompanied by qualitative and quantitative changes of the neurotransmitter enzymes and correlated with decreased invasiveness in vitro.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00884-03 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Ultrastructural organization of basal lamina

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

PI:	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI
	A. Modesti	Visiting Fellow	LP, NCI
	S. Scarpa	Visiting Fellow	LP, NCI
	T. Kalebic	Visiting Fellow	LP, NCI
	S. Togo	Guest Worker	LP, NCI

COOPERATING UNITS *(if any)*

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☐ (a1) Minors

☐ (a2) Interviews

B

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

The basal lamina has been ultrastructurally characterized as a continuous electron lucent layer (the lamina lucida) adjacent to the cell surface with an overlying electron dense layer (lamina densa), which interfaces with the mesenchymal stroma collagens and other matrix proteins). Biochemically, the basal lamina is known to contain type IV collagen, laminin, and basement membrane proteoglycan. The actual disposition of these constituents in the l. lucida l. densa, cell surface, and matrix is uncertain, various conflicting ultrastructural studies notwithstanding. Also, the relationship of type V collagen, a so-called cell surface collagen, to the basal lamina, is unknown. We are employing high resolution (ca. 5 nm) immunoelectron microscopy on tissue sections with purified antisera to laminin, type IV collagen, and type V collagen, using appropriate controls, to precisely localize these constituents of the basal lamina and neighboring extracellular matrix.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00899-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Small, round cell tumor monoclonal antibody reactivity

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

PI:	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI
OTHER:	P. Reynolds	Transplantation Unit, NNMC, USN	
	L. Donner	Pathology Resident, George Washington University Medical Center;	
	Fellow to be named		

COOPERATING UNITS *(if any)*

NMC Transplantation Unit

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4	3	1

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

Primitive childhood tumors (ie, classically Ewing's sarcoma, neuroblastoma, lymphoma, and soft tissue sarcoma), are frequently morphologically indistinguishable. Ultrastructural and immunocytochemical techniques are useful but not infallible. Monoclonal antibodies (MoAbs) which recognize neural, lymphoid (HLA-related), and tissue-specific determinants might be useful in distinguishing these entities. We have studied more than 20 cell lines by flow cytofluorometry and 2 tumors by frozen section immunocytochemistry with a panel of 12 MoAbs and find reproducible patterns of reactivity which serve to reliably distinguish all neural tumors and hematopoietic malignancies. Ewing's sarcoma is similar to rhabdomyosarcoma, but shows some reactivity with certain neural MoAbs. Peripheral neuroepithelioma is a unique tumor with reactivity intermediate between sarcomas and neural tumors, not unlike Ewing's sarcoma. Thus, most of the tumors are readily recognized, even in the absence of any distinguishing morphologic characteristic. These results have important diagnostic and therapeutic implications, but further study of more tumors is required.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB09125-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cytogenetic abnormalities and oncogene expression of small, round cell tumors

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

PI:	M. Israel	Senior Investigator	PB, NCI
OTHER:	T.J. Triche	Chief, Ultrastructural Pathology Section	LP, NCI
	C. Thiele	Research Associate	PB, NCI
	J. Whang-Peng	Chief, Cytogenetic Oncology Section	MB, NCI
	E. Gelmann	Senior Investigator	LTCB, NCI
	J. Miser	Expert	PB, NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Bethesda, MD 20205

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4	4	0

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have encountered a uniform rcp (11:22) translocation in Ewing's sarcoma. This is true of all lines and tumors examined to date (~ 20). It is not true of neuroblastoma, lymphoma, or soft tissue sarcoma. Interestingly, it is also present in a unique childhood tumor, peripheral neuroepithelioma. The break point on chromosome 22 is close to a known oncogene, c-sis. To date, no amplification or rearrangement of c-sis has been detected. In the case of peripheral neuro-epithelioma, c-sis is not amplified, but c-myc is. Unlike classic neuroblastoma, c-myc is not expressed. These results serve to emphasize the common abnormality found in Ewing's sarcoma, its distinction from other round cell tumors, and the unique character of peripheral neuroepithelioma.

HS 6040 (Rev. 1/84)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00508-07 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immune response of CBA/N mice to oligosaccharides coupled to protein carriers

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

PI:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
OTHER:	K. Stein	Senior Staff Fellow	DBP, BOB, FDA

COOPERATING UNITS *(if any)*

Aftab Ahmed, Merck Institute, Rahway, New Jersey

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NCI, NIH, Bethesda, MD 20205

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0.2	0.2	0

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☐ (a1) Minors

☐ (a2) Interviews

B

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

CBA/N mice are an inbred strain of animals that exhibit an X-linked deficiency in immune responsiveness to certain carbohydrate antigens including dextrans. Isomaltodextrins derived by partial enzymatic or acid hydrolysis of dextran were coupled as haptens to the protein carrier keyhole-limpet hemocyanin and were used as immunogens. These glycoconjugates were used to study formation of antibodies that bind dextran in normal adult and neonatal mice and in mice with the CBA/N defect. Of particular interest are studies of the size requirements for an oligosaccharide hapten to elicit a cross-reactive antibody response to the native polysaccharide and the ontogeny of the response to the polysaccharide following immunization with a glycoconjugate.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00510-06 LP

ERIOD COVERED

October 1, 1983 to September 30, 1984

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

Glucose-containing tetrasaccharide in human urine

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

PI:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
OTHER:	M. Ugorski	Visiting Fellow	LP, NCI
	P.A. Pizzo	Surgeon	PO, NCI

OOPERATING UNITS *(if any)*

Department of Clinical Chemistry, University of Lund, Lund, Sweden
(Dr. Arne Lundblad)

AB/BRANCH

Laboratory of Pathology

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Biochemical Pathology Section

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NCI, NIH, Bethesda, MD 20205

OTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

Antibodies raised against a glucose-containing tetrasaccharide-Glcα1-6Glcα1-4Glcα1-4Glc-coupled to KLH immunoassay to measure urinary excretion of the oligosaccharide in urine of patients with glycogenoses, pregnant women, and pediatric patients with soft tissue sarcomas. Preliminary data suggest that the rate of urinary excretion of this tetrasaccharide may be a useful indicator of the tumor mass present in certain patients. The oligosaccharide has been shown to originate from glycogen as a limit dextrin produced by the combined actions of alpha amylase and neutral alpha glucosidase in plasma.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00511-06 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Carbohydrate heterogeneity in alpha subunits of human polypeptide hormones

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

PI:	B. Nilsson	Visiting Scientist	LP, NCI
OTHER:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
	S.W. Rosen	Senior Investigator	CE, NIAMDD
	B. Weintraub	Senior Investigator	CE, NIAMDD

COOPERATING UNITS *(if any)*

Clinical Endocrinology Branch, NIAMDD

LAB/BRANCH

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NCI, NIH, Bethesda, MD 20205

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0.2	0.2	0

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Purified alpha subunits from human chorionic gonadotropin, TSH, FSH, and LH will be treated with neuraminidase and then subjected to alkaline borohydride degradation followed by trifluoroacetolysis. Oligosaccharides released by the alkaline borohydride step will be studied by gel filtration, methylation analysis and mass spectrometry of the permethylated oligosaccharide derivatives. Conditions for trifluoroacetolysis will be adjusted so as to destroy reducing amino sugars after release of oligosaccharides from chitobiosyl-asparagine linkages. Following removal of N-trifluoroacetyl groups from any remaining amino sugars in the mixture, oligosaccharides will be subjected to ion exchange chromatography to separate "high mannose" from "complex" type chains. The oligosaccharides obtained will be subjected to gel filtration chromatography, high voltage electrophoresis in borate buffer, and paper chromatography to investigate possible heterogeneity of carbohydrate chains. Fractions will be monitored by sugar analysis at each step.

HHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00523-05 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Complex carbohydrate released from mammalian cells by trifluoroacetolysis

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

PI:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
OTHER:	G.C. Hansson	Visiting Fellow	LP, NCI
	J. Cashel	Biologist	LP, NCI
	K. Nakahara	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

T.R. Chen, Research Scientist, ATTC, Rockville, MD

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NCI, NIH, Bethesda, MD 20205

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1.0	1.0	0

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Trifluoroacetolysis is a recently-developed method that releases oligosaccharides intact from glycoproteins and glycolipids. Carbohydrate chains released as a mixture from whole tissues, tissue fractions, or cells grown in culture, are easily recovered in nearly quantitative yield and reconstituted to their native form. Analysis of the majority of oligosaccharides containing six or fewer monosaccharide units is performed by combined gas chromatography and mass spectrometry of permethylated, N-trifluoroacetylated oligosaccharide derivatives. Analysis for certain specific oligosaccharides is carried out by radioimmunoassay using antibodies produced against purified oligosaccharides coupled to polypeptide carriers. It is anticipated that the repertoire of oligosaccharide chains produced by cells or tissues will reflect states of cellular differentiation and reveal potential cell surface markers.

HHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00525-05 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Analysis of oligosaccharides by combined gas chromatography-mass spectrometry

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

PI:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
OTHER:	J. Cashel	Biologist	LP, NCI
	E.A. Kabat	Consultant	IRP, NIADDK

COOPERATING UNITS *(if any)*

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NCI, NIH, Bethesda, MD 20205

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0.5	0.2	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Separation of reduced and permethylated oligosaccharides by gas chromatography can be facilitated by the use of a fused silica capillary column 100 meters long, coated with methyl silicon. The presence of N-acetylhexosamines in oligosaccharides increases their retention time and interferes with efficient GC separation. Transamidation of hexosamines by trifluoroacetolysis followed by reduction, removal of O-trifluoroacetyl groups and permethylation, dramatically reduces the retention time of hexosamine-containing oligosaccharides and permits separation of oligosaccharides containing up to six monosaccharide units, regardless of how many of these are hexosamines. The mass spectra of permethylated oligosaccharides with N-trifluoroacetylated amino sugars show unexpectedly high abundances of mass ions containing the N-trifluoroacetyl group. As many of these ions are large, they provide useful information regarding oligosaccharide structure.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00549-04 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Hybridoma antibodies to oligosaccharide haptens

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

PI:	K.R. Schroer	Senior Surgeon	LP, NCI
OTHER:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
	K. Wasniowska	Visiting Fellow	LP, NCI
	J. Phung	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

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LCI, NIH, Bethesda, MD 20205

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2.0	1	1

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Hybridoma antibodies that bind oligosaccharides are valuable reagents for analysis, localization, and purification of free oligosaccharides and glycoconjugates. We have developed immunization protocols and screening procedures, for producing hybridomas against oligosaccharides purified from human milk and urine. Many of these oligosaccharides are structurally identical with carbohydrate chains found in naturally-occurring glycolipids and glycoproteins. Hybridoma antibodies against a glucose-containing tetrasaccharide (G)4 with the structure Glcα1-6Glcα1-4Glcα1-4Glc have been used in a radioimmunoassay to study the metabolic origin of the tetrasaccharide. The same anti (G)4 hybridoma antibodies have been used for affinity purification of the free oligosaccharide.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00556-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Expression of glycolipids in lymphocyte subpopulations

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

PI:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
OTHER:	K. Schroer	Senior Assistant Surgeon	LP, NCI
	M. Ugorski	Visiting Fellow	LP, NCI
	K. Wasniowska	Visiting Fellow	LP, NCI
	J. Phung	Biologist	LP, NCI
	J. Cashel	Biologist	LP, NCI
	J. Fernandez	Biological Laboratory Technician	LP, NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Bethesda, MD 20205

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2.0	1.5	0.5

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Neutral glycolipids are differentially expressed in functionally distinct subpopulations of murine lymphocytes. Subpopulations of B cells can be studied by examining hybridoma lines derived from fusion of splenic B lymphocytes with the mouse myeloma SP2/0. We are analyzing total neutral glycolipids from hybridomas by thin layer chromatography and by GC/MS analysis of oligosaccharides after trifluoroacetolysis. Hybridomas from Balb/c splenocytes express glycolipids containing from two to five simple sugars. These include globoside and its precursors as well as asialo-GM2 and 2' fucosyllactosyl ceramide. The goal of this project is to correlate expression of oligosaccharide chains of glycolipids with functional parameters of B cell subsets such as responsiveness to Type I and Type II antigens.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00879-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Nucleotide sequencing of hybridoma antibodies

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

PI:	K.R. Schroer	Senior Surgeon	LP, NCI
OTHER:	J. Phung	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Bethesda, MD 20205

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1.0	1.0	0

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A substantial body of literature exists regarding the relationships of primary amino acid sequence to serological idiotypy and binding site characteristics of antibodies. This data is enormously weighted to reflect the repertoires of the Balb/c and NZB mice, the only strains in which spontaneous and elicited plasmacytomas have been derived. The CBA/N mouse which has a linked defect in expression of anticarbohydrate antibodies has been neglected in this regard, with only a single sequence of a hybridoma derived antibody published to date. This sequence demonstrated marked abnormalities in the pattern of VK, JH and DH segment utilization. We have assembled a panel of 200 unselected CBA/N B cell hybridomas for sequence analysis in order to investigate the role of combinatorial VDJH and VJK rearrangements in the expressed deficient repertoire of this strain of mouse.

Conventional anti-hapten or anti-protein antibodies will also be sequenced to investigate the combinatorial diversity and idiotypic correlates of several new families of antibodies (anti-type III pneumococcal polysaccharide, anti (G)4, anti-insulin and anti-LPS) in which shared idiotypic serologic markers have been observed.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00887-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immunochemical studies of cell surface glycoproteins

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

PI:	D.A. Zopf	Chief, Biochemical Pathology Section	LP, NCI
OTHER:	K.R. Schroer	Senior Surgeon	LP, NCI
	K. Wasniowska	Visiting Fellow	LP, NCI
	C.M. Reichert	Chief, Autopsy Service	LP, NCI
	M.H. McGinniss	Research Biologist	BB, CC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Biochemical Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Membrane glycoproteins behave as either carbohydrate or peptide antigens and occasionally express antigens constituted from specific structural elements present in both sugar and peptide moieties. Immune responsiveness to cell surface glycoproteins has not been studied systematically. We are characterizing the fine specificities of autoantibodies against glycoproteins of human erythrocytes from patients with altered immunologic states. In addition, we are preparing hybridomas that secrete monoclonal antibodies against various portions of the carbohydrate and peptide moieties of human glycophorin A, the major sialoglycoprotein of human erythrocytes.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00559-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cell matrix receptors role in metastases

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

PI:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI
OTHER:	C.N. Rao	Visiting Fellow	LP, NCI
	S.H. Barsky	Expert	LP, NCI
	G.J. Bryant	Senior Assistant Surgeon	LP, NCI
	P.H. Hand	Chemist	LTIB, NCI
	A.D. Thor	Medical Staff Fellow	LTIB, NCI
	J. Schlom	Chief, Laboratory of Tumor Immunol. and Biol.	LTIB, NCI

COOPERATING UNITS *(if any)*

Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Laminin, a glycoprotein of basement membranes, binds to a specific receptor on the surface of neoplastic and non-neoplastic cells. Laminin exhibits saturatable and competible binding to the surface of cultured living cells, or to isolated plasma membranes from cells or tissue. The binding coefficient is 2 nM with 50,000 receptors per cell. The receptor was isolated from murine and human carcinomas and melanomas. It has a molecular weight of approximately 67,000 daltons. The laminin receptor purified from human breast carcinoma plasma membranes was used as an antigen to generate monoclonal antibodies (mAbs). Using immunoblotting, the mAbs recognize a single ≈ 67,000 dalton protein among all the proteins extracted from breast carcinoma plasma membranes. The mAbs differed in their ability to block binding of laminin to the plasma membrane receptor. Antibody LR1 inhibited virtually 100% of the specific binding of laminin to both the isolated human breast - carcinoma plasma membranes or the living MCF-7 cells. In contrast, antibody LR2 had no effect on laminin binding under identical conditions. Thus, the two types of mAbs may recognize different functional domains on the laminin receptor. Preincubation of metastatic murine melanoma cells with syngeneic whole laminin followed by tail vein injection increased tumor cell retention in the lung and strongly stimulated metastases formation. The domain of the laminin molecule responsible for stimulating metastases was identified. Laminin is a cross-shaped molecule with three short arms and one long arm. All arms have globular end regions. Purified protease-derived fragments of laminin were prepared which a) lacked only the long arm of the molecule (alpha fragment), or b) lacked both the long arm and the globular end regions of the short arms (C1 fragment). Both types of fragments contained the laminin receptor binding region. The fragments had opposite effects on metastases. The alpha fragment stimulated metastases formation to the same extent as whole laminin. In contrast, the C1 fragment inhibited or abolished metastases formation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00877-02 LP

ERIOD COVERED

October 1, 1983 to September 30, 1984

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

Tumor desmoplasia: A study of the collagenous response to tumor invasion

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

PI:	S.H. Barsky	Expert	LP, NCI
OTHER:	T. Kalebic	Visiting Fellow	LP, NCI
	S. Togo	Visiting Fellow	LP, NCI
	C.N. Rao	Visiting Associate	LP, NCI
	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI

OOPERATING UNITS *(if any)*

AB/BRANCH

Laboratory of Pathology

ECTION

Tumor Invasion and Metastases Section

ISTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

OTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The study is designed to biochemically characterize the dense collagenous response to tumor invasion and by doing so, gain insight into the nature and purpose of this host response. Human breast cancer, because of its accessibility and because of its characteristic scirrhous or desmoplastic qualities will be the main tissue of investigation, but the study will be extended to other invasive tumors, which are, and are not associated with a desmoplastic response. Desmoplastic tissue is found to have a markedly increased content of type V collagen. It is proposed that myofibroblasts are recruited by mitogenic and chemotactic factors produced by the tumor cells. The myofibroblasts then contribute to the deposition of elastin and type V collagen. This hypothesis may extend to other situations in which unrestricted fibrosis may compromise host function.

