NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1986 through September 30, 1987

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00333-24 LB

PERIOD COVERED

October 1, 1986, to September 30, 1987

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
S.M. Wilson	Biologist	LB	NCI
M.G. Mage	Immunochemist	LB	NCI
O.W. McBride	Chief, Cellular Regulation Section	LB	NCI

COOPERATING UNITS *(if any)*

Hematology, Oncology Section, Walter Reed Army Medical Center

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Cellular Regulation Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20892

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: ZO1 CB 00366-17 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Structure and Expression of Endogenous Retroviral Elements

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
Z. Grossman	Visiting Fellow	LB	NCI
P. Arnaud	IPA	LB	NCI

COOPERATING UNITS *(if any)*
E. Leiter, Jackson Laboratory, Bar Harbor, ME; K. Ishizaka, Johns Hopkins School of Medicine, Baltimore, MD

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Biosynthesis Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

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

B

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

We have continued studies of the intracisternal A-particle (IAP) genes, a genetically distinctive family of retrovirus-like elements in the mouse. Nucleotide sequencing of a complete 7 kb IAP genomic element, designated MIA14, and analysis of the genomic organization and predicted protein products was completed during the past year. Homology to type-D monkey virus permits assignment of coding domains even though the IAP proteins are not processed to the usual retroviral components. The main 73 kDa IAP structural protein (P73) is analogous to the unprocessed gag precursor of immature retroviruses. Homology with the type-D retrovirus begins at the p27 coding domain. Upstream of p27, the sequence is unique to the mouse IAP genome. P73 N-terminates in a hydrophobic signal sequence which is probably the means by which the nascent particles are associated with the endoplasmic reticulum membrane. MIA14 contained two stop codons in the pol reading frame; these have since been corrected and now both gag and pol are open. The "env" region of the IAP genome contains multiple conserved stop codons in all reading frames, in agreement with the observed absence of a virally coded envelope protein in IAPs.

Sequencing was also completed for two very closely related protein-coding IAP cDNA clones, each isolated from thymus of a different inbred mouse strain. The clones are an order of magnitude more closely homologous to one another than to a number of other IAP clones for which comparable sequence is available. We plan experiments to determine whether these two clones represent allelic IAP elements specifically expressed in the thymus.

We have begun investigating the binding of nuclear factors to the cloned LTR of MIA14, previously shown to be active in promoting gene transcription. The aims of this work are (1) to define the mechanism by which DNA methylation inhibits the promoter activity, and (2) to analyze the reported enhancement of IAP LTR activity by nuclear oncogene products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 00945-14 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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
I. Oyamada	Visiting Fellow	LB	NCI
J. Palka	Visiting Fellow	LB	NCI
E. Schalk	Guest Researcher	LB	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Biological Interactions Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	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 have continued testing our model that proposes an indirect role for ascorbate in regulation of synthesis of the extracellular matrix, rather than through a direct role in the biosynthetic pathway of collagen. The model predicts that scurvy-induced fasting leads to changes in humoral factors involved in regulation of collagen and proteoglycan synthesis. Specific assays were performed for the somatomedins/insulin-like growth factors (IGF-I and II) and for somatomedin carrier protein in normal and scorbutic guinea pig sera. The results indicated that ascorbic acid deficiency in guinea pigs results in decreased levels of IGF-I as well as induction of an inhibitor of DNA synthesis in 3T3 cells and of extracellular matrix synthesis in cultured chondrocytes.

Further studies were carried out on two proα2(I)-like subunits of type I procollagen that are synthesized by a chemically transformed Syrian hamster fibroblast line. Normal procollagen type I is optimally secreted in a triple helical form, but the proα2(I)-like chains are secreted as single chains. Secretion of these chains was not affected by their level of proline hydroxylation or by incorporation of the proline analog, cis-hydroxyproline, into the chains. Since the chains were nonhelical, this result was not unexpected, but the finding that cis-hydroxyproline induced rapid, intracellular degradation of the chains did not conform with the assumed mechanism of action of the analog. The analog is thought to induce degradation by destabilizing the triple helical structure of the normal procollagen molecule and consequently rendering the denatured chains susceptible to proteolysis.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05202-20 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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
A. Bale	Biotechnology Fellow	LB	NCI
S. Olson	Biotechnology Fellow	LB	NCI
P. Burnett	Biologist	LB	NCI

COOPERATING UNITS *(if any)* J. Minna, F. Kaye, et al., F. Gonzalez, S. Kimura, D. Hatfield, S.A. Aaronson, S. Tronick, NCI; C. Kozak, M. Lerman, J. Chen, D. Nebert, et al., S. Detera-Wadleigh, NIH; R. Tukey, UCSD; M. Horowitz, Weizmann; R. Skoda, U. Meyer, Basal; R. Pirtle, NTS; D. Gajdusek, D. Goldgaber, CNSS; M. Smulson, Georgetown U.