S 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00888-01 LP

PERIOD COVERED

The genetic mechanism involved in the metastatic process & type IV collagenolysis

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

October 1, 1983 to September 30, 1984

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

PI:	U.P. Thorgeirsson	Visiting Scientist	LP, NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI
	T. Turpeenniemi-Hujanen	Visiting Fellow	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

1. The genetic mechanism for induction of the metastatic phenotype was studied by using a) somatic cell hybridization or b) DNA transfection technique. a) Cell hybrids were derived from fusions of high (B16-F10) or low (UV-2237) metastatic tumor cells with tumorigenic (K1735-C116) or normal cells (macrophages). Metastatic and tumorigenic cell hybridization resulted in augmentation of both metastatic capacity and collagenase IV activity. Metastatic and normal cell hybridization yielded suppression of both the metastatic capacity and collagenase IV activity. This study shows that collagenase IV activity correlates with the metastatic capacity in nude mice and may therefore be genetically linked with other factors required for metastases. b) NIH/3T3 cells transfected with human tumor DNA containing an activated N-ras oncogene were metastatic in 100% of NIH nude mice recipients. NIH/3T3 cells transfected with an oncogene alone (V-Harvey-ras) produced metastases in 50% of the mice. The control and spontaneously transformed 3T3 cells were non-metastatic. Both transfected 3T3 cell clones secreted augmented levels of collagenase IV, and invaded human amnion basement membrane *in vitro*. The transfectants were sensitive to natural killer (NK) cell or macrophage cytotoxicity *in vitro* but were able to produce metastases in NK stimulated nude mice. Southern blot and slot blot analysis of genomic DNA from the human tumor DNA transfected cells and the corresponding lung metastases revealed a low level (two-fold) amplification of the N-ras specific DNA sequences in the metastatic DNA. Human repetitive (Alu) sequences were also demonstrated in both the transfected and metastatic cells. This work shows that metastatic properties can be conferred upon NIH/3T3 cells by transfection with either an isolated oncogene or genomic tumor DNA. 2) Human type IV collagenase was purified from culture media of metastatic melanoma cells with a molecular weight of 70,000.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00889-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Laminin receptor: Biology and characterization

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

PI:	G.J. Bryant	Expert	LP, NCI
OTHER:	C.N. Rao	Visiting Associate	LP, NCI
	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI
	I.M.K. Margulies	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A cell surface receptor for the extracellular matrix glycoprotein laminin has been demonstrated via several methods in our laboratory. The proposed study is designed to biochemically characterize the receptor for laminin with regard to its location in the membrane and behavior after ligand binding. We have measured the number of receptors via live cell binding techniques to be 80-110,000 receptors per cell using various cell types (ie. human pancreatic carcinoma, breast carcinoma and bladder carcinoma). The major questions addressed by this study are a) Does the laminin receptor undergo internalization after ligand binding? b) Does the ligand (a part or whole) enter the cell? c) What endocytosis pathway is used by the cell? d) Is the receptor recycled? These questions will be addressed using temperature binding curves, immunoelectron microscopy (employing antibodies to laminin or to the receptor) or pharmacologic agents which block endocytosis.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00890-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Laminin receptor in leukocytes

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

PI:	G.J. Bryant	Expert	LP, NCI
OTHER:	E. Schiffmann	Research Biochemist	LP, NCI
	C.N. Rao	Visiting Associate	LP, NCI

COOPERATING UNITS *(if any)*

S.E. Martin and E. Magyarosy, Cytopathology Section, LP, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	.2	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Leukocytes (PMN) have been shown to use laminin preferentially to adhere to type IV-collagenous matrices. We now report that these cells contain laminin receptors that bind labelled laminin with high affinity (Kd = 6.16 x nM/L). An estimated 36,000 binding sites per cell are present. Monoclonal antibodies to the receptor inhibit the chemotactic response of PMN to peptide attractants. These findings suggest a major role for the laminin receptor in PMN attachment and migration. These characteristics are quite similar to those of highly metastatic tumor cells.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00891-01 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI:	S. Schiffmann	Research Chemist	LP, NCI
OTHER:	D.A. Katz	Senior Staff Fellow	SN, NINCDS
	R. Mandler	Graduate Student	DBS

COOPERATING UNITS *(if any)*

LDBA; NIDR; NIMH

LAB/BRANCH
Laboratory of Pathology

SECTION
Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A principal characteristic of metastatic tumors is their invasiveness. After the cells detach from the primary tumor, they must cross membrane barriers to reach other target sites. We are studying molecular events of an aspect of this process, the chemotactic responsiveness of these cells to certain stimuli. Using a human melanoma cell line, we have found that these cells migrate in response to a material in their conditioned media which has a Mr of about 20 KD and does not appear to be identical to other known attractants. The material may have plasminogen activator activity. These cells also respond to the peptides F Met-Leu-Phe and bombesin. Additionally, we have found that a highly metastatic murine melanoma cell line selected from a subline of cells is markedly more chemotactically responsive than a poorly metastatic line from the same subline.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00892-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Molecular biology of the metastatic phenotype

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

PI:	M.E. Sobel	Senior Investigator	LP, NCI
OTHER:	A.P. Claysmith	Biologist	LP, NCI
	P.S. Steeg	Guest Worker	LP, NCI
	T. Kalebic	Visiting Fellow	LP, NCI
	R.J. Muschel	Senior Staff Fellow	LP, NCI
	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI

COOPERATING UNITS *(if any)*

Dr. G. Vogeli, LMDBI, NEI
Dr. B. Smith, Veteran's Administration Outpatient Clinic, Boston, MA

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Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

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

B

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

We are studying the molecular biology of tumor metastasis and invasion. We are using a variety of techniques to identify specific genetic elements whose expression is altered in metastatic cells. Pulse-labeling studies of paired benign and metastatic cells reveal differences in the synthesis of specific proteins. RNA from cultured cell lines and tissues with varying metastatic potential is being analyzed by cell-free translation in a rabbit reticulocyte lysate and by hybridization analysis. In vitro translation studies indicate that the levels of several specific mRNAs are either markedly increased or decreased in metastatic murine melanoma cells and in metastatic human breast carcinoma cells. Rot curve analysis of human breast carcinoma cDNA confirms that specific gene sequences are present in abnormal amounts in more malignant cells. We are in the process of constructing and screening recombinant DNA libraries of metastatic cDNAs to isolate and study specific genes involved in the etiology and maintenance of the neoplastic state.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00893-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

DNA mediated transfer of metastatic potential

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

PI:	R.J. Muschel	Senior Staff Fellow	LP, NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.50	.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A cell line which is tumorigenic and transformed may not be capable of forming metastases. Using DNA mediated gene transfer, we will attempt to confer the metastatic potential upon benign tumor cells. We have found that NIH 3T3 cells transformed by certain viral oncogenes are metastatic. This result which indicates that metastatic potential can be transferred in this one instance should allow us to extend these observations to other cell types and to develop selection schemes for metastatic potential which will allow us to utilize gene transfer systems to isolate genes which may cause metastatic behavior.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00894-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Differential cDNA cloning of genes involved in metastasis

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

PI:	R.J. Muschel	Senior Staff Fellow	LP, NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Invasive tumor cells presumably express certain genes which are inactive in non-invasive cells. We are attempting to clone these genes by making cDNA from an invasive cell line and extensively hybridizing it against RNA from a non-invasive cell line. We then label the remaining single-stranded DNA to use as a probe of a cDNA library from an invasive cell line. This approach should identify those clones which are expressed in invasive cells but not benign cells.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00895-01 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of histocompatibility antigens in metastatic cells

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

PI:	R.J. Muschel	Senior Staff Fellow	LP, NCI
OTHER:	T. Kalebic	Visiting Fellow	LP, NCI
	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pathology

SECTION
Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

A series of lines of a common origin which differ in their metastatic potential have been analyzed for their expression of murine histocompatibility complex antigens (MHC). The benign, non-metastatic line has consistently been found to contain 5-10X more MHC specific RNA than the metastatic lines. We intend to further characterize this down regulation at the protein level and with more specific probes.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00896-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Development of highly metastatic cell lines & identification of metastatic markers

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

PI:	T. Kalebic	Visiting Fellow	LP, NCI
OTHER:	R.J. Muschel	Senior Staff Fellow	LP, NCI
	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The first requirement for studying regulatory mechanisms of metastatic phenotype is development of genetically related cellular systems containing the cell lines with low and high metastatic capacity. Starting from murine melanoma 1735 clone M2, a series of highly metastatic lines have been established using serial transplantation in the animals, serial passages of cells in culture and invasion through amnion and corion membranes.

Experiments demonstrate that the group of lines named TK (direct derivative from 1735-M2), TK-R (direct derivative from TK originating from metastatic nodules in the Rib), TK-L (derivative from TK, originating from metastatic nodules in the lung), TK-Li (derivative from TK line, originating from metastatic nodules in the liver) produce 10X more metastasis in the lung after intravenous infection than the parent line does. Syngeneic or NIH-nude mice injected with TK line and derivatives develop much higher numbers of metastatic nodules in the lung. The mice do not survive for more than 14 to 17 days. Injection of 1735 cell line does not affect the survival of the same animals for more than 4 to 5 weeks. According to previous findings, highly metastatic TK cell lines produce more collagenous type IV than low metastatic lines. Doubling time is approximately the same. In vitro migration assays demonstrate higher migratory capacity of TK cells than do metastatic cells.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08247-06 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Basement membrane degradation by normal and neoplastic cells

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

PI:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI
OTHER:	T. Kalebic	Visiting Fellow	LP, NCI
	T. Turpeenniemi-Hujanen	Visiting Fellow	LP, NCI

COOPERATING UNITS *(if any)*

Laboratory of Developmental Biology and Anomalies, NIDR, and Laboratory of Chemistry, NIAMDD

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

There are at least five genetically distinct collagen types whose degradation may be controlled independently. The initial step of collagen degradation is performed by collagenase. We were the first to find that type IV basement membrane collagen and type V collagen is not degraded by human skin collagenase suggesting that a separate collagenase may degrade types IV and V collagen. A collagenase which preferentially degrades type IV collagen has been derived from metastatic tumor cells and from mammary epithelium. This collagenase has been purified 1000-fold and its cleavage products have been partially characterized by rotary shadowing electron microscopy. We are further studying the secretion rate of this enzyme by a wide variety of cell types both normal and malignant. A collagenase which preferentially degrades type V collagen has been identified and purified from metastatic tumor cells and endothelial cells migrating toward an angiogenic stimulus. This collagenase has been purified and its cleavage products have been partially characterized by rotary shadowing electron microscopy. The enzyme cuts the type IV procollagen molecule at a single site 25% of the distance from the n-terminal end. We are further studying the secretion rate of this enzyme by a wide variety of cell types both normal and malignant. A collagenase which preferentially degrades type V collagen has been identified and purified from metastatic tumor cells. The type V collagenase has been purified. It has a molecular weight of 80 Kd and cleaves the type V collagen molecule at a single major site near one end. Membrane-associated forms of these enzymes have been discovered. Polyclonal monospecific antibodies and monoclonal antibodies to the type IV collagenase have been prepared. These antibodies react with human breast carcinoma cells in tissue sections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08266-04 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Structure and function of basement membrane molecules

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

PI:	C.N. Rao	Visiting Associate	LP, NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP, NCI
	I.M.K. Margulies	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

NIDR, Laboratory of Developmental Biology and Anomalies

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The nature and assembly of basement membrane constituents namely IV collagen, laminin and heparan sulphate proteoglycan were studied using a variety of in vitro binding assays. These basement membrane macromolecules were isolated from the EHS tumor grown in C57 black mice. Protease-derived fragments of laminin and IV collagen were characterized by rotary shadowing electron microscopy. The domains required for binding of laminin and IV collagen were identified. Laminin is a cross-shaped molecule with three equal short arms and one long arm. The cell binding region of laminin was also identified and found to reside at the intersection of the three short arms. The carbohydrate composition of laminin was obtained and the distribution of sugars on the long and short arms of laminin molecule was studied.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00543-06 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Characterization of the papillomaviruses

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

PI:	P.M. Howley	Chief, Viral Oncology and Molecular Pathology Section	LP, NCI
OTHER:	Y.-C. Yang	Visiting Fellow	LP, NCI
	B. Spalholz	Guest Worker	LP, NCI
	M. Rabson	Guest Worker	LP, NCI
	J.C. Byrne	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
39/12	30/12	9/12

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

There are currently recognized to be 27 human papillomaviruses and 6 bovine papillomaviruses. Each of these viruses is associated with distinct clinical entities which in humans include common warts, condyloma accuminata, laryngeal papillomatosis, and the macular pityriasis-like lesions of epidermodysplasia verruciformis. In cattle, these lesions are associated with cutaneous fibropapillomas and esophageal papillomatosis among other lesions. To date, no tissue culture system has been developed to propagate the papillomaviruses. There is a subset of papillomaviruses which are associated with cancers in their natural hosts. Among the human papillomaviruses, these include HPV-5 and HPV-8 in patients with epidermodysplasia verruciformis, and HPV-16 and HPV-18 in human cervical carcinomas. In cattle, it includes BPV-4 in cattle which feed on bracken fern. In the laboratory, we have focused our attention on the molecular biology of BPV-1, because it is capable of transforming susceptible rodent cells in culture. Transformation of rodent fibroblasts by papillomaviruses thus remains one of the only biological systems available to the study of the papillomaviruses. A unique feature of this papillomavirus transformation system is that the viral DNA does not integrate into the host chromosome necessarily upon transformation. The DNA may remain extrachromosomal as a stable multicopy plasmid. The factors involved in stable transformation as well as stable plasmid maintenance, are being extensively studied. A second feature associated with papillomavirus infection is the propensity of certain lesions caused by certain viruses to progress to carcinomas. What factors are involved in the progression of a benign lesion to a carcinoma, are not known. Studies involving the Shope papillomavirus and Shope papillomavirus-induced carcinomas are underway to define whether activated oncogenes in addition to the viral sequences may be associated with this progression.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00547-04 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

The use of papillomavirus DNAs as eukaryotic cloning vectors

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

PI:	P.M. Howley	Chief, Viral Oncology and Molecular Pathology Section	LP, NCI
OTHER:	M. Rabson	Guest Worker	LP, NCI
	C. Yee	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
14/12	8/12	6/12

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The bovine papillomaviruses are capable of transforming certain mouse fibroblast lines as well as certain rat fibroblast lines. The viral DNA in these transformed lines is maintained exclusively as extrachromosomal plasmids. The extrachromosomal nature of the viral DNA in these lines together with the selected malignant phenotypes has been utilized to develop the papillomaviruses as eukaryotic cloning vectors. We have shown that the complete genome cloned into a deletion derivative of pBR322 (pML2) is capable of serving as a shuttle vector between bacteria and eukaryotic cells. Eukaryotic and prokaryotic genes can be expressed in mammalian cells as part of BPV plasmids. We have shown that the human beta interferon gene can be inducibly regulated when maintained in a plasmid state in mouse cells. Using a stable plasmid containing the neomycin resistance gene (a phosphotransferase) from Tn5, we have the tissue range and host range of papillomavirus plasmid replication. This plasmid remains extrachromosomal in CV-1 cells (a monkey kidney cell line) and the cells do not exhibit any of the characteristics of transformed cells. When selected for drug resistance, specialized mouse cells including mouse epidermal cells, mouse hepatocytes, and mouse lymphocytes contain the DNA integrated.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00564-02 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Early events in VSV: host cell interaction

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

PI:	C.R. Schlegel	Senior Investigator	LP, NCI
OTHER:	R. Blumenthal	Chief, Membrane Structure and Function Section	LMMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pathology

SECTION
Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

B

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

VSV infects a wide variety of animal cells and has been used as a prototype for studying the mechanism of replication and assembly of enveloped viruses. Recently, there has been renewed interest in the study of virus uptake into host cells. Such studies have not only elucidated basic characteristics of cell function but have also given focus to new antiviral therapies. Our laboratory has been investigating the internalization of VSV into host cells in an attempt to (1) define the plasma membrane binding site for VSV, (2) determine the specificity characteristics of this binding, (3) dissect the mechanism by which VSV fuses with cell membranes, and (4) explore possible mechanisms for inhibiting or perturbing the early steps of infection.

We will use multiple approaches to study the internalization of VSV. Binding assays with purified S35-VSV will permit the detection of specific VSV binding. In addition, IF and EM techniques will be used to monitor the morphologic pathway of VSV entry. To analyze the fusion of virus and cell membranes, we will utilize liposomes containing VSV G protein (virosomes). These virosomes will be studied for their specificity of interaction with host cells and then used to study their interaction with other liposomes. Energy transfer fluorescence, fluorescence quenching, and EM will be used to quantitate and monitor the fusion process mediated by G protein. In addition, attempts will be made with circular dichroism and infra-red spectroscopy to follow possible conformational changes in G protein which occur during the fusion event. Neutralizing antibodies will be used to confirm relevant changes in protein conformation. Finally, synthetic peptides corresponding to conserved regions of the VSV G protein protein will be used to define the biological domains of G protein.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00565-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cell immortalization and transformation by papovaviruses

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

PI:	C. R. Schlegel	Senior Investigator	LP, NCI
OTHER:	J. Bolen	Senior Investigator	LBTV, NCI
	S. Schlegel	Expert	CIP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Cell transformation by papovaviruses requires the expression of several early viral gene products. Our laboratory is currently investigating the mechanism by which papillomaviruses (human and bovine) and polyomavirus perturb cell growth control and participate in tumorigenesis. The main research focus will be on human papillomaviruses (HPV). Although the role of HPV in benign human tumors (warts) is well established, it is only recently that an association between HPV and cervical cancer has been defined. More than 25 types of HPV exist (by DNA hybridization). Only a few of these HPVs are associated with cervical cancer, however. For example, type 16 appears to be found in various stages of cervical dysplasia (or "flat warts") as well as in carcinoma-in-situ and invasive carcinoma. Type 18 HPV is found only in invasive cervical carcinoma. The biological role of these viral genomes in tumor cells is unknown, but the ability of related bovine papillomaviruses (BPV) to transform cells in vitro suggests that HPV may have a role in either initiating or maintaining the transformed state. Our interest is to define the types of HPV in cervical squamous cell carcinomas, to determine whether their DNA is transcribed into mRNA, and hopefully to detect virus-specific proteins in the tumor cells. We will also attempt to transform cells in vitro with HPV DNA. Selected, formal studies with BPV will be performed for comcomparison of HPV and BPV transforming properties. Related to our attempts to transform cells with HPV is an effort to transform epithelial cells in vitro. Our laboratory has an interest in defining the progressive stages of carcinogenesis and, as part of this study, to establish an in vitro system for the transformation of epithelial cells. We have decided to focus on human and murine epidermal cells since much is known about the murine model for the induction of squamous cell carcinoma and since these cells can be propagated in vitro. Culture conditions for human epidermal cells are also well established. Our initial attempt will include transfecting murine epidermal cells with BPV and polyoma DNA and human epidermal cells with HPV-16 and HPV-18 DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00898-01 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of human papillomaviruses in human carcinogenesis

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

PI:	P.M. Howley	Chief, Viral Oncology and Molecular Pathology Section	LP, NCI
	C.R. Schlegel	Senior Investigator	LP, NCI
OTHER:	I. Hewlett	Visiting Fellow	LP, NCI
	C. Yee	Biologist	LP, NCI
	J.C. Byrne	Biologist	LP, NCI
	M. Wade	Biologist	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pathology