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Cellular Regulation Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

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.)*

Analysis of interspecific somatic cell hybrids segregating human chromosomes permits the localization of human genes to specific chromosomes. We have previously constructed a large panel of human-rodent hybrids and used them to chromosomally map protooncogenes and various other human genes by Southern analysis of hybrid cell DNAs with cloned DNA probes. In collaborative studies, these hybrids have now been used to chromosomally map several additional proto-oncogenes and putative chromosomal neoplastic breakpoints as well as genes for the β-amyloid polypeptide of Alzheimer's disease, glucocerebrosidase (Gauche's disease), thyroid peroxidase and TSH receptor β-polypeptide, multiple tRNAs, calmodulin, alpha2-HS glycoprotein, poly(ADP-ribose) polymerase, epoxide hydrolase, menadione oxiodoreductase, and multiple additional P450 genes. Many pseudogenes were also chromosomally localized, RFLPs identified, and genomic restriction maps of several of the active genes constructed. Nine probes identifying restriction fragment length polymorphisms (RFLPs) have been isolated from chromosome-specific DNA libraries, subcloned, and characterized. Construction of genetic linkage maps of human chromosomes 1 and 15 is underway using cloned gene and anonymous DNA probes with DNA samples from the CEPH pedigrees. DNAs have been isolated from 50 lymphoblastoid cultures of patients with DNA repair defects and analyses are in progress to detect involvement of β-polymerase and poly(ADP-ribose) polymerase genes in any of these diseases.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05203-19 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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
B. Nardelli	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 20892

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 methods for 1) the specific isolation of antigen-reactive cells for the study of the molecular interactions that occur in their immune reactions, and 2) their application to problems of tumor immunology. In order to be able to directly measure binding of antigen-specific receptors to MHC molecules, we have accomplished the synthesis of conjugates of anti-MHC antibodies and water-soluble polymers. In order to study targeting of tumors with MHC antigens, we have synthesized, by crosslinking different monoclonal antibodies, heteroconjugates that can bind two different class I MHC antigens. In studies aimed at understanding the enhanced immunogenicity of a xenogenized tumor cell line, we have found that the immune response to it is MHC restricted at the level of both helper and cytotoxic T cells. Furthermore, the xenogenized cell line, unlike the unmodified, unrejected tumor line from which it was derived, was found to have features characteristic of antigen presenting cells, i.e. surface expression of class II (Ia) antigens, and secretion of material with IL1-like activity.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05214-16 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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
B. Zmudzka	Visiting Associate	LB NCI
P. Kedar	Visiting Fellow	LB NCI
S. Widen	Hall-Shields Fellow	LB NCI
J. Abbotts	IRTA	LB NCI
C. Majumdar	Guest Researcher	LB NCI

COOPERATING UNITS *(if any)*
J. Mitchell, J. Oppenheim, H.R. Guy, NCI; G. Zon, F. Robey, FDA; K. Williams, Yale; R. Karpel, University of Maryland; J. Collins, Virginia Commonwealth University; F. Cobianchi, CNR, Pavia, Italy; S. Broder, NCI

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Nucleic Acid Enzymology Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	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 biochemical studies of mammalian DNA replication proteins. Our full length cDNA for human β-polymerase was subcloned in an expression vector. The protein was overproduced in E. coli and purified in mg quantities. Enzymological characterization indicated that the recombinant enzyme is similar to the natural enzyme in catalytic properties. In vitro DNA repair activities of the recombinant enzyme were studied. Genomic clones spanning the gene for human β-polymerase were isolated and characterized. The promoter region was sequenced and studied by deletion mutagenesis using a transient expression system. In other work, we found that the abundance of β-polymerase mRNA in cultured human cells is both cell cycle regulated and serum regulated. The β-polymerase coding sequence was stably transfected into human fibroblast and expression of the transfected gene was studied.

Steady-state kinetic analysis of the HIV DNA polymerase was conducted, and an overall kinetic scheme was derived. A DNA segment containing the coding region for this enzyme was subcloned in an expression vector and the enzyme was overproduced in E. coli.

Physical biochemical studies of a recombinant single-stranded nucleic acid binding protein termed A1 were conducted. The protein binds cooperatively to either RNA or DNA and the full-length protein binds much tighter than a truncated A1 protein lacking the glycine-rich COOH-terminal domain (residues 185-319). Proton NMR studies suggest the mechanism of A1 binding is similar to that of several prokaryotic ssDNA binding proteins in that binding involves close approach of aromatic amino acids with nucleotide bases. Indeed, we found that all 4 major sites of covalent A1 photocross-linking to $[^{32}P]d(pT)_8$ occur at Phe residues. No crosslinking sites were found within the COOH-terminal domain, yet this domain clearly makes a significant contribution to the overall free energy of binding of A1 to nucleic acids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05231-13 LB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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, Protein Biochemistry Section,	LB	NCI	
M.J. Hubbard	Visiting Fellow	LB	NCI	(3 months)
J.L. Foster	Guest Researcher	LB	NCI	
M.H. Krinks	Chemist	LB	NCI	
J.R. Miller	Technician	LB	NCI	(3 months)
T. Jean	Visiting Fellow	LB	NCI	
D. Guerini	Guest Researcher	LB	NCI	
J. Mackall	Guest Researcher	LB	NCI	

COOPERATING UNITS *(if any)* Dr. J. Schiloach, NIAMMD; Dr. P. Cohen, University of Dundee, Scotland; Dr. L. Heppel, Cornell University, Ithaca, NY; Dr. J. Wolff, NIAMDD; Dr. H. Plattner (University of Konstanz FRG); D.J. Tash and A.R. Means (Baylor University, Houston, Texas)

LAB/BRANCH: Laboratory of Biochemistry, DCBD

SECTION: Protein Biochemistry

INSTITUTE AND LOCATION: National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.5	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.)*

Several steps in the stimulus response coupling mediated by Ca^{2+} and calmodulin were investigated: (1) The existence of a GTP-specific diacylglycerol kinase which phosphorylates a specific pool of diacylglycerol has been demonstrated. This novel GTP-regulated protein may play a role in the release of Ca^{2+} from internal stores upon external stimuli. (2) Ca^{2+} binding by calmodulin is subject to a regulation by Mg^{2+} which appears to be mediated by a Mg^{2+}-induced conformational change in the central helix connecting the two halves of the calmodulin molecule. (3) The catalytic subunit of calcineurin, a calmodulin-regulated protein phosphatase, contains four distinct functional domains. The calmodulin-binding domain of calcineurin has been isolated and characterized. Genes for the two subunits of calcineurin have been cloned and their sequence is being determined. The availability of the amino acid sequence of the two proteins will permit mapping of the functional domains of the enzyme and further our understanding of the mechanism of activation by calmodulin. (4) In collaboration with Drs. Tash and Plattner we have obtained evidence for a role of calcineurin in the regulation of cell motility and exocytosis.

Stimulus response coupling by cAMP is being studied by J. Foster who has cloned, sequenced and mapped the gene for the catalytic subunit of cAMP-dependent protein kinase of Drosophila melanogaster. The gene encoding the cGMP-dependent kinase of the same organism has also been isolated and is being sequenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05244-10 LB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Organization of Repeated DNA Sequences in Primates

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

M. F. Singer	Chief, Laboratory of Biochemistry	LB	NCI
R. Thayer	Chemist	LB	NCI
T. Fanning	Expert	LB	NCI
S. Mongkolsuk	Visiting Fellow	LB	NCI
G. Humphrey	Guest Researcher	LB	NCI
V. Krek	Guest Researcher	LB	NCI
G. Swergold	PRAT Fellow	LB	NCI

COOPERATING UNITS *(if any)*

Jeffrey Sawyer, Clinical Diagnostic Foundation, Genetics Center, Corpus Christi, Texas; O. Wesley McBride, Laboratory of Biochemistry, DCBD, NCI

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Nucleic Acid Enzymology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.8	5.5	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors (lymphocytes) B

☐ (a2) Interviews

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

The goal of this work is to understand the structure and possible function of highly repeated DNA sequences and to elucidate the mechanisms by which such sequences are amplified either in tandem (satellite DNA) or through transposition to new genomic loci (interspersed repeats). One particular single copy sequence that is conserved in primates and rodents and is joined to species specific satellite DNA in each species analyzed is being characterized. This single copy sequence is located close to the Huntington's disease locus on human chromosome 4p16. Also, the extensive and rapid changes that occur in satellite DNA are being applied to taxonomic and evolutionary problems: in particular, evolutionary relationships in the carnivores are being investigated. To understand the significance of the LINE-1 family of interspersed repeats, the potential for some family members to be functional genes and encode a protein is being investigated. cDNA clones representing polyadenylated, cytoplasmic LINE-1 RNA from human teratocarcinoma cells have been isolated; they define a subset of LINE-1 sequences that appear to be specifically transcribed (or processed) in these cells and must thus be associated with specific transcriptional regulatory sequences. Comparison of the cDNAs indicates an overall structure that contains two open reading frames (ORFs) separated by two in-frame stop codons bracketing 33 bp. Conceptual translation of the 3' ORF (1284 codons) indicates a polypeptide with striking homologies to retroviral and retrotransposon reverse transcriptases. Sequence analysis of a feline LINE-1 allowed us to demonstrate that the reverse transcriptase homology is markedly conserved in LINE-1s from 4 mammalian orders. In vitro translation experiments demonstrate that at least one of the cDNAs can represent a functional mRNA for the 5'-ORF. Overall the data suggest that LINE-1 and retroviruses may share a common ancestor and that reverse transcriptase-encoding sequences have been preserved by selective pressure in mammals.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05258-08 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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	NCI	W. Quitschke	Visiting Fellow	NCI
J. Eldridge	Biochemist	NCI	L. DePonti	Visiting Fellow	NCI
J. Rodriguez	NRC Associate	NCI	Z.-Y. Lin	Visiting Fellow	NCI
B. Winter	Visiting Fellow	NCI			

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Biochemistry of Gene Expression Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	7	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.)*

Myogenic tissue culture systems provide extremely useful model systems in which to study gene regulation during tissue formation. We have isolated and characterized a variety of muscle specific and house-keeping genes that are differentially expressed during myogenesis. These include the genes for alpha skeletal, alpha cardiac, and beta cytoplasmic actin, and the myosin light chain 1-3 gene. The cis acting sequences responsible for the tissue specific regulated expression of these genes has been characterized and we are in the process of identifying proteins that interact with these regulatory regions.