SECTION
Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1 1/3	7/12	9/12

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The papillomaviruses are associated with naturally occurring human carcinomas in a variety of species, including man. HPVs 5 and 8 have been associated with cutaneous carcinomas in patients with epidermodysplasia verruciformis. HPVs 16 and 18 have been associated with carcinoma in situ as well as invasive carcinomas of the cervix in man. In addition, HPV-18 has been reported to be present within Hela cell DNA. Papillomaviruses in general cause benign tumors and there a number of animal systems where progression of the benign lesion into a carcinoma occurs. In general, this occurs after a period of time and often in the association with a second co-carcinogenic agents. The mechanism of this carcinogenic progression is unknown.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00550-04 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immunologic characterization of malignant lymphomas

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

PI:	E.S. Jaffe	Chief, Hematopathology Section	LP, NCI
OTHER:	J. Cossman	Senior Assistant Surgeon	LP, NCI
	R.I. Fisher	Senior Investigator	MB, NCI
	D.L. Longo	Senior Investigator	MB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

In order to assess the clinical and pathologic significance of the immunologic characterization of human malignant lymphomas, fresh biopsy tissues are obtained from patients referred to the Clinical Center for treatment. Biopsies are obtained with patient permission prior to therapy and processed in the Hematopathology Section. The neoplastic cells are characterized as to their origin from T cells, B cells, or histiocytes, and can in addition be identified as belonging to specific developmental and functional subpopulations. This data is then correlated with clinical and pathologic data.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00551-04 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Stimulation of phagocytosis by a peripheral T-cell lymphoma-derived lymphokine

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

PI:	E.S. Jaffe	Chief, Hematopathology Section	LP, NCI
OTHER:	C.R. Simrell	Senior Assistant Surgeon	LP, NCI
	L.M. Neckers	Expert	LP, NCI
	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

Certain patients with malignant lymphomas originating from peripheral T cells develop a rapidly fatal syndrome which mimics malignant histiocytosis. It is suspected that the pathogenetic mechanism of this phenomenon may involve a lymphokine produced by the neoplastic T cell which can stimulate the phagocytic cells of the reticuloendothelial system. In order to test this hypothesis, neoplastic cells from fresh biopsies of patients with malignant lymphoma are placed in overnight culture, and supernatants are tested for the presence of soluble factors which are able to affect human phagocytic cells in vitro.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00552-04 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Malignant lymphomas: Analysis with monoclonal antibodies and genetic probes

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

PI:	J. Cossman	Senior Assistant Surgeon	LP, NCI
OTHER:	L.M. Neckers	Expert	LP, NCI
	E.S. Jaffe	Chief, Hematopathology Section	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

A variety of monoclonal antibodies have been recently developed that distinguish among classes of normal human lymphocytes and identify discrete stages of differentiation. We are using a battery of these antibodies to determine the phenotypes of human malignant lymphomas using a Fluorescence Activated Cell Sorter (FACS-II). The phenotypic expression of these neoplastic lymphocytes is then related to normal lymphocytes and is useful in diagnosis and monitoring of patients' tumors during therapy. The diagnosis of B-cell neoplasms is aided by demonstrating monoclonality with anti-immunoglobulin light chain staining, cytoplasmic RNA blots and genomic DNA (Southern) blots.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00553-04 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Control of fibrinogen gene expression

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

PI:	G.R. Crabtree	Medical Officer	LP, NCI
OTHER:	E. Evans	Fogarty Fellow	LP, NCI
	J. Morgan	Fogarty Fellow	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

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Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We are studying the regulation and structure of the genes which code for fibrinogen, the major blood coagulation protein. We have found that fibrinogen mRNA levels are controlled through a complex feedback-like regulation involving the plasmin degradation products of fibrinogen and interleukin I. This same mechanism also appears to account for the induction of the acute phase reaction in response to injury or inflammation. This regulatory influence somehow coordinates the levels of each of the three fibrinogen mRNAs so that the genes are activated at the same time and to the same extent. We have begun studying the mechanisms underlying this coordinate regulation and have obtained cDNA and genomic clones for each of the three fibrinogen chains in the rat and human. Thus far, we have found that the three fibrinogen genes are linked on human chromosome four band q2, that the activation of the three genes occurs by increasing the rate of transcription of mRNA from each of the three genes, and that homologous sequences exist at the 5' ends of the genes which might account for this regulation.

We have also begun studying the hereditary human afibrinogenemias as models of defective fibrinogen production. Patients with these diseases do not make circulating fibrinogen. We have found that by examining the DNA using Southern blotting from patients with afibrinogenemias, that the three genes are present and that they are not rearranged or deleted at the level of Southern blot analysis.

The goals of our research are to understand the factors controlling and coordinating the expression of families of genes during differentiation and development.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00574-02 LP

ERIOD COVERED

October 1, 1983 to September 30, 1984

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

Anti-idiotype in the investigation and therapy of B-cell lymphoma and leukemia

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

PI:	J. Cossman	Senior Assistant Surgeon	LP, NCI
OTHER:	L.M. Neckers	Expert	LP, NCI
	J.B. Trepel	Biologist	LP, NCI
	R.M. Braziel	Medical Staff Fellow	LP, NCI
	M. Raffeld	Medical Staff Fellow	LP, NCI

OOPERATING UNITS *(if any)*

Medicine Branch, Clinical Oncology Program, NCI

AB/BRANCH

Laboratory of Pathology

ECTION

Hematopathology Section

NSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

OTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	3/4	1/4

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have developed an in vitro system to induce immunoglobulin secretion by malignant B cells (lymphomas and leukemias). The immunoglobulin secreted by these cells (IgM) is purified on an affinity column developed in our laboratory. This highly purified monoclonal immunoglobulin is used for the immunization of mice and development of monoclonal (hybridoma) antibodies specific to idiotypic determinants associated with the malignant cells. Antibodies have been produced and are being applied to investigations of differentiation and malignant transformation of B-cell neoplasms. Where appropriate, they will be administered to patients for imaging and passive immunotherapy. Preliminary evidence in another institution has shown that such antibodies may be efficacious in certain types of low-grade B-cell lymphomas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00850-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Regulation of differentiation in human B-cell lymphoma and leukemia

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

PI:	J. Cossman	Senior Assistant Surgeon	LP, NCI
OTHER:	L.M. Neckers	Expert	LP, NCI
	J.B. Trepel	Biologist	LP, NCI
	R.M. Braziel	Medical Staff Fellow	LP, NCI
	E. Lipford	Expert	LP, NCI
	S. Pittaluga	Visiting Fellow	LP, NCI
	R. McGlennen	Biol. Lab. Tech.	LP, NCI

COOPERATING UNITS *(if any)*

Metabolism Branch, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have found that under appropriate conditions human B-cell neoplasms can be induced to differentiate into immunoglobulin-secreting cells. Follicular (B-cell) lymphoma cells are often suppressed by nearby T cells, presumably as a host immune response. Induction is associated with a marked change in morphology characterized by both immunoblastic and plasmacytoid features. Abundant intracytoplasmic immunoglobulin accumulation occurs and cells secrete monoclonal immunoglobulin into the culture supernatants. A variety of cell surface markers have been analyzed and a loss of surface IgD is the only significant change seen during induction. The differentiation is regulated at least at a pretranslational level since there is significant and rapid accumulation of IgM mRNA. Like plasma cells, the activated cells selectively produce more secretory than membrane form of IgM mRNA by the developmentally regulated alternative processing of IgM mRNA.

The mechanism by which differentiation is activated in these cells is mediated by a calcium-dependent pathway, TPA-activated protein-kinase-C.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00851-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Mechanism of TPA-induced immunoglobulin secretion by CLL cells

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

PI:	L.M. Neckers	Expert	LP, NCI
OTHER:	S. Pittaluga	Fogarty Fellow	LP, NCI
	J. Cossman	Sr. Assistant Surgeon	LP, NCI
	J.B. Trepel	Biologist	LP, NCI
	R.M. Braziel	Medical Staff Fellow	LP, NCI
	R.C. McGlennen	Medical Student	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We have demonstrated that the phorbol ester TPA is capable of causing induction of immunoglobulin synthesis in chronic lymphocytic leukemia cells (CLL). This induction involves increased levels of mRNA coding for the secretory form of IgM. Our goal in this study is to discern the mechanism(s) whereby TPA exerts its effects on these CLL cells.

We have now expanded these studies to include the mechanisms by which immunoglobulin secretion normally occurs.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00855-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Pathologic features of HTLV-associated diseases

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

PI:	E.S. Jaffe	Chief, Hematopathology Section	LP, NCI
OTHER:	W.A. Blattner	Senior Investigator	EEB, NCI
	P. Bunn	Senior Investigator	DCT, NCI
	R.C. Gallo	Senior Investigator	LTCB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

Pathologic material from patients identified to be seropositive for HTLV is reviewed and correlated with clinical and epidemiologic features of disease. Material is derived from patients in the United States as well as other parts of the world. Where possible, immunologic phenotyping of the lymphomas is performed and tumor DNA is directly analyzed for viral genome.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00864-02 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Control of the interleukin II gene in normal and malignant cells

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

PI:	G.R. Crabtree	Medical Officer	LP, NCI
OTHER:	N.J. Holbrook	Guest Worker	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

T cell growth factor (interleukin II) is a 15,000 dalton polypeptide which is responsible for the clonal proliferation of normal T lymphocytes during the immune response. This small polypeptide is inducible by mitogen or antigen in normal human lymphocytes and recent evidence indicates that it may control the replication of certain human malignant T cells. Several years ago we found that production of T cell growth factor (TCGF) in normal cells could be completely inhibited by glucocorticoid, suggesting that glucocorticoid may be effective in treating certain human leukemias because of their effects on TCGF production.

The goals of our studies will be to define the factors controlling expression of the TCGF genes in normal and malignant cells, and to attempt to understand the mechanism through which these factors exert their effects.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00881-03 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Regulation of cell growth by transferrin receptors

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

PI:	L.M. Neckers	Expert	LP, NCI
OTHER:	J. Cossman	Sr. Assistant Surgeon	LP, NCI
	W. Funkhouser	Clinical Associate	SB, NCI
	E. Grimm	Expert	SB, NCI
	S. James	Clinical Associate	I, NCI
	G. Yenokida	Clinical Associate	I, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI; Immunology Branch, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

All cells studied to date require transferrin for growth. We and others have shown that antibodies to the transferrin receptor block the growth of lymphoblastoid cell lines. In mitogen-stimulated lymphocytes, these antibodies block proliferation. We are studying the processes which regulate the appearance of these receptors in lymphocytes and lymphoblastoid cell lines, and the function of these receptors in cell growth and metabolism.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00883-03 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Malignant lymphomas: analysis with monoclonal antibodies on tissue sections

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

PI:	S.-M. Hsu	Medical Staff Fellow	LP, NCI
	E.S. Jaffe	Chief, Hematopathology Section	LP, NCI
OTHER:	M. Raffeld	Medical Staff Fellow	LP, NCI
	C.R. Simrell	Senior Assistant Surgeon	LP, NCI
	J. Cossman	Senior Assistant Surgeon	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

A variety of monoclonal antibodies (hybridomas) have been recently developed that distinguish among classes of normal human lymphocytes and identify discrete stages of differentiation. We are using a battery of these antibodies to determine the phenotypes of human malignant lymphomas using an immunohistochemistry technique. The phenotypic expression of these neoplastic lymphocytes is then related to normal lymphocytes and is useful in diagnosis and monitoring of patients' tumors during therapy.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB00517-43 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Report from the Pathological Technology Section

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

PI: B.J. Coolidge Chief, Pathological Technology Section LP, NCI

OTHER:

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Pathological Technology Section

INSTITUTE AND LOCATION

NCI, FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	0	4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05003-19 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cell-Mediated Cytotoxicity

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

PI:	J. R. Wunderlich	Senior Investigator	IB, NCI
Others:	C. C. Ting	Medical Officer	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Mouse effector cells mediating broadly reactive antitumor cytotoxic activity, induced under syngeneic conditions in vitro with polyinosinic acid and/or phorbol-induced lymphocyte growth factors, destroy tumor cells not only in vitro but also in tumor neutralization (Winn) assays in vivo. In both assays T-cell depletion of responding cells increases the antitumor response, whereas T-cell depletion of effector cells abrogates the response. In vitro analysis of the genetic control of the antitumor response shows that it is regulated by multiple genes even when ancillary cell effects are depleted. Genetic regulation of the antitumor response correlates well with regulation of the in vitro generation of T cells, suggesting that most of the new T cells have antitumor activity or that the antitumor response represents a phase of T cell differentiation. The relevant phorbol-induced growth factor(s) from mouse cells appears to be closely related to highly purified human IL2, because 1) human IL2 can substitute for the mouse-derived growth factor(s) in generating antitumor responses in vitro, and 2) the strain distribution patterns of levels of cytotoxicity induced by the two preparations are the same.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05018-14 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI:	P. A. Henkart	Senior Investigator	IB, NCI
Others:	M. Henkart	Expert	IB, NCI
	P. Millard	Biologist	IB, NCI
	C. Yue	Medical Staff Fellow	IB, NCI
	W. Munger	Investigator	IB, NCI
	T. Soares	Microbiologist	IB, NCI
	C. W. Reynolds	Investigator	BTB, FCRF, NCI
	R. P. Blumenthal	Chief, Membrane Structure Sect.	LMMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The mechanism of target cell lysis by LGL tumor granule cytolysin has been studied by several different approaches. Since previous results had suggested a complement-like protein insertion and pore formation, we first showed that liposomes were targets for the cytolysin in a rapid, calcium-dependent reaction leading to carboxyfluorescein release. Cylindrical pore-like structures were shown to be inserted into liposomes which also displayed penetration of negative stain. Similarly, electrical measurements on artifical membranes showed that cytolysin induced a calcium-dependent ionic permeability increase which was highly voltage dependent and identical in properties to that previously described with lymphocytes in an ADCC model. Confirming the protein insertion model, it was shown that liposomes and lipoproteins were highly inhibitory to the lytic activity of cytolysin. Antibodies raised against purified granules specifically stained LGL granules in fluorescent microscopy, and F(ab')2 fragments specifically neutralized cytolytin activity as well as ADCC and NK activity. These results strongly suggest that a granule component is involved in the cell-mediated cytotoxic process. Additional studies were directed at the differences in sensitivity to the cytolysin of various nucleated target cells. The resistant cells are capable of inactivating cytolysin in a calcium-independent process that is not understood, but which appears to account for the differences in sensitivity to the cytolysin. Cytolysin has been purified by solubilization in 2M NaCl and gel filtration on ACA 54, where it elutes at the position of a 60 kd. protein. Further purification can be accomplished by DEAE-HPLC, but the preparation still contains several bands on SDS gels.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05021-13 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigens Determined by the Murine Major Histocompatibility Locus

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

PI:	D. H. Sachs	Chief, Transplantation Biology Section	IB, NCI
Others:	J. A. Bluestone	Laboratory Leader	IB, NCI
	S. L. Epstein	Senior Staff Fellow	IB, NCI
	S. Chatterjee-Hasrouni	Visiting Fellow	IB, NCI
	N. Shinohara	Expert	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION
Transplantation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.5	1.5

CHECK APPROPRIATE BOX(ES)

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

B

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

Studies are being directed toward understanding the major histocompatibility complex, the structure and function of the products of this complex, and manipulations of immune responses to these products. Current studies include: 1) Characterization of major histocompatibility antigens: Congenic resistant strains of mice are developed, maintained, and used in serologic and immunochemical analyses of the MHC products of the mouse; 2) Studies of monoclonal antibodies to H-2 and Ia antigens: Hybridoma cell lines are produced by fusion of immune mouse spleen cells with mouse myeloma cells. The monoclonal anti-H-2 and anti-Ia antibodies produced by these hybridomas are analyzed by serologic and immunochemical means and are used to further characterize the fine structure of the MHC; 3) Characterization of receptor sites for histocompatibility antigens: Anti-idiotypic antisera are produced against anti-H-2 and anti-Ia hybridoma antibodies, and the effects of these antisera on in vitro and in vivo parameters of histocompatibility are assessed; and 4) Mechanism of tolerance to H-2 and Ia antigens: The humoral and cellular responses of radiation bone marrow chimeras are examined, and the mechanism for maintenance of tolerance in these animals is studied.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05023-13 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI:	D. H. Sachs	Chief, Transplantation Biology Section	IB, NCI
Others:	S. A. Rosenberg	Chief, Surgery Branch	SB, NCI
	M. D. Pescovitz	Medical Staff Fellow	IB, NCI
	L. R. Pennington	Medical Officer	IB, NCI
	F. Popitz	Visiting Fellow	IB, NCI
	D. S. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*
NIH Animal Center, Poolesville, Maryland
Joan K. Lunney, Research Chemist, USDA Animal Parasitology Institute, Beltsville, Maryland

LAB/BRANCH
Immunology Branch

SECTION
Transplantation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

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

B

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

A breeding program has been carried out starting with two miniature pigs from different sources and selecting offspring according to tissue typing procedures aimed at defining the major histocompatibility complex of this species. By this procedure three herds of miniature swine, each homozygous for a different set of histocompatibility antigens at the MHC have been developed. Current projects include: 1) Assessment of survival of organs and tissue transplants among and between members of these herds as a model for tissue typing and transplantation; 2) Purification and characterization of the major histocompatibility antigens of this species, and isolation and characterization of peptides from these antigens for sequence analyses and for assessment of immunologic reactivity; 3) Assessment of the immunologic parameters involved in tolerance to allografts in this species; 4) Detection and characterization of intra-MHC recombinants. Two intra-MHC recombinants have been obtained and bred to homozygosity. Kidney transplants utilizing these new recombinants have shown that selective matching for Class II antigens frequently permits long-term kidney graft survival across a Class I difference. The mechanism of this apparent tolerance is under further study; and 5) Bone marrow transplants in miniature swine. The effect of mixing autologous plus allogeneic marrow in the reconstituting inoculum are being examined. This modality is being assessed as a specific preparative regimen for allogeneic organ transplantation.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05033-13 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immunotherapy of Human Cancer

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

PI:	R. J. Hodes	Chief, Immunotherapy Section	IB, NCI
Others:	S. A. Rosenberg	Chief	SB, NCI
	R. I. Fisher	Senior Investigator	MB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

Immunotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

D

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

A controlled, randomized trial comparing immunotherapy to chemotherapy in stage I and stage II malignant melanoma has been initiated. A total of 181 patients have entered the trial, which is closed to further accrual of patients. Preliminary evaluation of data has demonstrated no significant effect of adjuvant therapies on clinical course.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB05035-12 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Function of B Lymphocyte Surface Membrane Molecules

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

PI:	H. B. Dickler	Senior Investigator	IB, NCI
Others:	M. C. Lamers	Postdoctoral Fellow	IB, NCI
	S. Heckford	Postdoctoral Fellow	IB, NCI
	F. Uher	Postdoctoral Fellow	IB, NCI