PC12 cells undergo neuronal differentiation in response to NGF. We have isolated the full-sized cDNA and corresponding gene for an mRNA sequence that is induced 50-80 fold in response to NGF. Antibodies to the polypeptide, prepared with lac fusions and various ORFs in the cDNA, demonstrate the protein induction in vitro and its localization in various neuronal tissues in vivo. Preliminary studies suggest that the promoter of this gene responds to NGF induction when joined to a reporter gene (CAT). Regulation of this gene and identification of the protein are under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05262-07 LB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Eukaryotic Gene Regulation and Function: 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				
M. Wong	Visiting Fellow	LB	NCI	D. Thiele	Guest Researcher	LB	NCI
M.J. Walling	Microbiologist	LB	NCI	P. Furst	Guest Researcher	LB	NCI
J. Trnovsky	Visiting Fellow	LB	NCI	R. Hackett	Staff Fellow	LB	NCI
J. Imbert	Visiting Fellow	LB	NCI	S. Hu	Chemist	LB	NCI
M. Ernoult	Visiting Fellow	LB	NCI				

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Biochemistry of Gene Expression Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	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 to study how eukaryotic genes are regulated and how the gene products allow cellular adaptation and homeostasis. A mouse nuclear factor that binds to the metal control sequences of the mouse metallothionein-I gene has been identified and is being purified. Developmental regulation of the mammalian genes is being studied by transfection and transgenic animal experiments. Using yeast as a simple model system, trans-acting genes involved in metallothionein gene expression are being identified and cloned. Structure function studies of yeast metallothionein suggest the presence of preferred nucleation sites for the cooperative folding of this protein.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05263-06 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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 Scientist	LB	NCI				
B. Walker	Staff Fellow	LB	NCI	B. Davis	Laboratory Worker	LB	NCI
S. Wilson	Research Assistant	LB	NCI	L. Brown	Visiting Fellow	LB	NCI
V. Zimarino	Visiting Associate	LB	NCI	C. Tsai	Howard Hughes Fellow	LB	NCI
H. Ueda	Visiting Fellow	LB	NCI				

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Developmental Biochemistry and Genetics Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	7.0	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 analysis of sequence specific DNA binding proteins in Drosophila has been continued by this group. The heat shock activator protein is now routinely purified to homogeneity, and mouse antibodies against the protein have been prepared. This reagent will be extremely useful for further studies on the function of the protein, which pre-exists in normal cells, and is post-translationally modified in response to heat shock to be a sequence-specific binding protein. NFftz1, a nuclear protein found in Drosophila embryos, has been extensively purified and characterized biochemically. This protein may negatively regulate the transcriptional activity of the segmentation gene fushi tarazu.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05264-06 LB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Research Chemist	LB NCI
Edward 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 20892

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.)*

Studies on the structural and functional organization of type II intracisternal A-particle retrovirus elements at the molecular level have continued. We have shown that methylation and association with two defined repetitive sequences appear to play a role in controlling expression of these genes in mouse cells. On the basis of sequence, a peptide representing the viral integrase has been synthesized and used to raise antibodies in rabbits. The antibody will permit us to study the integrase function in a myeloma cell line in which extensive amplification of these IAP sequences has occurred. A cDNA library has been made from this cell line. Clones for further study have been selected on the basis of IAP protein expression as well as sequence homology. Cloning of the integrase gene will allow characterization of this protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05265-05 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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
N.-D. Vu	Staff Fellow	LB	NCI
A. Carroll	Biologist	LB	NCI
H. Foster	Lab Technician	LB	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry, DCBD

SECTION
Protein Biochemistry Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	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.)*

Interactions between cytoplasmic myosin and actin are responsible for a variety of cellular motile activities. As in muscle contraction, hydrolysis of ATP by myosin provides the required energy. While the actin-activated ATPase activities of vertebrate smooth muscle and nonmuscle myosins are regulated by phosphorylation, there is disagreement as to the mechanism of this regulation. It has been reported that unphosphorylated gizzard myosin is inactive and that phosphorylation causes a large increase in its actin-activated ATPase activity. However, we have found that unphosphorylated calf thymus cytoplasmic myosin and unphosphorylated calf aorta smooth muscle myosin are active. The main effect of phosphorylation on these myosins is to increase their affinities for actin. At high actin concentrations, the MgATPase activities of thymus and aorta myosins are almost independent of phosphorylation. As it seemed unlikely that actin could stimulate unphosphorylated aorta myosin but not unphosphorylated gizzard myosin, we reexamined the ATPase activity of gizzard myosin. We found that both unphosphorylated and phosphorylated gizzard myosins needed to be filamentous for their MgATPase activities to be activated by actin. Under conditions where the unphosphorylated myosin was filamentous, its MgATPase activities were stimulated 10-fold by actin. Under conditions typically used by other investigators, the unphosphorylated myosin was monomeric, and its MgATPase was not activated by actin. Active unphosphorylated smooth muscle myosin helps explain the ability of smooth muscles to maintain tension in the absence of myosin phosphorylation. We have also used limited proteolysis to identify regions on the heavy chain of thymus myosin which appear to be involved in actin and ATP binding and in the regulation of its interaction with actin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 CB 05267-03 LB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mechanisms of Plasmid Maintenance

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

M. Yarmolinsky Chief, Developmental Biochemistry & Genetics Section, LB, NCI

D. Chattoraj	Research Chemist	LB NCI	E. Hansen	Visiting Fellow	LB NCI
B. Funnell	Visiting Scientist	LB NCI	S. Pal	Visiting Fellow	LB NCI
S. Jafri	Guest Researcher	LB NCI	K. Tilly	Sr. Staff Fellow	LB NCI
K. Muraiso	Guest Researcher	LB NCI			
	R. Mason-Simmons	Technician LB NCI			