COOPERATING UNITS *(if any)*

Dr. F. D. Finkelman, Dept. Medicine, USUHS, Bethesda, MD

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.00	3.00	1.00

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The goal of this project is to characterize the function of B lymphocyte membrane molecules. Previous findings indicate that the Fcγ receptors of B lymphocytes interact with: a) the lymphocyte cytoskeleton, b) Ia antigens and LyM antigens, c) surface IgM, and d) surface IgD. Each of these interactions is distinct, specific, and non-random. Initial experiments with purified monoclonal anti-Fcγ receptor antibodies showed induction of B lymphocytes to both proliferate and secrete antibody. However, it was subsequently shown that the B lymphocyte triggering activity was due to a copurified low molecular weight factor produced by the hybridoma. This result suggests the possibility that certain B lymphocytes may produce factor(s) with helper activity. Recent studies have indicated that antigen-antibody complexes in antigen-excess are very effective at inhibiting B lymphocyte antibody production in response to $F(ab')_2$ anti-mu plus lymphokine containing supernatants, while not affecting proliferation. Direct evidence was obtained that this inhibition was mediated by B lymphocyte Fcγ receptors. Kinetic data also suggested the possibility that this inhibition might be due to interference with utilization of a helper lymphokine. Monoclonal anti-Fcγ receptor antibodies on a Sepharose matrix but not in soluble form also inhibited B lymphocyte antibody production in response to anti-mu plus lymphokines. No inhibition of proliferation was seen and this inhibition appeared specific. Thus, B lymphocyte Fcγ receptors deliver a negative signal to B lymphocytes at a particular stage of development when cross-linked by their specific ligand or specific monoclonal antibody.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05036-12 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Control of the Immune Response to Staphylococcal Nuclease

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

PI:	D. H. Sachs	Chief, Transplantation Biology Section	IB, NCI
Others:	R. J. Hodes	Chief, Immunotherapy Section	IB, NCI
	A. Finnegan	Guest Worker, Immunotherapy Section	IB, NCI
	C. A. Devaux	Visiting Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION
Transplantation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

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

B

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

Antibodies directed against idiotypic determinants on anti-Staphylococcal nuclease antibodies from different mouse strains have been produced in rats and in pigs. The idiotypes are detected by ELISA assays and by the inhibition of antibody-mediated inactivation of nuclease. By screening a variety of strains and offspring from appropriate matings between strains for the presence of idiotypes and other markers, it has been shown that idiotype expression is linked to the heavy chain allotype markers. By means of an in vitro anti-TNP nuclease plaque-forming cell response, idiotypic markers have been demonstrated on T helper cells. Administration of anti-idiotypic antibodies to mice has been found to induce idiotype expression in the serum of these animals. This effect appears to involve T cells, since it is not observed in nude mice, and since idiotype-bearing T helper cells for in vitro anti-TNP nuclease response have been found in spleens from such treated animals. Several hybridomas reactive with nuclease and/or anti-idiotype have been produced. Syngeneic anti-idiotypes have also been produced and are presently being characterized in both antibody and T cell systems. Competitive binding studies are used to determine epitopes of nuclease as defined by available monoclonal antibodies. Site-directed mutagenesis of the nuclease gene has provided numerous point mutants of nuclease which are being studied for changes in immune reactivity.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05038-12 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cell-Mediated Immunity to Hapten Modified Syngeneic Lymphocytes in Mice

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	D. Segal	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Mouse spleen cells were modified with trinitrobenezene sulfonate (TNP), and the TNP-self modified cells were tested in two different ways. First, several monoclonal antibodies (mAbs) specific for class I H-2 antigens were tested for binding to TNP-modified spleen cells. Second, the modified cells were used as stimulator and target cells for in vitro tests for cytotoxic T lymphocyte (CTL) responses to TNP-self. A number of anti-H-$2K^k$ antibodies not not mAbs of other specificities exhibited enhanced binding to cells that normally express K^k. Furthermore, these same mAbs bound to H-2^b cells modified with TNP. These results parallel the patterns of preferential CTL recognition in H-2^k mice and of crossreactive CTL in H-2^b mouse strains.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB05050-10 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Immunologically Relevant Cell Surface Phenomena

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

PI:	D. M. Segal	Senior Investigator	IB, NCI
Others:	B. Karpovsky	Medical Staff Fellow	IB, NCI
	P. Perez	Visiting Fellow	IB, NCI
	G. Shearer	Senior Investigator	IB, NCI
	J. Bluestone	Laboratory Leader	IB, NCI

COOPERATING UNITS *(if any)*

S. K. Dower, Immunex Corporation, Seattle, Washington

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

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

B

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

1. Cell:cell interactions have been examined using a novel flow cytometric technique. Two systems have been studied and compared: the FcγR-mediated aggregation of P388D_1 cells with antibody-coated mouse spleen cells and the formation of conjugates between cloned CTL and splenic target cells. Many of the processes involved in the specific recognition and lysis of a target cell have been defined.

2. A survey of the expression of MHC class I molecules on mice of different strains has been made by measuring the binding of radiolabeled anti-class I monoclonal antibodies to mouse spleen cells. The study suggests that the levels of expression of class I molecules are strictly controlled, and vary by only small amounts between individual animals of the same or different strains.

3. Effector cells have been generated using heteroaggregates of anti-FcγR and anti-target cell antibodies. Unlike ADCC effector cells, these cells are not inhibited by immune complexes. These studies demonstrate that FcγR must be brought into close proximity to the target cell in order for lysis to occur.

4. The distribution of FcγR on mouse T-cells has been studied by dual parameter flow cytometry. At least 2 subsets of $Fc\gamma R^+$ T cells have been identified, $Lyt2^+$ and $Lyt2^-$. The $Lyt2^+$, $Fc\gamma R^+$ subset increases in size in CMV-infected mice and may be responsible for suppression of allogeneic CTL responses in infected mice.

PHS 6040 (Rev. 1/84)
GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05058-09 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immunoregulation by Anti-idiotype Antibodies

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

PI:	H. B. Dickler	Senior Investigator	IB, NCI
Others:	H. Weissberger	Postdoctoral Fellow	IB, NCI

COOPERATING UNITS *(if any)*

Dr. Seth Pincus, University of Utah

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The goal of this project is to characterize the mechanisms by which anti-idiotype antibodies regulate immune responses and lymphocyte function. A system has been developed in which, for the first time, soluble antibody responses to the synthetic polypeptide (T,G)-A--L can be generated and detected in vitro using antigen-primed lymph node cells. Responses are antigen dependent and specific, and H-2 linked Ir gene regulated. Antibodies specific for the idiotypes of anti-(T,G)-A--L antibodies induce antigen-independent anti-(T,G)-A--L antibody responses. These responses are specific at the levels of the anti-idiotype reagent, the antigen-priming, and the antibody produced. The anti-idiotype antibodies stimulate function from antigen-primed T lymphocytes in the form of soluble helper lymphokines, and function from both primed and unprimed B cells in the form of specific antibody secretion. Unprimed B cells, in addition to anti-idiotype, require either primed T cells or idiotype or unrelated antibody complexes to be present in order to obtain function. Responses to anti-idiotype antibodies, in contrast to those to antigen, appear not to be regulated by Ir genes. A monoclonal anti-idiotype which reacts with a public idiotope present on the majority of anti-(T,G)-A--L antibodies has been obtained, and is being evaluated for functional effects.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05062-09 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Application of Rapid Flow Microfluorometry to Cell Biology

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

PI:	J. R. Wunderlich	Senior Investigator	IB, NCI
	S. O. Sharrow	Chemist	IB, NCI
Others:	D. M. Segal	Senior Investigator	IB, NCI
	J. Bluestone	Lab Leader	IB, NCI
	J. Titus	Chemist	IB, NCI
	D. H. Sachs	Chief	IB, NCI
	A. Singer	Senior Investigator	IB, NCI
	P. Morrissey	Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

J. Jones, T. Jefferson Univ., Phil., Pa; F. Finkelman, USUHS; M. Lotze, Surgery Br., NCI; S. Rosenberg, Chief, Surgery Br., NCI; J. Berzofsky, Metabol. Br., NCI; B. Mathieson, FCRF.

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	0.2	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Using rapid flow microfluorometry (FMF) for analysis and sorting of cells, aspects of the following projects have been supported during the previous year: (1) analysis of early events which occur in the process of cells specifically binding to one another, (2) characterization of subclasses of mouse splenocytes on the basis of immunoglobulin Fc receptor expression, (3) characterization of subclasses of human peripheral blood lymphocytes on the basis of cell surface differentiation antigens, (4) changes in human peripheral blood lymphocytes associated with interleukin-2 therapy, and (5) changes which occur in maturing mouse T cell which are relevant to development of tolerence.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB05064-08 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Genetic Control of the Immune Response In Vitro

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

PI:	A. Singer	Senior Investigator	IB, NCI
Others:	R. J. Hodes	Chief, Immunotherapy Section	IB, NCI

COOPERATING UNITS *(if any)*

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Immunology Branch

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The possibility that B cell-macrophage interactions are genetically restricted was assessed in vitro for responses stimulated by TNP-Ficoll. Under conditions in which TNP-Ficoll responses did not require T cells, it was observed that B cells from F1 --> parent and fully allogeneic (A --> B) radiation bone marrow chimeras were only triggered by macrophages expressing host H-2 determinants, and were not triggered by macrophages expressing donor H-2 determinants. This genetic restriction was not overcome by the addition of T cells. Indeed, it was observed that the activation of TNP-Ficoll responsive B cells by macrophages was genetically restricted requiring B cell recognition of macrophage H-2 determinants.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05067-09 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Human In Vitro Cellular Immune Responses

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

PI:	S. Shaw	Senior Investigator	IB, NCI
Others:	W. E. Biddison	Senior Investigator	NI, NINCDS
	R. Hoffman	Medical Staff Fellow	IB, NCI
	M. Sanchez-Perez	Visiting Fellow	IB, NCI

COOPERATING UNITS *(if any)*
T. A. Springer, Department of Membrane Immunochemistry, Dana-Farber Cancer Institute, Boston, Massachusetts

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Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.4	0.7

CHECK APPROPRIATE BOX(ES)

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

B

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

Studies are continuing on the process of recognition of foreign antigen by human T cells, particularly with respect to the role of T cell surface molecules in cytotoxic T cell (CTL) interaction. Recognition of the SB antigens has been used as a model system. In order to analyze functional heterogeneity among CTL clones a panel of SB2-specific CTL clones has been derived. Analysis by monoclonal antibody blocking demonstrates that susceptibility to inhibition by antibodies against some T cell surface markers (anti-T3 and anti-T4) varies markedly from one clone to another; in contrast, inhibition varies little or none with other antibodies (anti-T11 and anti-LFA-1). Clonal 'avidity', inferred from analysis of the capacity of competitor cells to disocciate preformed effector-target cell conjugates, correlates with clonal susceptibility to inhibition by both anti-T3 and anti-T4. This correlation was confirmed for anti-T4 but not for anti-T3 when inhibition was compared for the same CTL clone on: a) a SB2-positive target and b) a cell line which expressed a 'crossreactive' specificity with which the effector interacted with lower avidity. Partitioning of the assay into conjugate formation vs delivery of the lethal hit demonstrated that anti-T4 inhibits the former and that anti-T3 inhibits the latter. Thus, the T4 molecule may be functionally involved principally in conjugate formation by clones of relatively low avidity while there must be a different biological basis for the heterogeneity of anti-T3 inhibition.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB05069-08 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Expression of Ia Antigens on Functional Cell Subpopulations

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

PI:	R. J. Hodes	Chief, Immunotherapy Section	IB, NCI
Others:	B. Needleman	Medical Staff Fellow	IB, NCI
	D. H. Sachs	Chief, Transp. Biol. Sec.	IB, NCI
	D. H. Lynch	Investigator	IB, NCI

COOPERATING UNITS *(if any)*

Centre d'immunologie, INSERM-CNRS de Marseille - Luminy
Marseille, France

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Immunology Branch

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Immunotherapy Section

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

It has been demonstrated that the T cell proliferative response to Con A and the T cell dependent antibody responses to the soluble antigens TNP-KLH, TNP-T,G-(A-L), and TNP-Nuclease require the participation of adherent, radio-resistant, non-T, non-B and accessory cells which express Ia (I region associated) determinants. In addition, I-A and I-E positive cells within the splenic adherent cell population are the predominant stimulators of the one way murine mixed lymphocyte response when responder and stimulator cells differ either at H-2 or the Mls locus. Studies designed to analyze the functional importance of specific determinants on Ia molecules were carried out employing a battery of monoclonal anti-I-E reagents specific for different epitopes on the same I-E product molecule. Inhibition studies demonstrated that different clones of antigen-specific and I-E restricted T cells recognize antigen in association with different epitopes on a given I-E molecule.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05083-06 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evolutionary Variations in Murine MHC Gene Organization

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

PI:	D. S. Singer	Senior Investigator	IB, NCI
Others:	M. J. Rogers	Senior Staff Fellow	LG, NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The organization of MHC genes from a collection of wild mice has been examined. These mice, collected from all over the world, have been separated in evolution for periods of up to 15 million years, and represent 4 sub-genera, 3 species, and 2 sub-species. It has been demonstrated that changes in the number of class I MHC genes can be observed to occur over short periods of evolutionary time, namely between closely related species. Further, these changes do not occur uniformly throughout the class I gene family, but can be restricted to sub-sets of these genes. In contrast, the class II genes (A_α, E_α, A_β, and E_β) have been conserved with respect to number and also relatively well conserved with respect to restriction fragment length polymorphisms. Of particular interest was the finding that the genomic fragment corresponding to the $E_\beta 2$ gene was highly conserved in all of the animals tested. This fragment could also be identified by a human SB_β probe. Based on these observations we predict that the $E_\beta 2$ gene is is a genetically functional gene.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05085-06 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Development of Syngeneic Tumor Immunity

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	L. Joseph	Medical Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

J. Hochman, Department of Biology, Hebrew University, Jerusalem, Israel

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Mice of the BALB/c strain injected with a line of the syngeneic T cell lymphoma S-49 which grows in suspension accept the tumor and die within two weeks. BALB/c mice injected with a plastic adherent (7.3) line of the same tumor are not killed. Furthermore, mice injected first with the 7.3 line and subsequently challenged with TAS are protected from the syngeneic tumor. Spleen cells from mice protected with 7.3 and challenged with TAS can be adoptively transferred to naive BALB/c mice which then protects these recipients. The 7.3 cell line appears to produce a factor in culture that stimulates growth of B lymphocytes. This may be a factor involved in immune protection against the metastatic TAS line.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05086-06 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immune Response Gene Regulation of the Immune Response In Vitro

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

PI:	R. J. Hodes	Chief, Immunotherapy Section	IB, NCI
Others:	D. H. Sachs	Chief, Transp. Biol. Sec.	IB, NCI
	A. Finnegan	Investigator	IB, NCI

COOPERATING UNITS *(if any)*

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Immunology Branch

SECTION

Immunotherapy Section

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The cellular expression of immune response (Ir) gene function was studied in both primary and secondary in vitro antibody responses to the TNP conjugates of (T,G)-A--L and (H,G)-A--L. It was demonstrated that the function of accessory cells in responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L is under the control of genes which also map to I-A. In contrast, the expression of Ir gene function by B cells is related to the B cell activation pathway; Ir gene function is expressed by B cells activated under conditions involving MHC-restricted T-B interaction. In vitro augmented primary and secondary responses to TNP-nuclease (TNP-NASE) have also been established and documented to be under the control of H-2 linked Ir gene(s) mapping to the I-B subregion. For these responses, accessory cell function was shown to be under Ir gene control. Recent data employing monoclonal anti-Ia reagents have suggested that genes in the I-A subregion may also be involved in regulating responses to TNP-NASE. In order to further analyze the genetic regulation of T cell responses to NASE, a series of cloned lines were generated in BALB/c ($H\text{-}2^d$) as well as ($H\text{-}2^b$ x $H\text{-}2^a$)F_1 T cells. Individual BALB/c clones were restricted to recognizing NASE in the context of either $I\text{-}A^d$ or I-E products. Individual F_1 clones were specific for NASE in association with either $H\text{-}2^a$ or $H\text{-}2^b$ antigen-presenting cells and subregion mapping studies are currently in progress.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05088-06 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Effects of Graft Vs. Host Reactions on Cell-Mediated Immunity

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	L. Joseph	Medical Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

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Immunology Branch

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The intravenous injection of F_1 hybrid mice with parental spleen cells resulted in a loss in the ability of the F_1 mice to generate T-cell mediated cytotoxic responses in vitro to TNP-self and alloantigens. The loss of response potential depended on the H-2 type of the parental cells, since $H\text{-}2^{k,a}$ spleen cells induced unresponsiveness, whereas $H\text{-}2^{b}$ spleen cells did not. The phenomenon is dependent on recognition of F_1 I-A alloantigens by grafted parental cells (GVH). Suppressor T cells were found to be responsible for loss of immune potential. The immune responses of F_1 mice immunized with other antigens at the same time or prior to inoculation with parental cells were not susceptible to suppression. Spleen cells from F_1 mice suppressed by parental lymphocyte inoculation were defective in their ability to make Il-2 in culture, and spleen cells from GVH mice appeared to have lost the Il-2 receptors. The immune systems of F_1 mice suppressed by GVH were not reconstituted by whole body irradiation and repopulation with F_1 spleen or bone marrow.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05090-06 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Role of Accessory Cells in B Cell Activation

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

PI:	H. Golding	Visiting Fellow	IB, NCI
Others:	A. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

It was demonstrated previously that macrophages specifically interact with a distinct B cell subpopulation which is characterized as Lyb5+. Current experiments have demonstrated that Lyb5- B cells can be stimulated by the mitogen LPS. To gain further insights into the activation requirement of B cells which comprise the Lyb5- B cell subpopulation, the ability of lipoprotein free (phenol extracted) and lipoprotein rich (butanol extracted) LPS to stimulate Lyb5- B cells was examined. We used the bromodeoxyuridine (BUdR) + light technique to specifically eliminate B cells which respond to one type of LPS and determine whether the remaining B cells can respond to the other LPS extract. This approach allowed us to identify in normal mice a subset of B cells which respond to butanol-extracted LPS but not to pheno-extracted LPS. A similar subset was found in neonatal (less than 2 weeks old) mice which have not yet developed $Lyb5^+$ B cells. Thus, the pool of $Lyb5^-$ B cells in normal mice can be divided into two subsets: one which responds both to pheno-extract and to butanol-extract LPS, and a separate cell pool which respond to the butanol-extract but not to the phenol-extracted LPS. In contrast, none of the $Lyb5^-$ cells which are found in Xid CBA/n mice to phenol-extract LPS, indicating that the Xid defect affects the differentiation of $Lyb5^-$ cells as well as the development of $Lyb5^+$ B cells.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB05093-05 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Environmental Influences on Self-Tolerance

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

PI:	P. J. Morrissey	Senior Staff Fellow	IB, NCI
Others:	A. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

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Immunology Branch

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The induction of immunological tolerance in T cells can possibly occur prior to their entry into the thymus, during thymic differentiation or after the cells have emigrated from the thymus. Experimental systems have been constructed to investigate the susceptibility of T cells or their precursors to tolerance induction during the various phases of their differentiation. The basic model consists of murine thymus engrafted radiation bone marrow chimeras in which the cell surface alloantigens can, in theory be specifically localized in the extra-thymic or intra-thymic differentiation environments. The results demonstrate that tolerance to MHC encoded antigens can occur pre-thymically and intra-thymically and that tolerance to Mls encoded antigens can occur both intra-thymically and post-thymically but not pre-thymically. In addition, it has been shown that in chimeric mice in which the engrafted thymus is the only MHC allogeneic element, thymically induced tolerance to MHC encoded alloantigens is not sufficient to prevent autoreactivity since peripheral T cells were reactive to the thymic MHC encoded antigens. In sum, these results demonstrate that tolerance to various cell surface antigens occurs at different stages of T cell development and that the thymus is not a unique site of tolerance induction for maturing T cells.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05094-05 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Role of the Thymus in Generation of the Self-MHC Specific T Cell Repertoire

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

PI:	J. Keene	Staff Fellow	IB, NCI
Others:	A. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

To determine the mechanism by which T cells are educated in the thymus, neonatal mice were chronically treated with monoclonal anti-IA^k antibodies in vivo. The results of these studies demonstrate that such mice are virtually devoid of Ia+ cells. The T cells from these mice were found to be deficient in their recognition of either syngeneic or allogeneic class II MHC determinants, but were not deficient in their recognition of either syngeneic or allogeneic class I MHC determinants. The defect in Ia recognition correlated precisely with the intra-thymic suppression of Ia antigen expression but did not correlate with the extra-thymic suppression of Ia antigen expression. It is concluded that Ia-specific and K/D-specific T cells are educated on different intra-thymic elements.

PHS 6040 (Rev. 1/84)

GPO 904-917

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05095-05 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Regulation of Cell-Mediated Immunity by Germ Cells

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	K. Tung	Guest Researcher	IB, NCI

COOPERATING UNITS *(if any)*

Laboratory of Immunology, NINCDS

LAB/BRANCH

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Autologous mouse testicular cells derived from the seminiferous tubules activate suppressor T cells which inhibit mixed cell reactions in vitro. Generation of cytotoxic T cells in vitro is reduced in the presence of syngeneic germ cells. Mice were repeatedly injected intrarectally with xenogeneic semen (swine), and the cytotoxic T lymphocyte potential of the mice was followed with time. Depressed immune responses were seen early in the course of injection (first 5 weeks), but responses returned to normal levels as the mice continued to be stimulated. No evidence for Kaposi's-like lesions were detected in the skin of these mice.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05099-04 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Immunogenetic Effects of Murine Cytomegalovirus on Induced and Natural Immunity

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	J. Titus	Chemist	IB, NCI
	D. Segal	Senior Investigator	IB, NCI
	S. Sharrow	Chemist	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Mice injected with sublethal doses murine cytomegalovirus (MCMV) exhibit rapid and dramatic changes in their ability to generate in vitro cytotoxic T lymphocyte responses to hapten-self and to alloantigens. Within three days after intraperitoneal injection of (MCMV), the CTL responses to hapten-self and alloantigens are abrogated or severely reduced. This is followed by rapid recovery to a normal level of CTL potential. Injection of F_1 hybrid mice with either MCMV or parental spleen cells resulted in rapid and severe immunosuppression. Inoculation of either the virus or parental cells were selected so that they would be below the threshold for severe immunosuppression. However, when these two inocula were combined, severe immunosuppression was observed. These studies permit the investigation of the immunosuppression of MCMV infection and the possibility consequences of CMV infection coupled with a graft-versus-host reaction (GVHR).

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05100-04 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

The Role of HLA Genes in Human Disease

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

PI:	S. Shaw	Senior Investigator	IB, NCI
Others:	R. Hoffman	Medical Staff Fellow	IB, NCI
	T. J. Lawley	Investigator	DB, NCI
	S. I. Katz	Chief, Dermatology Branch	DB, NCI

COOPERATING UNITS *(if any)*

D. Glass, Brigham and Women's Hospital, Boston, MA; J. Hansen, Director, Histocompatibility Laboratory, Seattle, WA

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

We have defined an HLA locus (SB) which maps centromeric to the other known genes of the HLA complex. We are analyzing the importance of the genetic region marked by this gene in human disease. Family studies are continuing in dermatitis herpetiformis and are confirming the concept that the SB1 allele is part of an entire HLA haplotype which occurs in increased frequency in affected individuals. Studies have been initiated on juvenile rheumatoid arthritis, specifically patients with the DR5 allele, in order to determine whether there is an extended HLA haplotype (including an SB allele) which confers disease susceptibility. Finally, a study has been started in bone marrow donor-recipient pairs in order to: a) identify additional SB/DR recombinant individuals; and b) determine if SB-mismatching between otherwise HLA-identical donor-recipient pairs predisposes to morbidity or mortality.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05101-04 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI:	S. Shaw	Senior Investigator	IB, NCI
Others:	W. E. Biddison	Senior Investigator	NI, NINCDS
	E. Long	Investigator	LIG, NIAID
	D. Monos	Investigator	DCBD, NCI

COOPERATING UNITS *(if any)*
A. Ziegler, Medizinische Klinik, Univ. of Tubingen, Germany; R. DeMars, Lab of Genetics and Dept. of Human Oncology, Univ. of Wisconsin, Madison, WI; J. Trowsdale, P. Austen & W. Bodmer, Imperial Cancer Research Foundation, London, England

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.4	0.7

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

B

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

Using two different cell-mediated responses (secondary lymphocyte proliferative responses and secondary cell-mediated cytotoxicity) we have continued to probe the complexities of the alloantigenic differences between normal human donors. Development of SB2-specific cytotoxic T cell (CTL) clones has facilitated detailed analysis of SB-region determinants. Seventy anti-Ia monoclonal antibodies have been studied systematically for their ability to inhibit SB-specific cell-mediated cytotoxicity. Both inhibition and enhancement have been seen, which suggests a complex relationship between the epitope recognized by antibody and that recognized by T cells. Binding and inhibition studies indicate that one of the monoclonal antibodies studied identifies a new SB-related gene product. Analysis of the specificity of a panel of 10 SB-specific CTL clones demonstrates homogeneity of the SB2 phenotype in populations of normal Caucasians but reveals striking diversity of SB2-specific T cell recognition on HLA-deletion mutant cell lines. Some differences between mutant cell lines is apparently controlled by a new HLA gene which is being identified. In addition, one CTL clone detects a variation between donors in the population which is consistent with structural heterogeneity of SB2-related alleles. Studies of human minor histocompatibility antigens are continuing but have been hampered by technical difficulties.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05103-03 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of Cytotoxic T Lymphocyte Granules

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

PI:	P. A. Henkart	Senior Investigator	IB, NCI
Others:	T. Soares	Microbiologist	IB, NCI
	P. Frederikse	Microbiologist	IB, NCI
	J. Bluestone	Laboratory Leader	IB, NCI
	M. Henkart	Expert	IB, NCI
	C. Yue	Medical Staff Fellow	IB, NCI
	R. P. Blumentahl	Chief, Membrane Structure Sect.	LMMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Cytoplasmic granules from cytotoxic T lymphocytes and other lymphoid cells were purified by the Percoll gradient technique previously shown to yield pure cytoplasmic granules from cytotoxic LGL tumors. Such granules purified from cloned CTL lines were shown to be of comparable cytolytic activity (on a per cell basis) to the LGL tumor granules. Cytolytic activity is associated with lysosomal enzymes in the Percoll gradient, and is seen on a variety of nucleated cells as well as on red cells. The lytic process is rapid, occurring within 15 minutes at room temperature, and is absolutely dependent on calcium in the medium. Addition of calcium to purified cloned CTL granules give rise to membrane-associated ring structures with internal diameters of 5-10 nM as seen in the EM. These are smaller than those produced by the LGL cytolysin, and there are other subtle differences in the lytic activity. CTL granules caused carboxyfluorescein release from liposomes in a rapid and calcium-dependent process. Granules prepared from primary CTL cultures gave a calcium dependent cytolytic activity, but this was about 10-50 fold less than the cloned CTL on a per cell basis. Normal lymphocytes from spleen, thymus and the T cells from peripheral blood gave granules with no cytolytic activity, as did most lymphoid tumor cells. The mouse T cell tumor BW5147 gave a weakly cytolytic granule preparation. Two cloned CTL lines having no cytolytic activity gave granules of comparable cytolytic capacity to the CTL lines, but other cloned T lymphocytes gave non-cytolytic granules. CTL granule-mediated lytic activity was specifically neutralized by rabbit antibodies raised against the LGL tumor granules, but these antibodies did not inhibit CTL activity.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05104-03 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Detection and Analysis of H-2 Variant Cell Lines from Murine T Cell Lymphomas

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	L. Joseph	Medical Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Various lines of the S-49 T cell lymphoma of BALB/c origin are being studied for the expression of H-2 antigens. Normal BALB/c lymphocytes express H-2K^d, H-2D^d, and H-2D^d antigens. We have found that the five lines of the S-49 lymphoma thusfar studied do not express all of these cell surface H-2 antigens. The patterns of expression of H-2 antigens using these cells as targets for: (a) antibody and complement; and (b) cytotoxic T lymphocytes (CTL) exhibit four different patterns of H-2^d expression in the five lines tested. This system may be of value for investigating regulation of expression of major histocompatibility complex (MHC) antigens, and raises the possibility of a relatively high rate of modulation of these antigens among tumor cell lines of the same origin.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05105-03 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Specificity of Human Cytotoxic Effector Cells Generated by Stimulation with ConA

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	S. Payne	Biologist	IB, NCI
	S. Rosenberg	Chief, Surgery Branch	NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1		0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Peripheral blood leukocytes (PBL) from normal donors stimulated with Conconavalin A (Con A) generate cytotoxic effector cells (EC) which lyse allogeneic PBL from sarcoma patients but not PBL from normal donors. These EC also lyse allogeneic Epstein-Bar virus (EBV)-transformed cell lines, but not T cells from the same donors. They also lyse Daudi cells, which do not express Class I but do express Class II MHC antigens. These findings raise the possibility that Con A activated EC are detecting unique antigens expressed by virus-transformed cells and found in cancer patients but not normal leukocytes. These antigens could be modified Class II MHC antigens. Note: Due to recent emphasis on AIDS-related research, there has been no progress on this project during the past year. However, note Proposed Course of Project for AIDS-related work in future.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05106-03 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Analysis of the T Cell Alloreactive Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. J. Hodes	Chief, Immunotherapy Section	IB, NCI
Others:	R. Gress	Senior Investigator	IB, NCI
	D. H. Lynch	Investigator	IB, NCI
	B. Needleman	Medical Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI

LAB/BRANCH

Immunology Branch

SECTION

Immunotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The alloreactive T cell repertoire has been analyzed for responses to two categories of alloantigens: mutant K^b determinants and non MHC-encoded Mls antigens. It was demonstrated by limiting dilution techniques and slope analysis that proliferating F_1 T cell populations contain distinct subsets capable of recognizing Mls^c encoded determinants in the context of parental MHC products. These findings demonstrate that Mls^c determinants are recognized by responding T cells in association with MHC encoded determinants. T cell clones specific for Mls^a were recently generated, and the MHC restriction of recognition by these clones was evaluated. The specificity of these clones for Mls^a products was first established employing a variety of inbred strains including recombinant inbred lines. Studies of H-2 congenic strains demonstrated that cloned T cells recognize Mls^a in the context of H-2 determinants expressed by some but not all haplotypes. It was also observed that a high proportion of clones specific for soluble antigens (antigen-specific), foreign I region products (alloreactive), or syngeneic I products (autoreactive) showed cross-reactive recognition of Mls^a in an H-2 restricted fashion. Thus H-2 restricted recognition of Mls^a products occurs in high frequency in the T cell repertoire. Cloned T cells specific for Mls^a were capable of mediating skin graft graft rejection in nude (T-deficient) recipients, suggesting that Mls determinants may serve as targets for graft rejection in vivo.

Responses to K^b mutant determinants were also evaluated employing radiation bone marrow chimeras, neonatal tolerization, and cold target inhibition in assays of cell mediated lympholysis (CML). The results of such studies demonstrated that the generation of the T cell repertoire to these mutant MHC determinants was not the result of T cell genotype alone or of maturation environment alone, but rather represented the outcome of unique interactions between these two variables. This T cell repertoire appears to reflect, at least in part, the activation requirements of cytotoxic T cell precursors, and to be regulated by genes in the K region of the MHC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05107-03 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

T Cell Responses to Minor Histocompatibility Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Rosenberg	Medical Staff Fellow	IB, NCI
Others:	A. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ability to generate cytotoxic T lymphocyte responses to minor H antigens offers a potent tool for the study of self-tolerance and self-recognition. Results obtained in this system have thus far demonstrated 1) that the self + X T cell repetoire is highly cross-reactive for allogeneic MHC determinents suggesting that the response to allogenic MHC antigens is comprised of multiple self + X specificities and 2) that self minor H determinents tolerize T cells only in association with self MHC determinents so that tolenance induction to non MHC self components is restricted by MHC encoded products. We are currently examining the role of antigen processing in the generation of the cytotoxic response to minor-H antigens. Results so far indicate that 1) Macrophages are requisite for minor H specific CTL generation in vitro. 2) The minor antigens need not be synthesized by the antigen presenting cell but can be acquired in vitro by macrophages and subsequently presented in an immunogenic fashion. 3) The generation of the CTL by non antigen bearing APC's is inhibitable by antibodies to the T4 molecule expressed by Ia-restricted T helper cells.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05108-02 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

T Cell Regulation of B Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. J. Hodes	Chief, Immunotherapy Section	IB, NCI
Others:	Y. Asano	Visiting Associate	IB, NCI
	B. Needleman	Medical Staff Fellow	IB, NCI
	A. Finnegan	Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

Immunotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Suppression of B cell response was shown to be mediated by regulatory T cells functioning through two distinct pathways distinguishable by the involvement of different Lyt-defined T cell subpopulations. Both pathways were MHC-restricted and antigen-specific in their activation requirements. An Lyt 1^+2^- population functioned through an antigen non-specific effector pathway requiring the participation of an Lyt 1^-2^+ unprimed T cell. An Lyt 1^-2^+ T cell functioned through an antigen-specific and MHC restricted effector pathway without requirement for participation of additional T cells. Cloned lines of T cells were derived which function as suppressors of MHC-restricted T cell-dependent antibody responses. These cloned T cells express an Lyt1^+2^- L3T4$^+$ phenotype and proliferate in response to specific antigen plus the appropriate I-A or I-E encoded product. Cloned T suppressors inhibit responses of heterogeneous T helper cells and B cells in an MHC-restricted and antigen-specific manner. These cloned suppressor cells are also able to inhibit responses mediated by cloned T helper cells in the absence of other T cells, indicating that they can function as direct effectors of suppression.

A series of autoreactive T cell clones was also generated which proliferate in response to syngeneic I-A or I-E products without apparent involvement of foreign antigen. Certain of these autoreactive T cells functioned as T helper (T_H) cells to activate antibody response by B cells. Autoreactive T_H cells functioned through two distinct pathways: one pathway was polyclonal and MHC unrestricted at the level of T_H-B cell interaction and the other was MHC restricted and dependent upon antigen-specific triggering of responding B cells.

It was demonstrated that the optimal antibody responses generated by cloned antigen-specific T_H cells were substantially augmented by populations of unprimed Lyt 1^+2^- T cells. These T augmenting (T_A) cells were MHC-restricted in their ability to cooperate with cloned T_H cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB05109-02 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cyclophosphamide Effects on Murine T Cell Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	M. Miller	Biologist	IB, NCI
	J. Richardson	Microbiologist	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

F_1 mice undergoing GVH-associated immunosuppression as a result of parental T cell inoculation were treated with cyclophosphamide (Cy) at the time of parental cell inoculation. Such treatment prevented the development of immunosuppression. Furthermore, mice injected 37 days earlier with parental spleen cells were "rescued" in that their immune response potential was restored by injection of Cy. Inbred strains of mice injected with spleen cells from allogeneic strains and Cy exhibited antigen-specific reduced response potential to the H-2 antigens expressed by the strain used for injection.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05110-02 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immune Studies in Homosexual Men at Risk for Acquired Immune Deficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Others:	S. Payne	Biologist	IB, NCI
	W. Biddison	Senior Investigator	NINCDS
	S. Jacobson	Postdoctoral Fellow	NINCDS
	L. Joseph	Medical Staff Fellow	IB, NCI
	W. E. Biddison	Senior Investigator	NI, NINCDS
	K. Tung	Guest Researcher	IB, NCI
	R. C. Gallo	Chief	LTCB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	0.6	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

A

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Peripheral blood leukocytes (PBL) were drawn from a number of age matched heterosexual and homosexual men from the Washington, DC area, and New Mexico. The PBL were sensitized in vitro to influenza virus and to HLA alloantigens. These sensitized cultures were tested for the generation of cytotoxic T lymphocytes (CTL) specific for influenza virus and alloantigens. Assays were also run for OKT4:OKT8 ratios (i.e., helper:suppressor cell), and for interferon production in culture in the presence of influenza virus. In the Washington group, anti-influenza CTL responses were reduced in approximately 25% of the donors without any detectable loss in reactivity to HLA alloantigens. Abnormalities were also detected in interferon in this group, although all of these donors except one exhibited normal OKT4:OKT8 and thymosin α 1 levels. Heterosexuals generated CTL responses within the normal range to influenza virus. Approximately 40% of the New Mexico homosexual donors exhibited elevated CTL activity to HLA alloantigens without any reduction in CTL to influenza. The one donor from the Washington group that had a OKT4:OKT8 reversal and that exhibited no CTL to influenza developed AIDS after 10 months in our study. His sera had antibody activity to HTLV-III and cultures of his lymphocytes exhibited elevated levels of reverse transcript activity.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05111-02 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Generation of Allospecific CTL

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Golding	Visiting Fellow	IB, NCI
Others:	A. Singer	Senior Investigator	IB, NCI
	T. Muziochi	Visiting Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role performed by macrophages during activation of alloreactive cytotoxic T lymphocytes was investigated. Using an experimental approach in which both stimulator and responding populations were depleted of accessory cells, reconstitution of the response could be achieved using accessory cells of either stimulator or responder origin, but the mechanisms of activation differed fundamentally depending on the H-2 type of macrophages used. Using the lysosomal disruptive drug chloroquine it was found that activation via responder macrophages required processing of class I alloantigens shed by the stimulator cells. In addition this activation pathway was extremely sensitive to blocking by monoclonal anti-Ia antibodies. Reconstitution by stimulator macrophages was chloroquine insensitive and completely resistant to blocking by anti Ia mAb. We have thus identified two CTL activation pathways (Ia-dependent vs Ia-independent) and demonstrated that macrophages play central yet different roles in initiating these alternative pathways.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB05112-02 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Recognition Structures on T Cells and B Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Bluestone	Laboratory Leader	IB, NCI
Others:	D. H. Sachs	Chief, Transplantation Biology Section	IB, NCI
	O. Leo	Visiting Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The recognition structures of both B cells and T cells have been examined using anti-receptor antibodies prepared against monoclonal anti-H-2 antibodies (mAb) and cytotoxic T cell (CTL) clones. Anti-idiotypic antibodies (anti-Id) were prepared against several monoclonal anti-H-2 antibodies. In one case, anti-idiotypic antibodies prepared against an anti-H-$2K^b$ mAb detected a public idiotype expressed on a majority of anti-H-$2K^b$ alloantibodies. In contrast, no reactivity could be detected between these anti-idiotypes and a series of cytotoxic T cell clones of a similar specificity. The results suggested that either the recognition structures of T cells and B cells are substantially different, the allo-determinants recognized by these cells are not the same, or the anti-idiotypes used did not detect appropriate idiotopes. To expand the idiotypic repertoire recognized by the anti-idiotypic reagents, rabbits were immunized with conventional anti-H-$2K^b$ alloantibodies purified by affinity chromotography on an anti-28-13-3 Id column. These new anti-idiotypic reagents will be used to examine T cell recognition structures and to manipulate immune responses in vivo. In addition, anti-receptor antibodies specific for the CTL clones have been produced and attempts are being made to generate mAbs that recognize the T cell receptor.