COOPERATING UNITS *(if any)*

Dr. S. Wickner, LMB, NCI; Dr. N. Sternberg, DuPont

LAB/BRANCH

Laboratory of Biochemistry, DCBD

SECTION

Developmental Biochemistry and Genetics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	6.0	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 continue our studies of the replication and partition mechanisms that account for the stable inheritance of unit-copy bacterial plasmids for which P1 plasmid prophage serves as exemplar. In the period of this report we have (1) provided evidence for a role of DNA looping in the control of plasmid replication, (2) demonstrated a biphasic relationship between the concentration of a plasmid-determined initiator and plasmid copy number, (3) established an in vitro system for mini-P1 plasmid replication with which to deepen our understanding of replication requirements and control, (4) characterized a lytic replicon of P1 that may possibly serve as an auxiliary plasmid replicon, (5) demonstrated an aggressive interference with plasmid maintenance by an overproduced partitioning protein, (6) constructed indicator plasmids for the identification of host factors involved in plasmid partitioning and (7) completed a comprehensive review of the biology of bacteriophage P1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 03663-11 LCO

PERIOD COVERED

October 1, 1986 through September 30, 1987

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:	D. R. Lowy	Chief, Lab of Cellular Oncology	NCI
OTHER:	J. T. Schiller	Senior Staff Fellow	LCO NCI
	W. S. Sawchuk	Medical Staff Fellow	LCO NCI
	K. H. Vousden	Visiting Fellow	LCO NCI
	T. J. Velu	Visiting Fellow	LCO NCI
	P. Hawley-Nelson	Biotechnology Fellow	LCO NCI
	N. L. Hubbert	Microbiologist	LCO NCI
	A. G. Papageorge	Microbiologist	LCO NCI

COOPERATING UNITS *(if any)*

See next page.

LAB/BRANCH

Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.00	6.00	3.00

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.)*

This program studies oncogenes and papillomaviruses.

Oncogene studies have involved ras and EGFR. Some ras experiments are directed towards determining how ras proteins are stimulated to become biologically active and to identify their cellular target(s). Mutations that have been found previously not to affect cell transformation by a highly oncogenic ras gene have been engineered into a proto-oncogene version of ras. Some of these mutations have a significant effect on the ability of the proto-oncogene to induce cellular transformation. In vivo studies with Harvey murine sarcoma virus variants indicate that the target cell for tumor formation is determined, at least in part, by the transforming activity of ras in the virus. Experiments with a full-length EGF receptor proto-oncogene placed in a retrovirus vector have shown that established mouse cells transfected with the viral DNA or infected with a corresponding retrovirus, developed a fully transformed phenotype in vitro that required functional EGFR expression and presence of EGF in the growth medium. These results demonstrate that increased numbers of EGF receptors can contribute to the transformed phenotype.

In papillomavirus studies, the E2 open reading frame has been shown to bind to a specific motif present several times in BPV and other PVs. This motif is an enhancer whose activity depends upon E2. Enhancement requires two or more copies of the motif. Anti-E2 sera have detected two E2 protein products in BPV transformed cells. The smaller form of E2, which may competitively inhibit enhancement by the full-length E2 product, contains the DNA binding activity but lacks enhancer activity. In HPV studies, E6 protein has been identified in human cervical carcinoma cell lines known to express HPV 16 RNA and in mouse cells morphologically transformed by HPV 16 DNA. These results support the hypothosis that E6 can contribute to the transformed phenotype in human cervical cancers.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09051-02 LCO

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Biology of fps/fes oncogenes

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

PI: R. A. Feldman Senior Staff Fellow LCO NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	0.00

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.)*

This project aims at elucidating the biological function(s) of the normal human c-fps/fes proto-oncogene, and to understand the molecular basis of its oncogenic potential. We have generated a cDNA copy of human c-fps/fes from a genomic DNA by means of a retroviral shuttle vector, and have begun characterization of its biological and biochemical properties. The rescued cDNA encoded an NCP92 protein that was indistinguishable from myeloid cell NCP92, providing direct evidence that this 92 Kda cellular tyrosine kinase is the gene product of human c-fps/fes. We also showed that human c-fps/fes is susceptible to oncogenic activation by N-terminal linkage with viral gag sequences.

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 CB 05550-18 LCO

PERIOD COVERED

October 1, 1986 through September 30, 1987

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:	K. S. S. Chang	Medical Officer	LCO NCI
OTHER:	L.-C. Wang	Visiting Associate	LCO NCI
	C. Gao	Guest Researcher	LCO NCI

COOPERATING UNITS *(if any)*

Laboratory of Molecular Virology and Carcinogenesis, FCRF
Biotech Research Laboratories, Rockville, Maryland

LAB/BRANCH

Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	2.00	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.)*

1. Certain cultured lines as well as tumor tissues of human embryonal carcinoma (EC) showed amplification and enhanced expression of c-Ki-ras2 proto-oncogene. Retinoic acid treatment of EC cell lines resulted in down-regulation of c-Ki-ras2 gene expression and morphological differentiation into neuron-like cells. This would suggest that epigenetic changes may influence the oncogenicity of EC cells.