GPO 904-917

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB05113-01 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Swine Genomic Repetitive DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. S. Singer	Senior Investigator	IB, NCI
Others:	L. Abelson	Biologist	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A moderately repeated interspersed DNA sequence family consisting of approximately 7×10^4 members has been identified in the swine genome. Three of these elements have been isolated from a swine genomic library and their DNA sequence determined. The repeat length of the element is 130 bp.; it terminates at the 3' end in a stretch of 20-30 A residues, and is flanked on either side by short direct repeats. The three family members analyzed are 70-80% homologous to one another. Structurally, these repeated DNA sequence elements resemble interspersed repetitive elements from other species, such as the Alu elements of human and monkey. However, there is no significant DNA sequence homology between this repeat and other known repetitive elements. Although there are two GT-rich regions within the swine repeated DNA segment which display sequence homology with viral enhances sequences, no enhancer activity can be detected.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05114-01 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sequence Organization of Class I Major Histocompatibility Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. S. Singer	Senior Investigator	IB, NCI
Others:	S. Rudikoff	Senior Investigator	LG, NCI
	M. L. Satz	Visiting Fellow	IB, NCI
	R. Ehrlich	Visiting Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this work is to determine the DNA sequence organization of class I genes contained in the swine major histocompatibility complex (SLA). It has been demonstrated that there are a total of 10-15 class I MHC genes in the swine genome. A series of genomic clones containing MHC-homologous DNA sequences has been isolated from both genomic lambda and cosmid libraries. Detailed DNA sequence analysis of two of the MHC genes is currently in progress. Our studies to date indicate that swine class I genes consist of 8 exons encoding distinct functional domains of the SLA antigen: leader polypeptide, three extracellular domains, a transmembrane domain, and intracytoplasmic domains. Comparison of the two swine class I genes inter se indicates a strong conservation of both over-all organization and DNA sequence. Comparison of these two sequences with known human MHC genes suggests different regions of the gene are subject to different evolutionary constraints: either allele specific or species specific. Further analysis of these gene sequences should shed light on the evolution of this multigene family and the generation of polymorphism.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB05115-01 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Expression of Class I MHC Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. S. Singer	Senior Investigator	IB, NCI
Others:	M. L. Satz	Visiting Fellow	IB, NCI
	R. Ehrlich	Visiting Fellow	IB, NCI
	L. Abelson	Biologist	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this work is to investigate the mechanisms controlling the expression of class I MHC genes. It has been demonstrated that there are 10-15 class I genes in the genome of the miniature swine. A series of genomic clones containing MHC-homologous DNA sequences has been isolated and introduced into mouse L cells. Two categories of MHC genes have been identified in this way: a set of closely related genes which are expressed in L cells and appear to represent the genes encoding the major locus products and a set of more distantly related genes which are not expressed in L cells. The regulation of expression of the swine MHC DNA segment, PD1, in mouse L cells has been used as a model system to study the regulation of expression of a single member of the multigene family. We have demonstrated that the pig DNA segment is subject to regulatory constraints indistinguishable from endogenous sequences with respect to chromatin structure, differential transcription of the segment and transcriptional enhancement by the exogenous inducer, interferon. We are now attempting to identify regulatory sequences within the gene by examining the expression capability of a series of deletion mutants. In addition, the expression vectors, pSV2cat and pSV0cat, are being used to identify any regulatory swine sequences. Differential patterns of MHC gene expression in different tissues have been examined. Preliminary data indicate that the various MHC genes display differing patterns of methylation and DNAse I sensitivity in chromatin in different tissues.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05116-01 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Graft-Versus-Host Disease Prophylaxis in Allogenic Bone Marrow Transplantation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. E. Gress	Senior Investigator	IB, NCI
Others:	R. R. Quinones	Medical Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts are being directed towards the prevention or control of graft-versus-host disease in human allogeneic bone marrow transplantation. Such graft-versus-host disease is mediated by alloreactive T cells in the inoculated marrow. With respect to the prevention of graft-versus-host disease then, reagents and techniques have been developed to remove these T cells from the marrow inoculum and to measure the success of that depletion. To this end, several murine monoclonal antibodies specific for antigens expressed on human T cells have been developed, three of which are cytotoxic. These antibodies have been utilized, in conjunction with other depletion techniques, for complement-mediated lysis of T cells in marrow. By a clonogenic assay now available, residual T cells in marrow following such a depletion are at a level of less than 0.1% of the total cell population. With respect to the control of alloreactive T cells mediating graft-versus-host disease, studies on the origin or generation of such alloreactivity have been undertaken in murine radiation bone marrow chimeras. It has been shown that the generation is influenced by a unique interaction of T cell genotype and the T cell maturation environment. The control by specific suppressor cells of alloreactive T cells resulting from the generation of this repertoire has been further studied in human alloreactive and putative suppressor T cell clones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05117-01 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Allodeterminants of Class I Major Histocompatibility Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: J. A. Bluestone Laboratory Leader IB, NCI

COOPERATING UNITS *(if any)*
S. G. Nathenson and S. Geier, Dept. Microbiology & Immunology, Albert Einstein Col. of Med., Bronx, NY; H. Allen and R. A. Flavell, Biogen Research Corp., Cambridge, MA

LAB/BRANCH
Immunology Branch

SECTION
Transplantation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Anti-receptor antibodies produced against monoclonal anti-H-2 antibodies do not appear to detect determinants on alloreactive T cells. One possible explanation is that T cells and B cells do not recognize the same allodeterminants on Class I molecules. Therefore, current efforts have been devoted to examining the nature of the allo-determinants recognized by cloned T cell populations as compared to those determinants recognized by alloantibodies. To examine this question, H-2 structural mutants have been isolated from a somatic cell line by mutagenesis and immunoselection using monoclonal anti-H-2 antibodies. Examination of alloantigen-specific CTL clones on these mutants suggest that the majority of CTL clones recognize determinants different from those which elicit antibody production. In addition, the regions of the MHC molecule involved in CTL recognition were studied using L cells transfected with H-2 genes constructed by shuffling exons between the H-2K^b and D^b genes. The findings suggest that unlike mAbs which can recognize individual epitopes on different domains, CTL recognition is influenced by the interaction of the two external domains.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05118-01 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Responses to Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. C. Ting	Medical Officer	IB, NCI
Others:	M. E. Hargrove	Microbiologist	IB, NCI
	S. S. Yang	Investigator	LCO, NCI
	T. R. Malek	Investigator	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Studies of the mechanisms for the induction of in vivo tumor immunity: T cells isolated from FBL-3 ascites growth were highly cytotoxic in vitro but lacked long lasting in vivo anti-tumor immunity. Further studies showed that the lack of in vivo protective effect of these T cells was attributed to the presence of suppressor macrophages which were able to suppress the in vivo protective effect of immune T cells against tumor challenge. After removal of the macrophages, then the in vivo protective effect of the T cells from progressor tumors became readily demonstrable.

2. Regulation of T cell-mediated immunity by prostaglandins and antigens: Two immunoregulatory suppressor circuits are involved in the generation of cytotoxic T lymphocyte (CTL) response. 1) In the absence of antigen, endogenous production of prostaglandins regulates the activation of cytotoxic lymphocyte precursors and prevents the "spontaneous activation" of the cytotoxic precursors. 2) During antigen sensitization, both antigen-specific and antigen-nonspecific suppressor T cells are generated. The antigen specific suppressor T cells help to determine the magnitude of CTL response and the antigen-nonspecific suppressor T cells help to determine the specificity of CTL response.

3. Regulation by lymphokines of the cell mediated immunity: Initial endogenous production of Interleukin 2 (IL2) is essential for the differentiation and pro liferation of cytotoxic T lymphocyte precursors into CTL. The production of higher levels of IL2 at a later time induces the generation of antigen-nonspecific suppressor T cells and augments the generation of antigen specific suppressor T cells. These suppressor T cells help to determine the specificity and magnitude of CTL response.

4. Generation of lymphokine-induced cytotoxic cells (LICC): LICC are generated by culturing normal spleen cells with Interleukin 2 (a T cell product) and a novel lymphokine, the cytotoxic cell differentiation factor (a macrophage product). The LICC selectively kill tumor cells in vitro and also possess strong in vivo anti-tumor activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05119-01 I

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Helper T Cells in Allogeneic Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Mizuochi	Visiting Fellow	IB, NCI
Others:	A. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Precursors of class I specific allo-CTL were found to be activated by at least two distinct populations of helper T cells: (1) $L3T4^{+}$ $Lyt2^{-}$ helper T cells which were class II restricted, and (2) $L3T4^{-}$ $Lyt2^{+}$ helper T cells which were class I restricted. The mechanism by which these two T_H populations functioned were distinct since monoclonal antibody against the IL-2 receptors expressed by pCTL preferentially blocked the activation of pCTL by class II restricted helper T cells rather than class I restricted helper T cell. Thus, these results demonstrate the existence of two different classes of T_H cells and two distinct helper mechanisms in the induction of class I specific allo-CTL responses.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB05120-01 I

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Regulation of Lymphocyte Proliferation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K. Kelly Senior Investigator IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lymphocyte metabolism and effector function expression are regulated by antigen/mitogen and lymphokine binding to cell surface receptors. We are investigating the physiological consequences of mitogen and lymphokine mediated signals by isolating and characterizing genes which are transcriptionally regulated by these events. We expect that genes induced within a few hours after antigen or mitogen activation of lymphocytes will be fundamentally important for the initiation of proliferation and effector function expression in these cells. We have shown that the c-myc onogene is transcriptionally induced as early as one hour after the activation of murine B cells with LPS or T cells with Con A. Thus, the c-myc oncogene is a member of the family of those genes that are regulated by mitogen binding to the surface of lymphocytes. The identification and characterization of additional members of this inducible gene family is currently in progress utilizing PHA stimulated human peripheral blood T cells and subtraction cDNA cloning methodology.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08525-08 LIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunotherapy of Primary Autochthonous Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Zbar	Chief, Cellular Immunity Section	LIB NCI
OTHER:	K. Nakanishi	Guest Worker	LIB NCI
	Y. Tanio	Visiting Fellow	LIB NCI
	T. Borsos	Chief, Humoral Immunity Section	LIB NCI

COOPERATING UNITS *(if any)*

John Langone, Department of Medicine, Baylor University School of Medicine, Houston, Texas

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Cellular Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rats with experimentally-induced primary autochthonous mammary cancer are being studied as guides to the immunotherapy of human cancer. Rats with primary mammary adenocarcinomas have been treated by intravenous injection of plasma from tumor bearing rats. Before injection, plasma was absorbed with protein A Sepharose, Sepharose, or CNBr-Sepharose.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08528-08 LIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Delayed Hypersensitivity and Tumor Graft Rejection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Zbar	Chief, Cellular Immunity Section	LIB NCI
OTHER:	N. Terata	Visiting Fellow	LIB NCI
	Y. Tanio	Visiting Fellow	LIB NCI
	K. Nakanishi	Guest Worker	LIB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Cellular Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.5	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is the purpose of this project to develop methods for altering the antigenicity of nonimmunogenic tumors and to analyze the cellular and molecular basis of tumor rejection. The current areas of interest are: a) the induction of transplantation antigens by in vitro treatment of tumor cells with mutagen; b) the basis of recurrence of retrovirus-infected fibrosarcoma cells; and c) the role of Ia antigens in the rejection of a B cell leukemia.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08550-10 LIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Modification of Tumor Cells and Immune Cytolysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S. H. Ohanian Research Microbiologist LIB NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pretreatment of guinea pig tumor cells with chemotherapeutic agents, metabolic inhibitors, enzymes or hormones modifies the susceptibility of the cells to killing by immune attack. Human and mouse tumor cells in asynchronous growth show variations in sensitivity to killing by antibody plus C. The purpose of this investigation is to determine the attributes of cells which influence the cells' ability to modify or resist cellular and humoral cytotoxic mechanisms.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08552-18 LIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Complement Fixation and Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Borsos	Chief, Humoral Immunity Section	LIB NCI
OTHER:	A. Circolo	Visiting Associate	LIB NCI
	P. Battista	Visiting Fellow	LIB NCI

COOPERATING UNITS *(if any)*

Department of Biochemistry, University of Lausanne

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

NCI, NIH, FCRF, Frederick Md, 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a long-range project investigating the mechanism of complement fixation and action. In particular the interaction of antibody-antigen complexes with the first component of complement and the result of this interaction on the other components are investigated. The relation between antibody action and complement activation is also explored. Finally, the significance of complement in the humoral immune defense mechanism is studied.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB08575-12 LIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Leonard	Chief, Immunopathology Section	LIB NCI
OTHER:	Enrica Alteri	Visiting Fellow	LIB NCI
	Antal Rot	Visiting Fellow	LIB NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Immunopathology Section

INSTITUTE AND LOCATION

NCI, NIH, FCRF, Frederick Md, 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this work is to study the cells that participate in the effector arm of the immune response. The current emphasis is on chemotaxis, which is a mechanism by which cells can be attracted to inflammatory sites, delayed hypersensitivity reactions and growing tumors. The project includes chemistry of lymphocyte derived chemotactic factors, identification of substances that modulate chomotactic and phagocytic responses and definition and separation of functional subpopulations of leukocyes.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB03200-15 LCBGY

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Factors Influencing the Induction, Growth and Repression of Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L. W. Law, Chief Lab. of Cell Biology NCI

COOPERATING UNITS *(if any)*

Sloan-Kettering Cancer Center, New York, NY
Yale University, New Haven, CT

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	2.00	4.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Major emphasis is placed upon the study of tumor antigens of the transplantation rejection type (TATA), and of tumor antigens (TA) assayed by in vitro techniques and of the immune responses they evoke. As a corollary to this study the biologic properties in vitro and in vivo of alien histocompatibility (H-2) antigens and of variant antigens in several neoplasms are under study. Solubilization and methods of purification of TATAs are under investigation with the ultimate purpose of defining these membrane and cytosol antigens after purification in physicochemical, biologic and molecular terms.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB03229-15 LCBGY

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Analysis of Histocompatibility and Tumor Antigens and T-cell Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ettore Appella Medical Officer (Res.) Lab. of Cell Biology NCI

COOPERATING UNITS *(if any)*
Fox Chase Cancer Center, Philadelphia, PA
Wistar Institute, Philadelphia, PA
Lab. of Dev. and Mol. Immunol., NICHD
Lab. of Immunology, NIAID

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Chemistry

INSTITUTE AND LOCATION
NIH, NCI, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.25	6.25	1.50

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the molecular structure of histocompatibility antigens, tumor antigens, and T-cell receptors. A combination of protein and DNA sequencing, in conjunction with peptide and nucleotide synthesis, is being used in order to reach a better understanding of the molecular architecture of these important biological molecules. Oligonucleotide directed site mutagenesis has been employed to elucidate the role of individual amino acids on the function and expression of histocompatibility class I and II antigens. Synthetic peptides corresponding to oncogene structures such as myc and erb are being generated and antibodies are being made to study the role that these proteins play in transformation and in normal growth control.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09100-01 LCBGY

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunogenicity of Melanoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

V. J. Hearing, Jr. Research Biologist LCBGY NCI

COOPERATING UNITS *(if any)*

Douglas M. Gersten Dept. of Pathology Georgetown Univ. Wash., D.C.

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.72	0.61	.11

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed at elucidating the host immune response(s) to malignant melanoma, and the role these response(s) play in the progression of tumor growth and metastatic spread. Our preliminary results indicate that various murine melanomas (of spontaneous, ultraviolet light induced, and chemically induced origin) share common cell surface antigens which are capable of eliciting a tumor rejection response. These antigens appear to have specificity restricted to melanoma cells based on immunization protocols with other neoplastic cell lines. Initial purification attempts have shown that aqueous butanol solubilizes this antigen(s) quantitatively from the melanoma cell surface.

Other related projects are covered in the Annual Report of the Dermatology Branch.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05190-04 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Monoclonal Antibodies Reactive with Human Mammary and Colon Carcinoma Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jeffrey Schlom	Chief	LTIB, DCBD, NCI
Patricia Hand	Chemist	LTIB, DCBD, NCI
Peter Fuhrer	Expert	LTIB, DCBD, NCI
Maureen Weeks	Chemist	LTIB, DCBD, NCI
Ann Thor	Medical Staff Fellow	LTIB, DCBD, NCI
Raffaella Muraro	Visiting Fellow	LTIB, DCBD, NCI
David Colcher	Research Microbiologist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
W. Johnston, Dept. of Pathology, Duke University, Durham, NC
D. Kufe, Dana Farber Cancer Institute, Boston, MA
L. Liotta and C. Rao, Lab. of Pathology, NCI; Phil Noguchi, FDA

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.0	2.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies (MAbs) have been generated and characterized using membrane enriched fractions of human carcinoma metastases. These antibodies have been characterized as to reactive antigen, and the presentation of antigen on human mammary and colon carcinoma cell populations and normal human tissues. MAb B72.3 has been shown to be reactive with a 220,000 to 400,000d glycoprotein complex expressed in approximately 50% of human mammary tumors, greater than 85% of human colon carcinomas, but is not expressed to any significant extent in any adult human tissues thus far tested. MAb B72.3 is currently being evaluated for its use in the detection of occult adenocarcinoma cells in pleural effusions, and for the in situ radioimmunodetection of metastases in carcinoma patients. Antigenic phenotyping with MAb B72.3 revealed that little correlation exists between the expression of the B72.3 reactive antigen in primary tumor cells of a colon carcinoma mass and its expression in tumor cells of regional node or distal metastases. A correlation was shown to exist, however, between the expression of this antigen in tumor cells of regional nodes and those in distal metas-metastases. These findings suggest that the effective use of monoclonal antibodies for diagnostic imaging of distal metastases may require the evaluation of the antigenic expression on tumor cells in regional lymph nodes rather than the primary lesion. Monoclonal antibodies have recently been generated to human laminin receptor and the p21 human ras gene product. These are currently being evaluated to determine if any correlations exist between the expression of the laminin receptor and/or ras p21 in specific cell populations and the processes of carcinoma initiation, promotion, or progression.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB09008-03 LTIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Localization of Human Tumors Using Radiolabeled Monoclonal Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David Colcher	Research Microbiologist	LTIB, DCBD, NCI
Joel Lundy	IPA	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)* J. Carrasquillo, A. Keenan, S. Larson, J. Reynolds, Nuclear Medicine Dept., CC, NIH; F. Monex, O. Gansow, Radiation Oncology, NCI, NIH
W. Kaplan and D. Kufe, Dana Farber Cancer Inst., Boston, MA
S. DeNardo, Dept. of Nuclear Medicine, Univ. of Calif., Davis, CA

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Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.5	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

D

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

IgG has been purified from monoclonal antibodies B6.2 and B72.3. F(ab')2 fragments, and Fab' fragments of monoclonal antibody B6.2 have also been generated. The IgG and its fragments were radiolabeled with I-125 and I-131 without loss of immunoreactivity and were injected into athymic mice bearing human mammary tumor transplants or human colon carcinomas. The radiolabeled B6.2 antibody localized in the tumor within 24 hours with tumor to tissue ratios rising over a 96 hour period. The F(ab')2 was better than the IgG and gave tumor to liver and spleen ratios of 15 to 20:1, and tumor to muscle and brain ratios of 50 to 110:1. No localization was observed in mice bearing human melanomas, or with radiolabeled normal murine IgG in mice bearing human mammary tumors or colon carcinomas. The ability of the radiolabeled antibody to localize in mammary and colon tumors was sufficient to give high quality gamma scans of tumor bearing mice. Monoclonal antibody B72.3 was shown to localize human colon cancer xenografts in athymic mice and showed an increase in uptake in the tumor over the first two days post inoculation of the antibody and stayed constant over the 19 day period of study. Monoclonal antibodies B72.3 and B6.2 are being labeled with several isotopes to test for appropriateness for clinical studies for carcinoma localization.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05233-03 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification and Purification of Human Carcinoma Associated Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David Colcher	Research Microbiologist	LTIB, DCBD, NCI
Andrew Paterson	Visiting Fellow	LTIB, DCBD, NCI
Virginia Johnson	Visiting Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI
Peter Fuhrer	Expert	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

A

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies to human mammary tumor metastases were tested for reactivity to novel and known tumor associated antigens. The monoclonals were used to immunoprecipitate antigens from a radiolabeled breast tumor metastasis extract. Monoclonal antibody B72.3 immunoprecipitated a high molecular weight complex of approximately 220,000d-400,000d . This complex is composed of high molecular weight glycoproteins containing high levels of sialic acid. B6.2 and four other antibodies immunoprecipitated a 90,000d polypeptide. The four other antibodies cross-react in radioimmunoassay for monoclonal B6.2 but differ in their ability to compete with the binding of B6.2. Two antibodies, B1.1 and F5.5, were shown to differentially react with carcinoembryonic antigen. The high molecular weight complex identified by monoclonal B72.3 has been purified from both the breast tumor metastasis extract and the LS174T colon tumor cell line, using molecular sieving and antibody affinity chromatography, with minimal loss of immunoreactivity. Radioimmunoassays have been established for the quantitation of the antigen detected by monoclonal antibody B72.3. These assays are being used to study human serum and other biological fluids.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09009-03 LTIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigenic Heterogeneity and Modulation of Human Mammary and Colon Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Patricia Horan Hand	Chemist	LTIB, DCBD, NCI
David Salomon	Expert	LTIB, DCBD, NCI
David Colcher	Research Microbiologist	LTIB, DCBD, NCI
John Greiner	Senior Staff Fellow	LTIB, DCBD, NCI
Arnaldo Caruso	Visiting Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

P. Noguchi, National Center for Drugs and Biologics, FDA
D. Kufe, Dana Farber Cancer Institute, Boston, MA

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SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antigenic variation was observed in the expression of specific tumor associated antigens within individual human mammary tumor masses using monoclonal antibodies. This variation was demonstrated by both the pattern and cellular localization of reactivity with a given antibody. This diversity was also observed in human mammary tumor cell lines grown in vivo and in vitro. Analyses of DNA content and cell surface binding of monoclonal antibodies during logarithmic growth phase, and at density-dependent arrest, demonstrated that the expression of some tumor associated antigens is related to S-phase of the cell cycle. Membrane expression of the reactive antigens appeared to be stable despite prolonged exposure to antibody. Antigenic drift was observed with continued passage of mammary tumor cell lines; consistent with this finding, the "same" mammary tumor cell line obtained from different sources exhibited distinct antigenic phenotypes. Single-cell clones derived from the MCF-7 mammary tumor cell line exhibited at least four distinct antigenic phenotypes; a change in cell surface phenotype of some of the clones was seen during subsequent passage. Antigenic modulation as well as heterogeneity was also observed for the expression of the antigen reactive with monoclonal B72.3. Monoclonal antibody B72.3 reacts with the majority of breast and colon carcinomas obtained from biopsy but with only 1/28 breast carcinoma cell lines and 2/19 colon carcinoma cell lines. Growth of one of the positive colon carcinoma cell lines, LS-174T, as tumors in athymic mice, caused a 100-fold increase in the antigen reactive with B72.3 in cell extracts and a 10-fold increase on the cell surface. Consistent with this finding, increased expression of the antigen reactive with B72.3 was observed when LS-174T cells were grown under culture conditions that promote three-dimensional growth of the cells.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB9000-02 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Recombinant Interferon-Induced Enhancement of Carcinoma Antigen Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John W. Greiner	Senior Staff Fellow	LTIB, DCBD, NCI
Patricia Hand	Chemist	LTIB, DCBD, NCI
Martin Tobi	Medical Staff Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
P. Noguchi, FDA; P. Fisher, Columbia University, New York, NY; S. Pestka, Roche Institute of Molecular Biology, Nutley, NJ

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies have found that certain types of recombinant human interferon can increase the expression of tumor antigens on the surface of human carcinoma cells. Treatment of human breast or colon carcinoma cells with leukocyte clone A increases the binding of several monoclonal antibodies to 3 surface tumor antigens. Other human tumor cells (e.g. melanoma) and normal fibroblasts that do not express these antigens remain negative after treatment with up to 10,000 units of interferon. Other clones of human leukocyte interferon exert a wide range of activities for both antiproliferation and enhancement of surface antigen expression. Clones of the human breast carcinoma cell line, MCF-7, were examined for their responsiveness to human leukocyte clone A interferon. Three of 10 MCF-7 clones were unresponsive to the interferon-mediated increase of surface antigen expression. Upon examination of the number and affinity of the surface interferon receptors, no difference was detected between the responsive and nonresponsive cloned cell lines. These findings indicate that two biological events of human leukocyte interferon, antiproliferation and enhancement of surface antigens, can be functionally separated. Furthermore, the data suggest that additional transcriptional and/or post-translational events are required for the increase in tumor antigen expression induced by interferon. Such findings implicate the possible use of recombinant leukocyte interferon as an adjunct to overcome tumor cell heterogeneity. These studies may also lead to an increase in the efficiency of monoclonal antibodies for localization and treatment of human carcinomas.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB09012-01 LTIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monoclonal Antibodies to Define Carcinoma Cells in Effusions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ann Thor	Medical Staff Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Drs. W. Johnston, C. Szpak, C. Lottich - Duke University, Dept. of Pathology, Durham, NC

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

A

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several monoclonal antibodies have been evaluated for their reactivity to benign and malignant serous effusions using immunohistochemical techniques. Monoclonal B72.3 which is reactive against a 220,000-400,000 d glycoprotein complex was uniformly reactive with malignant effusions containing adenocarcinoma of the breast, ovary and lung. This monoclonal antibody was routinely negative with squamous cell carcinoma, lymphoma, leukemia and benign effusions. Other monoclonal antibodies exhibited staining which did not differentiate mesothelium from carcinoma. The preliminary results of this study present evidence that monoclonal antibody B72.3 may function as a highly selective marker in recognizing a cancer cell versus a mesothelial cell, and an adenocarcinoma cell versus other malignant tumor cells.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09014-01 LTIB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monoclonal Antibodies Reactive with Human Colon Carcinomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Raffaella Muraro	Visiting Fellow	LTIB, DCBD, NCI
David Wunderlich	Microbiologist	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

P. Noguchi, FDA, Bethesda, MD

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	1.1	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies have been generated that are reactive with human colon carcinomas. The rationale for the studies was to utilize extracts from patient biopsy material (and not colon cancer cell lines) as immunogen to increase the probability that any monoclonal antibody generated be reactive with colon carcinomas in a clinical setting. Five immunization protocols were used employing extracts and membrane enriched fractions from both primary and metastatic colon carcinoma lesions. Seventeen monoclonal antibodies from double-cloned hybridoma cultures have been characterized; all are of the IgG isotope. Preliminary results indicate that the monoclonal antibodies can be placed into at least 6 groups on the basis of their differential reactivies to six colon carcinoma extracts, the surface of three colon carcinoma cell lines and five partially purified CEA preparations from bloods of colon cancer patients. Some of the monoclonal antibodies were shown to bind from one to all of the five CEA preparations tested, while others showed no anti-CEA reactivity. None of the monoclonal antibodies selected for further study reacted with extracts of 21 normal tissues including livers, spleens, kidneys, red blood cells, (of several blood groups), or polymorphonuclear leukocytes. All the monoclonal antibodies could be distinguished from antibodies previously generated in our laboratory. Further immunohistochemical and radiolocalization studies will further define the potential clinical utility of the monoclonals described.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB9013-01 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Monoclonal Antibody to a Mammary Differentiation Antigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ann Thor	Medical Staff Fellow	LTIB, DCBD, NCI
Joel Lundy	IPA	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
D. Kufe - Dana Farber Cancer Institute, Boston, MA
W. Johnston, C. Szpak - Duke University, Durham, NC
W. Hartman - Vanderbilt University, Nashville, TN

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have defined a human mammary differentiation antigen using murine monoclonal antibody (MAb DF3) prepared against a membrane enriched fraction of a human breast carcinoma. This antigen has a molecular weight of 290,000, and is detectable on the cell surface of human breast carcinoma cells using a live cell radioimmunoassay and fluorescence flow cytometry. More importantly, immunoperoxidase staining with MAb DF3 distinguishes malignant and benign breast lesions via cellular distribution of reactive antigen. A cytoplasmic staining pattern has been observed with a majority of breast carcinomas, and only one of 13 fibroadenoma or fibrocystic disease specimens. In contrast, reactivity of benign breast lesions with MAb DF3 primarily occurs along apical borders. These results demonstrate that the DF3 antigen is present on apical borders of more differentiated secretory mammary epithelial cells and in the cytosol of less differentiated cells. This novel monoclonal antibody may be employed to distinguish less differentiated from better differentiated mammary carcinomas.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09003-02 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transforming Growth Factors in Human Mammary Tumors and Human Milk

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David S. Salomon	Expert	LTIB, DCBD, NCI
Robert Bassin	Chief, Biochem. of Oncogenes Sec.	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
W. Kidwell and M. Bano, LPP, DCBD, NCI

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transforming growth factors (TGF's) are heat and acid-stable peptides which have been isolated from a variety of rodent and human carcinomas and from the conditioned medium (CM) of rodent and human tumor cell lines and from retrovirus transformed cells. TGF's are able to reversibly confer upon normal cells certain properties ascribed to the transformed phenotype, namely a reduced serum requirement in monolayer culture and a loss of anchorage-dependent growth. TGF's have been isolated from the CM of a human mammary carcinoma cell line (MCF-7) and from the CM of several clones derived from this cell line. Comparable TGF's have also been isolated from a transplantable human mammary adenocarcinoma (Clouser), biopsies of human breast tumor and human milk. These TGF's are: 1. able to compete with epidermal growth factor (EGF) for receptor binding; 2. able to induce the anchorage-independent growth of rat fibroblasts and MCF-7 cells in soft agar and 3. potent mitogens for rat fibroblasts and normal mammary epithelial cells. The TGF's present in the MCF-7 CM, the human tumor biopsies and human milk exhibit a pI of 4.0. The TGF activity which is present in human milk has been partially purified. This activity has a molecular weight of approximately 6000 and is present in milk at a concentration of approximately 25 micrograms/liter. The biological activity associated with this species is inactivated by reduction but is stable to heat and acid treatment. The TGF activity is apparently not human EGF (pI 4.6) since polyclonal antibodies raised against human EGF fail to detect any EGF in the TGF preparations. Moreover, human EGF and the milk-derived TGF elute at different positions with acetonitrile following reverse phase high performance liquid chromatography.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09002-02 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Tumor Promoters and Growth Factors on Protein Kinase C Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David S. Salomon	Expert	LTIB, DCBD, NCI
Atul Sahai	Visiting Fellow	LTIB, DCBD, NCI
Nili Feuerstein	Visiting Fellow	LTIB, DCBD, NCI
Herbert Cooper	Chief, Cell.& Mol. Phys. Sec.	LTIB, DCBD,NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X☐ (c) Neither
B
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), and epidermal growth factor (EGF) inhibit the growth of human A431 epidermoid carcinoma cells within 24 to 48 hours after exposure of the cells to these agents. Addition of TPA and EGF inhibit cell growth in an additive or synergistic manner. These effects on cell growth are preceded by a change in the activity of a calcium-dependent, cyclic nucleotide-independent and phospholipid-dependent protein kinase (protein kinase C). Specifically, EGF produced a 2- to 3-fold stimulation in protein kinase C activity within 30 to 60 minutes following exposure to the cells. TPA alone had no effect on protein kinase C activity. However, TPA attenuated the increase in protein kinase C activity that was induced by EGF. In EGF treated cells (250 ng/ml, one hour) there was a three to four-fold increase in the phosphorylation of a cytosolic protein at 17-20 Kd (pp17-20, pI approximately 5.5) and a moderate increase in the phosphorylation of other proteins at molecular weights of 27,40,45 and 70-80 Kd as detected by two-dimensional gel electrophoresis. Treatment of the cells with TPA (10^{-7}M, one hour) resulted in a similar effect on the phosphorylation of pp17-20 as well as on pp27 and pp70-80. However, TPA in contrast to EGF did not affect the phosphorylation of pp40 and pp45. In combination with EGF, TPA attenuated the EGF-induced phosphorylation of pp40, but did not affect the phosphorylation of the other proteins. In vitro studies demonstrated three bands following one-dimensional gel electrophoresis at 17, 26, and 47 Kd which are phosphorylated in the presence of phospholipids and might therefore be substrates for protein kinase C and modulated by EGF.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05148-05 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Study of Neoplasia of Outbred Colonies of Feral Species of the Genus Mus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert Callahan	Chief, Oncogenetics Sect.	LTIB, DCBD, NCI
Chantel Theillet	Visiting Fellow	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
Christine Kozak, LVD, NIAID; Michael Potter, LG, DCBD, NCI
Daniel Gallahan, Biologist, Lab. of Genetics, DCBD, NCI

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Oncogenetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In previous studies we identified a pedigreed breeding colony of feral Mus musculus musculus (designated Czech II) which does not contain mouse mammary tumor virus (MMTV) proviral genomes in their germline. We have now completed a study of the affect of the chemical carcinogen dimethyl-benzanthracene (DMBA) on the incidence of mammary gland neoplasia in Czech II mice. Three percent of the breeding females have developed mammary tumors whereas no tumors have been observed in virgin females up to two years age. Treatment of the mice with DMBA significantly increased the frequency of tumors (30-50 percent of the treated mice) and decreased the latency in tumor development (average 11 months). Most of the chemically induced and all of the spontaneous mammary tumors were type A adenocarcinomas. In an independent study we obtained evidence that some lactating females contain MMTV gp52 envelop protein in their milk. Analysis of tumor cellular DNA revealed the presence of MMTV proviral DNA in many but not all mammary tumors. The corresponding liver cellular DNA from MMTV positive tumor bearing mice lacked MMTV proviral DNA. This suggests to us that the Czech II colony is infected with an exogenous MMTV. Restriction enzyme analysis of the MMTV proviral genomes in the mammary tumor cellular DNA showed that the viral genome was not that of common laboratory strains of MMTV since it contains several restriction site polymorphisms. Analysis of the restriction pattern of MMTV proviral genomes in mammary tumor cellular DNA showed that four out of 18 virus positive tumors contained common virus-host junction fragments. In each case the MMTV common integration regions (designated Int-1 and Int-2) defined in mammary tumors of inbred mice were unoccupied. We tentatively conclude that the common virus-host junction fragments identified in this study represent a new common integration region for MMTV in mammary tumors.