2. Further studies on v-mos expression in S+L-mink cells superinfected with a novel retrovirus showed that the state of anchorage-independent, transformed cells growing in suspending fluid medium, is associated with a high oncogenicity and metastatic ability. This is related to greatly increased v-mos gene integration and expression accompanied by decreased production of vimentin, a component of intermediate filaments. However, ouabaine-induced adherent revertant cells exhibited a lowered oncogenicity without diminishing v-mos expression. Thus, an elevated level of v-mos expression is necessary but not sufficient for high oncogenicity.

3. Further studies on the amplification of c-abl-related sequence accompanied by new insertions of ecotropic provirus in the DNA of spontaneous reticulum cell neoplasms of SJL/J mice is in progress. Attempts are made to clone the 9-19 kb fragments of HindIII cleaved DNA, and to make cDNA of the mRNA.

4. A serological study using ELISA, Western blot, and immunofluorescence tests was conducted, and it was found that a high proportion of I.V. drug abusers is found to be infected with both HTLV-I and HIV (human immunodeficiency virus). Since IgM antibody against HIV was found more frequently than that against HTLV-I in these dually infected persons, the time of infection with HIV must have been more recent than that with HTLV-I.

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 CB 04834-11 LCO

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Genetic mechanism of neoplastic transformation

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

PI:	S. S. Yang	Research Chemist	LCO NCI
OTHER:	J. V. Taub	Biolab Technician	LCO NCI
	R. Modali	Biologist	LCO NCI

COOPERATING UNITS *(if any)*

B. J. Park, LMCB, NCI
E. Butler, LDN, NICHD

LAB/BRANCH

Laboratory of Cellular Oncology

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.50	1.00	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.)*

The thrust of this project is to elucidate the genetic mechanism of neoplastic transformation of normal cells. The experimental system centers on a 3.1 kilobasepair, poorly cell-transforming human oncogene, hHC^M, isolated from an African hepatocellular carcinoma cell line, Mahlavu. In a survey of 24 additional hepatoma DNAs (17 Korean, 3 Taiwan Chinese, 2 Caucasian, and 2 African), 20 hepatomas had DNA sequences homologous to hHC^M. Seven clones of these hepatocellular carcinoma oncogenes were isolated from a Chinese and a Korean hepatoma. Their cell-transformation capability was ascertained and their structural relationship analyzed. The presence of the integration site for human hepatitis B virus DNA within the hHC^M was found and the relationship of HBV to hHC^M was critically examined. As an on-going interest, the aflatoxin B_1 dG targets on hHC^M were predicted by computer analysis. By a combination of recombinant DNA technology and DNA-mediated cell-transformation assay, the possible role(s) of these poorly cell-transforming oncogenes, hHC, and their relationship(s) with HBV and afltoxin B_1, in neoplastic transformation of cells are currently being examined.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 CB 05596-18 LGN

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

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

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

P.I.:	M. Potter	Chief, Laboratory of Genetics	LGN,NCI
	E.B. Mushinski	Bio. Lab. Technician	LGN,NCI
	L. D'Hoostelaere, S. Brust, R. Duncan	Biologists	LGN,NCI
	E. Shacter, K. Huppi	Staff Fellows	LGN,NCI
	B. Mock	Hall-Shields Fellow	LGN,NCI
	K. Sanford	Chief, In Vitro Carcin. Sect.	LCMB,NCI
	K. Kohn	Chief	LMPH,NCI

COOPERATING UNITS *(if any)* Dr. H.C. Morse, III, NIAID; Dr. F. Wiener, Karolinska Institutet, Stockholm, Sweden; Dr. R. Parshad, Howard Univ., Wash. DC; Dr. K. Marcu, SUNY, Stony Brook, NY; Dr. L. Blankenhorn, Hahneman Medical School, Phil., PA

LAB/BRANCH: Laboratory of Genetics

SECTION:

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.0	5.0	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.)*

The major project in the laboratory is to determine the pathogenetic mechanisms involved in the development of parrafin oil (pristane) induced plasmacytomas in BALB/c mice. BALB/cAn mice are highly susceptible to developing these tumors while most other strains are resistant. Over 95% of plasmacytomas induced by pristane have chromosomal translocations [rcpt(12;15), rcpt(6:15)] involving directly or indirectly the c-myc locus on chr 15. One aim of our work is to identify the origin of mutagenic substances associated with the chronic inflammatory tissue evoked by pristane. We hypothesize these are oxygen and lipid radicals generated by inflammatory cells. A major clue in this work is provided by the finding that indomethacin inhibits plasmacytoma formation. Studies are aimed at determining the mode of action of indomethacin. We have developed model genetic systems for finding the genetic basis of susceptibility and resistance to plasmacytoma development by using BALB/cAn.DBA/2 (C.D2) and BALB/cAn.BALB/cJ (C.J) congenics. Several genes have been identified that confer partial resistance. Congenic mice constructed by combining two weakly active resistance genes have produced even stronger resistance. In collaboration with Dr. K. Sanford, we are studying genes associated with DNA repair deficiencies in BALB/cAn mice and C.D2 and C.J congenics. We have identified two genes in DBA/2.