HS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB04829-10 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Genetic Organization and Role of Endogenous Retroviruses in Neoplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert Callahan	Chief, Oncogenetics Section	LTIB, DCBD, NCI
Renato Mariani-Costantini	Visiting Associate	LTIB, DCBD, NCI
Jacqueline Fetherston	Staff Fellow	LTIB, DCBD, NCI
Toby Horn	Staff Fellow	LTIB, DCBD, NCI
Charles Theillet	Visiting Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI
Igbal Ali	Visiting Scientist	LTIB, DCBD, NCI
Robert Bassin	Chief, Biochem. Oncogenes Section	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Dr. Ing-Ming Chiu, Dr. Steven Tronick, and Dr. Stuart Aaronson; LCMB, DCCP, NCI

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Oncogenetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	3.25	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have confirmed and extended our earlier findings on the evolutionary relationship between different oncovirus genera and oncoviral related sequences in human cellular DNA. By the combined criteria of low stringency blot hybridization and comparative nucleotide sequence analysis, we have established the existence of two major pol gene families in the evolution of oncoviruses. One family is composed of mammalian type C viruses. The other family includes type A,B,D, avian type C and human T-cell leukemia virus (HTLV). The major region of homology between members of the latter family could be localized to the 3' end of the pol gene. In the avian type C, Rous Sarcoma virus, this region corresponds to a pp30 peptide which has endonucleolytic activity that is highly specific for closed circular proviral DNA. Nucleic acid sequence homology could also be demonstrated between the env genes of mammalian type C and type D oncoviruses. This data provides evidence for the genetic interaction between the progenitors of mammalian type C and type D oncovirus genera and suggests that this phenomenon may be an active force in the evolution of oncoviruses.

In previous work we have described recombinant clones of human cellular DNA which contained MMTV related sequences. The major region of homology corresponds to the pol gene of MMTV. The corresponding nucleotide sequence of one human recombinant clone (designated HLM-2) has been determined. Within a 524 base pair segment HLM-2 shares 51,50,44, and 37 percent homology with respectively MMTV (type B), SMRV (type D), RSV (avian type C) and HTLV oncoviruses. There was no significant nucleotide sequence homology with the Moloney leukemia virus pol gene. These results confirm our earlier findings using low stringency blot hybridization.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB04848-12 LTIB

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of Flat Cellular Revertants to Study the Functions of Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert H. Bassin	Chief, Biochem. Oncogenes Sec.	LTIB, DCBD, NCI
Robert Callahan	Chief, Oncogenetics Sec.	LTIB, DCBD, NCI
Herbert Cooper	Chief, Cell. & Mol. Phys. Sec.	LTIB, DCBD, NCI
David Salomon	Expert	LTIB, DCBD, NCI
Wayne Anderson	Research Chemist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Makoto Noda, Keio University, Tokyo, Japan

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Biochemistry of Oncogenes Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	3.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Flat cellular revertants which are resistant to transformation by ras and certain other oncogenes have been described. Resistance to transformation can be demonstrated both by cell fusion of revertant cells to transformed cells or by direct infection with transforming retroviruses. Resistance of the revertants to transformation is specific -- ras, fes, and src-related oncogenes do not transform the revertant cells efficiently, while cells transformed by a number of other oncogenic agents including the retroviral oncogenes fms, sis and mos, SV40, polyoma, and a number of chemically transformed cell lines retain their transformed phenotype after fusion. Revertant cells secrete a transforming growth factor into their culture medium which is capable of inducing NIH/3T3 and NRK test cells to grow in semisolid agar. Revertants do not respond to exogenous transforming growth factors, indicating that the revertant phenotype may be due to a cellular alteration affecting the function of TGF or some later stage in the biochemical pathway leading to cell transformation.

Initial studies indicate that the revertant phenotype is transmissible to recipient cells by DNA transfection procedures, an indication that it may be possible to identify the molecular basis for reversion. Finally, 2 transformation-sensitive proteins, which are present in normal NIH/3T3 cells, disappear in transformed cells, and reappear in the revertant cell lines, have been detected in 2-dimensional gel electrophoresis studies.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08256-05 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Retinoids and Hormones in Mediating Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wayne B. Anderson — Research Chemist — LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
D. Evain-Brion, Unite INSERM 188, Paris, France
S.P. Nissley, Metabolism Branch, NCI, NIH; G.R. Grotendorst, LDBA, NIDR, NIH
H.L. Nakhasi, LLP, DCBD, NCI, NIH; M. Sherman, Roche Inst. Molec. Biol., Nutley, NJ

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Biochemistry of Oncogenes

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Exposure of undifferentiated embryonal carcinoma stem cells to retinoic acid (RA) previously has been shown to induce differentiation to parietal endoderm, and to rapidly increase cyclic AMP-dependent protein kinase (cAMP-PK) activity. Two stem cell mutants (provided by M. Sherman) which are insensitive to RA have been used to study the mechanism of RA action. One (PCC4-RA) lacks the intracellular RA binding protein (cRABP); treatment with RA was found to cause a decrease in cytosolic cAMP-PK and R_I subunit. The other (Nulli-RA) does have the cRABP; RA treatment was observed to enhance cAMP-PK activity even though later RA-mediated events are defective in this cell type. Treatment of F9 stem cells with RA also markedly alters the ability of calcitonin and parathyroid hormone to stimulate adenylate cyclase activity. Results indicate that F9 cells secrete immunoreactive calcitonin (iCT) into the culture medium while PYS (parietal endoderm-like) cells secrete immunoreactive parathyroid hormone (iPTH). Retinoid-induced differentiation of F9 cells to endoderm results in a progressive reduction in iCT production, while there is an increase in the level of iPTH found in the conditioned medium. Thus, iCT is produced by undifferentiated F9 cells which possess a calcitonin responsive adenylate cyclase system, while iPTH is produced by endoderm cells which respond to PTH with increased cAMP synthesis. These results raise the possibility that embryo production of these two hormones at specific stages in development may contribute to the regulation of subsequent steps of differentiation. Exposure of F9 cells to RA also provokes a 4-fold induction of cell surface N-acetylglucosamide B (1-->4) galactosyltransferase (GT) activity. The RA-induced GT activity was further enhanced by treatment of the cells with 8-bromo cAMP. The ability of RA in combination with cAMP to induce GT activity was inhibited by both actinomycin D and cycloheximide, indicating that the increase in GT activity noted involved de novo synthesis of new enzyme protein.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09015-01 LTIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification Of Cellular Targets Of Oncogene Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wayne B. Anderson	Research Chemist	LTIB, DCBD, NCI
Robert Bassin	Chief, Biochem. Oncogenes Sec.	LTIB, DCBD, NCI
Patricia Horan Hand	Chemist	LTIB, DCBD, NCI
Thomas P. Thomas	Visiting Scientist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

A. Spiegel, Metabolic Diseases Branch, NIADDKD, NIH
W. Farrar, Lab. Md. Immunoregulation, NCI, FCRF, Frederick, Maryland

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Biochemistry of Oncogenes

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NIH/3T3 fibroblasts, Kirsten sarcoma virus (Ki-MuSV) transformed NIH/3T3 cells, and cellular revertants of these cells which are resistant to transformation by specific oncogenes have been utilized to determine possible cellular components involved in the malignant transformation of cells. A solid phase radioimmunoassay was developed to measure the levels of 53 K cellular protein which is elevated in several types of malignant cells. NIH/3T3 cells were found to have elevated levels of p53 protein relative to other normal cell types, and transformation of these cells by Ki-MuSV caused a 2-to-5 fold increase in p53. The revertant cells, which are resistant to transformation by ras p21 oncogene product, exhibit levels of p53 protein only 1/3 that of the NIH/3T3 cells. Studies indicate that p53 protein is elevated in normal cells within 3 to 6 hours after treatment with phorbol ester tumor promoter.

Calcium activated, phospholipid-dependent protein kinase (PK-C) appears to be involved in regulating cell growth. This kinase serves as the cellular phorbol ester receptor to mediate early events of tumor promotion. PK-C activity is found to be elevated in the particulate fraction of cells under conditions of phorbol ester tumor promotion and low population density, rapid cell growth. Ki-MuSV transformed NIH/3T3 cells also have an increased amount of PK-C activity in the particulate fraction when compared to control, growing NIH/3T3 cells. The revertant cells exhibit low membrane-associated PK-C activity when compared to the parent NIH/3T3 cells. Thus, transformation-induced and phorbol ester-induced changes in p53 protein and in association of PK-C with the plasma membrane may be critical events in mediating eventual malignant transformation.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09016-01 LTIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Mechanisms in Expression of Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Herbert L. Cooper	Chief, Cell. & Mol. Phys. Sect.	LTIB, DCBD, NCI
Elwood McDuffie	Bio. Lab. Tech.	LTIB, DCBD, NCI
Richard Braverman	Chemist	LTIB, DCBD, NCI
Robert Bassin	Chief, Biochem. of Oncogenes Sect.	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Cellular & Molecular Physiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Biochemical events associated with transformation due to expression of viral oncogenes in NIH/3T3 cells were studied. Expression of v-ras and its product, p21 were associated with reduction or suppression of synthesis of a number of cellular proteins. Revertant lines which still expressed v-ras and p21 but were not transformed showed restoration of synthesis of most, but not all of these proteins. Two proteins were of special interest - p37/pI-4.8 and p41/pI-4.8. Synthesis of these proteins was strongly suppressed not only by v-ras, but by all of the 5 other oncogenes examined, regardless of relation to v-ras. These findings suggest that a final common pathway for oncogenesis by many oncogenes involves suppression of synthesis of p37/4.8 and p41/4.8, and that normal levels of synthesis of these proteins is essential for maintenance of the normal growth pattern in 3T3 cells.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09005-02 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Posttranslational Formation of Hypusine and Control of Protein Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Herbert Cooper	Chief, Cell. & Mol. Phys. Sec.	LTIB, DCBD, NCI
Richard Braverman	Chemist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Cellular & Molecular Physiology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.35	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study investigates the role of a unique posttranslational modification, hypusine formation, in a single protein of all eukaryotic cells, which we have identified as protein synthesis initiation factor 4D (eIF-4D). The enzymatic mechanism involved in the modification and the importance of the modification in the function of eIF-4D are currently being studied.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CB09006-02 LTIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Events in Phorbol Ester Effects on Normal and Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Nili Feuerstein	Visiting Fellow	LTIB, DCBD, NCI
Herbert L. Cooper	Chief, Cell. & Mol. Phys. Sect.	LTIB, DCBD, NCI
Elwood McDuffie	Bio. Lab. Tech.	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Cellular & Molecular Physiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.45	1.2	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phosphorylation of proteins during response of cells to treatment with phorbol esters (PMA) was studied. In HL-60 promyelocytic leukemia cells, growth arrest and differentiation after PMA exposure are associated with rapid phosphorylation-dephosphorylation of proteins pp17 and pp27. Cell-free studies suggest that this may involve the activation and cooperation of two classes of protein kinase, calcium-phospholipid-dependent kinase and cAMP-dependent kinase. Significantly, enhanced phosphorylation of pp17 and pp27 was found only in cell lines where PMA caused growth arrest and differentiation. The effect was minimal in cells where PMA was mitogenic.

PMA also induces aggregation of platelets, during which increased protein phosphorylation occurs. Elevated phosphorylation of class-I HLA molecules was documented, together with evidence suggesting association of HLA in a complex with myosin and actin and implicating modification of HLA as a component of platelet activation.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB09007-02 LTIB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms in HLA Function and Polymorphism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dimitri Monos	Visiting Fellow	LTIB, DCBD, NCI
Herbert L. Cooper	Chief, Cell. & Mol. Phys. Sect.	LTIB, DCBD, NCI
Elwood McDuffie	Bio. Lab. Tech.	LTIB, DCBD, NCI
Richard Braverman	Chemist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Immunology Branch, NCI; Lab. of Microbial Immunity, NIAID

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Cellular & Molecular Physiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.70	1.45	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of Class I HLA antigens in cell-cell interactions was studied. Rapid turnover of HLA proteins in human peripheral lymphocytes was inhibited by conditions that minimized cell-cell contact. HLA antigens in human platelets were phosphorylated under conditions of platelet aggregation. These findings suggest that HLA molecules are metabolically responsive to cell-cell and cell-environment interactions.

Peripheral lymphocytes were shown to synthesize and secrete Calmodulin continuously. The secreted molecules may play a role in modulating cell-cell interactions.

The biochemical basis for the genetic predisposition of NZB mice to autoimmune disease is being studied. A developmental derangement was found in expression of certain proteins by splenic lymphocytes in which proteins normally synthesized at high levels only in young animals were re-expressed as animals aged and developed autoimmune disease.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00944-22 LTIB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Total Metabolism of Cancer Cachexia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Seoras D. Morrison — Research Physiologist — LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
Jeffrey A. Norton, Surgical Metabolism Section, Surg. Branch, NCI

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project concerns the causes and mechanisms of the nutritional depletion and general deterioration of the cancerous host, known as cancer cachexia. The object is to find ways of blocking or reversing the cachectic effects of cancer so that the cancer patient would become more accessible and less vulnerable to anti-cancer therapies. Premature satiety is the immediate cause of the reduction in food intake possibly operating through an enhanced cephalic phase of satiety. The host body mass can be conserved by TPN but at the cost of acceleration of tumor growth. The body mass can be conserved and the voluntary food intake increased by insulin treatment without acceleration of tumor growth. The mass conserved is not lost on withdrawal of insulin. Insulin administration during cold exposure (a non-pathological inducer of weight loss) synergistically increases food intake conserves body mass but all the mass conserved is lost immediately on withdrawal of insulin.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB08212-10 OD

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
From Gene to Protein: Structure Function and Control in Eukaryotic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shelby L. Berger	Research Chemist	OD NCI
Other:	Robert S. Puskas	Senior Staff Fellow	OD NCI
	William H. Eschenfeldt	Senior Staff Fellow	OD NCI
	Marc Krug	Staff Fellow	OD NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
OD, DCBD

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

A/B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methods have been developed for quantifying messenger RNA and for efficient cloning of cDNA. (1) The mole percent polyadenylated RNA in a preparation contaminated with rRNA has been determined by labeling with poly(A) polymerase and cleaving the product with ribonuclease H in the presence of oligothymidylate. The absolute concentration of submicrogram quantities of mRNA can also be ascertained. (2) Single stranded cDNA has been prepared with reverse transcriptase in 400% yield by modifying the template RNA. (3) Double stranded cDNA has been synthesized in 70 to 80% yield by priming the polymerization of the second strand with RNA fragments generated by the ribonuclease H associated with reverse transcriptase. (4) The conditions under which 3'-ends of cDNAs are extended with homopolymeric DNA have been altered. Tails of uniform length at both ends of each molecule have been synthesized in virtually 100% yield. (5) Cerenkov radiation has been used to monitor reactions performed in small volumes and to quantify precious biological materials with losses of less than 0.5 µl.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05526-16 OD

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Common Protein in Embryonic Differentiation and in Cellular Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter T. Mora, Chief, Macromolecular Biology Section		OD DCBD NCI
Other:	Usha S. Thathamangalan	Visiting Fellow	MBS OD DCBD NCI
	C. Dale Smith	Visiting Fellow	MBS OD DCBD NCI

COOPERATING UNITS *(if any)*
Daniel Simmons, University of Delaware; H. J. Westphal, LMG, NICHHD, NIH; K. Chandrasekaran, IRSC, Villejuif, France; F. V. Roy, University of Ghent, Belgium

LAB/BRANCH
OD, DCBD

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Immunoaffinity chromatography was developed to isolate the cellular phosphoprotein p53 from a mouse neuroblastoma cell. This p53 was stable, not complexed to other protein and had methionine labeled tryptic peptides very similar to the p53 isolated from mouse embryo cells. A method was developed for the quantitation of the p53 mRNA employing a cDNA clone and Northern blot hybridization. A single polyadenylated mRNA species migrating at ca 18S was found in SV40 transformed mouse fibroblasts in the neuroblastoma cells, in embryonal carcinoma cells and also in mouse embryo cells. The level of p53 mRNA was measured in different stages of mouse embryogenesis. Several SV40 transformed cells were found in which the p53 is not in complex with the T antigen. These cells included human placenta cells, human osteosarcoma cells and a set of mouse embryo fibroblast cells. In these latter cells it was shown that the absence of complexing correlates with a low degree of phosphorylation of the p53.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB00941-28 OD

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Other Factors Affecting Marrow Transplantation in Irradiated Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Delta E. Uphoff Research Biologist OD DCBD NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
OD, DCBD

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B/D

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The investigation of physical factors affecting the success of bone marrow transplantation experiments was continued and include both radiobiological effects of the direction of the exposures and subtle changes in physical factors that alter the survival of irradiated inbred mice with and without marrow graft. Basic concepts of radiation biology were demonstrated to be invalid and physical factors, considered of little consequence when applied to biological systems, were critical for the reproducibility of these experiments. Consequently the concept of the dose-rate effect can no longer be limited to fractionated and protracted irradiation where cell proliferation and repair are major factors in improved survival but must also include exposures at low dose rates over time spans too short to allow for cell proliferation and little if any repair. New parameters for "defining" x-ray treatment are required along with more precise reporting of experimental conditions as a result of these investigations.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05097-3 OD

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure of Thyroid Proteins in Human Thyroid Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Sidney Shifrin	Chemist	OD	DCBD	NCI
Other:	Leonard D. Kohn	Medical Officer		LBP	NIADDK
	Michele De Luca	Visiting Scientist		LBP	NIADDK
	Pilar Santisteban	Visiting Scientist		LBP	NIADDK
	William Coleman	Res. Microbiologist		LBP	NIADDK

COOPERATING UNITS *(if any)* William A. Valente, Univ. of Md.; Eduardo Consiglio, Salvatore Aloj, Paolo Laccetti, Univ. of Naples; Ephraim Yavin, Weizmann Inst. of Science, Rehovot; Annalisa Tanini, Roberto Toccafondi, Univ. of Florence; Mario Andreoli, Univ. of Rome; Richard Montali, National Zoo, Wash., D.C.

LAB/BRANCH
OD, DCBD

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

A

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to examine the physicochemical properties of the proteins which are extracted from the thyroid glands of a variety of animals and from humans who suffer from Graves' disease and Hashimoto's thyroiditis - two autoimmune disorders and from thyroid cancer.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CB05544-14 OD

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Surface Changes in Transformed Mouse Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Samuel W. Luborsky Chemist MBS OD DCBD NCI
Other: Peter T. Mora, Chief, Macromolecular Biology Section OD DCBD NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
OD, DCBD

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

B

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

SV40 transformed mouse embryo cells were labelled in culture with ^{35}S-methionine, protein was extracted and aliquots of the extract treated with hamster anti-T antigen serum, or with buffer, to produce SV40 T antigen, or control (N), immunoprecipitates (IP), respectively, which were analyzed by SDS-polyacrylamide gel electrophoresis. Three specific bands were found in T reactive solutions, of molecular weights about 94,000 and 19,000 and 53,000 daltons, for SV40 large T and small t antigens, and the cellular 53K protein, respectively. A similar procedure is being used to attempt to detect RNA in these IP proteins, after incubating the cultures with ^{3}H-uridine, an RNA precursor. The IP from these cells possessed low levels of radioactivity, which was not found on the gels. Work is continuing to determine the nature of the radioactivity associated with the IP, which seems to be absent from the gels used to analyze them.

PHS 6040 (Rev. 1/84)
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