Plasmacytomas can be induced with short latent periods in BALB/cAn mice by the infection of pristane conditioned mice with retroviruses containing oncogenes. Our recent studies demonstrate the potent cooperative action of myc and ras (in collaboration with K. Marcu, Stony Brook).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08727-10 LGN

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

P.I.: J.F. Mushinski	Medical Director	LGN,NCI
G.L.C. Shen-Ong, L. Wolff	Senior Staff Fellows	LGN,NCI
K. Huppi	Staff Fellow	LGN,NCI
B. Mock	Hall Shields Fellow	LGN,NCI
J. Kurie	Biotech. Training Fellow	LGN,NCI
S.R. Bauer, L. D'Hoostelaere	Biologists	LGN,NCI
L. Miribel	Visiting Fellow	LGN,NCI
M. Potter	Chief, Lab. of Genetics	LGN,NCI

COOPERATING UNITS *(if any)*

D. Givol, Meloy Labs; K. Marcu, Dept. of Biochemistry, SUNY, Stony Brook, NY; H.C. Morse, III, LVD, NIAID; M.C. Sneller, LCI, NIAID, J. Ashwell, BRMF, NCI

LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.0	8.0	2.0

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.)*

The overall goal of this research is to study the activation of particular genes in diseased and normal cells in order to understand which genes may play important roles in the development of malignancies, autoimmune diseases and normal differentiation. The immune system has been chosen as the central focus of this research, and we have concentrated on the expression of "oncogenes," especially myc, myb and ras, as well as immunoglobulin and T cell receptor genes. To this end we have been studying the lymphoid tumors (particularly the plasmacytomas) that are regularly induced in BALB/cAnN mice by intraperitoneal injections of alkane mineral oils, such as pristane. These tumors represent immortalized lines of B lymphocytes or myeloid cells at different stages of differentiation. Currently we are using this model system of tumors to learn how the genes involved in myeloid and B cell carcinogenesis are organized and regulated. Generally oil-induced plasmacytomas arise only after a long latent period, typically 12 months. In such a long time period many genetic changes could have accumulated, one or more of which could be causally involved in the carcinogenic process. The latent period can be drastically shortened by injecting certain retroviruses, e.g., Abelson virus complex or viruses incorporating avian v-myc genes. The latency period is shortened presumably by supplying one of the genetic lesions that by chance arose in the oil-treated peritoneal cells. We have also studied how endogeneous proto-oncogenes are expressed and found the frequent elevation of steady state levels of RNA from the proto-oncogenes c-myc and c-myb in certain tumors, autoimmune cells, and in dividing normal lymphocytes. Recent studies have shown changes in some of the ras family of oncogenes in a large number of these tumors. The details and consequences of these mutations are currently being analyzed. Another putative oncogene, bcl-2, has been found to be expressed at only certain periods during B cell differentiation. An extensive effort has been made to characterize the structure and control of expression of these oncogenes in normal and abnormal cells.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05553-18 LGN

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

P.I.:	Stuart Rudikoff	Microbiologist	LGN, NCI
	W. Davidson	Visiting Associate	LGN, NCI
	R. Nordan	Staff Fellow	LGN, NCI
	J. Hyde	Postdoctoral Fellow	LGN, NCI
	A. Cuddihy	Graduate Student	LGN, NCI
	D. Hilbert	Guest Researcher	LGN, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION
NIC, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	5.0	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.)*

1) In the last several years a number of lymphokines have been described which play important roles in the growth and/or differentiation of B- and T-lymphocytes. Studies from this laboratory have identified an activity in supernatants from macrophage cell lines which is required by early plasmacytomas for growth and viability in vitro. As part of our current interest in control of cell growth, a major effort is being made to characterize this factor and determine its role in vivo. Ultimately, we plan to extend these studies to the receptor for this factor in order to assess the potential biological role of this factor-receptor system in normal cell growth and/or possibly plasmacytomagenesis. 2) A second major interest in the area of growth and differentiation is directed toward an attempt to isolate and characterize genes (and their protein products) involved in ontological differentiation of lymphoid cells. To approach this question, we have developed new or selected existing cell lines which can be induced to differentiate. The strategy of these experiments is to prepare subtractive cDNA libraries using the parental line and the differentiated derivatives. The resulting clones will then be analyzed with the eventual aim of transfecting isolated genes back into the parental cell type to attempt to reproduce the differentiated phenotype in the absence of inducing agent. 3) Mutational processes operating during gene evolution may range from relatively simple, such as point mutation, to considerably more complex, such as recombination, gene conversion, expansion and contraction. Most evolutionary studies in higher organisms have involved comparisons of similar protein or gene sequences from two or more species which are generally widely separated in evolutionary time. We have attempted to assess the role of such processes in a more dynamic context by examining gene structures among different wild mice species. We have therefore initiated experiments to examine single copy genes, pauci-gene families and multi-gene families among our wild mice colony representing the evolutionary spectrum of this species.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05552-18 LGN

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	F. Curcio	Visiting Scientist	LGN,NCI
	R. Swerdlow	Staff Fellow	LGN,NCI

COOPERATING UNITS *(if any)*

Dr. M. Luisa Brandi, NIDDKD, MD; Dr. F. Saverio Ambesi-Impiombato, Istituto di Patologia Generale, Naples, Italy

LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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 systems for cell culture. We are now pursuing intensively a single cell system: culture of cells from the neonatal rat olfactory epithelium (OLFE). The projects involving thyroid gland reconstruction and thyroid cell genetics have been postponed. We hope to continue these timely experiments as soon as our principal collaborator, Dr. F. S. Ambesi, can return to the laboratory. Meanwhile, our progress with the OLFE has been sufficiently exciting during the past year that it has merited our full attention. Using complex media and substrates we have succeeded in culturing several cell types from the OLFE. The mixed, mass cultures of these cells provide an appropriate conditioned medium that has permitted the isolation of 20 clonal cell strains from 3rd to 6th passage cultures. We have continued the hybridoma screen using OLFE as antigen and fluorescent anti-mouse IgG staining of frozen sections of OLFE for selection. These results, too, are very encouraging; some of the hybridoma supernates appear to identify small patches of cells selectively in the OLFE, and others identify only the apical structures on either the sensory cells or the sustentacular cells. The sensory neurons of the olfactory epithelium are renewed throughout life from a stem cell population. Development of this system would make available the first mammalian neuroblast to neuron cell culture system and provide a means to study the growth and differentiation dichotomy common to all blast cell systems. It is hoped that basic issues in olfactory sensory physiology can be explored with this system.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08950-05 LGN

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	Sr. Staff Fellow	LGN,NCI
C. Mainhart	Microbiologist	LGN,NCI
T. B. Lavoie	Biologist	LGN,NCI
E. Vacchio	Biologist	LGN,NCI
R. Duncan	Biologist	LGN,NCI
C. Mallett	American Cancer Society Fel.	LGN,NCI
P. Hamel	Fellow, NCI of Canada	LGN,NCI
P. Rousseau	Student Volunteer	LGN,NCI

COOPERATING UNITS *(if any)*
A.B. Hartman, Dept. of Biologics, Walter Reed; K. Dorrington, Dept. of Biochem., Univ. of Toronto, Toronto, Canada; D. Davies, LMB, NIADKD; B. Brooks, DCRT, NIH; A. Basten, University of Toronto, Toronto, Canada

LAB/BRANCH
Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

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

Monoclonal antibodies directed against the model protein antigen, lysozyme c, are used as probes to study antibody-protein interactions and structure-function relationships, and to study developmentally regulated antigens in normal and neoplastic development. The interaction of antibodies with the epitopes are modeled and ultimately refined by protein crystallography studies. One antibody-lysozyme complex has been refined to 2.5 Å by X-ray crystallography. The results confirm the serologically predicted epitope and indicate a high degree of complementarity between the opposing surfaces. Another antibody-lysozyme complex has been studied in detail utilizing a new computer method for efficiently examining the interaction between 2 proteins. The conclusions from both studies are being tested with peptide binding experiments, by site-specific mutagenesis of in vitro expressed cloned immunoglobulin genes, and utilizing transgenic mice expressing the cloned lysozyme and/or antibody genes. The development of specificity for lysozyme from an apparently multispecific available antibody repertoire is currently being examined in detail utilizing large panels of hybridoma antibodies. In addition, monoclonal antibodies are being generated against bacterially expressed mouse c-myc protein; these antibodies will be used to purify and characterize structure-function relationships in the myc protein, applying the principles derived from the model protein studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08951-05 LGN

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular basis for the acute erythroleukemias induced by murine retroviruses

PRINCIPAL INVESTIGATOR *(List othar professional personnal 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
	S.-W. Chung	Visiting Fellow	LGN,NCI

COOPERATING UNITS *(if any)*

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

The Friend strain of the spleen focus forming virus (SFFV) causes an acute erythroleukemia in mice. Studies have been aimed at defining the area(s) of the viral genome responsible for pathogenicity and erythroid cell specificity and determining the mechanisms by which SFFV alters erythroid cell growth and differentiation.

Using a Moloney MuLV-based retroviral vector, we have demonstrated that the SFFV env gene, when introduced into mice in the absence of other SFFV genes, is pathogenic. The disease induced is indistinguishable from that induced by the entire SFFV genome, proving that the primary effect of the virus, which is to alter the requirements of erythroid cells for erythropoietin (Epo), is due solely to the product of its env gene. To further understand how the viral env gene product alters erythroid cells, we have continued our studies of variants of the virus, designated $SFFV_P$ and $SFFV_A$, both of which induce acute erythroleukemia but differ in their effects on erythroid cells as well as in the processing of their env gene products. We were previously able to localize the biological and biochemical differences between the two viruses to a 678 bp region in the 3' half of the env gene. We have now further localized the critical region to a 120 bp fragment from the extreme 3' end of the env gene which encodes the p15E-related transmembrane domain of the protein. Finally, we have attempted to understand how the SFFV envelope glycoprotein alters the requirements of erythroid cells for Epo by comparing normal and virus-infected cells for Epo receptors. Our results indicate that spleen cells from $SFFV_P$-infected mice, which proliferate and differentiate in the apparent absence of Epo, have the same number of Epo receptors as normal erythroid cells, whereas spleen cells from $SFFV_A$-infected mice, which proliferate better in the presence of Epo and which require Epo for differentiation, have 4 times as many Epo receptors. Cross-linking studies show no obvious quantitative differences in the receptors on normal and SFFV-infected cells.

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 CB 08952-01 LGN

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oncogenes and their cooperative effects in myeloid leukemias

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

P.I.:	L. Wolff	Senior Staff Fellow	LGN,NCI
	L. Pasamontes	Guest Researcher	LGN,NCI
	K. Nason-Burchenal	Graduate Student	LGN,NCI
	G. Shen-Ong	Senior Staff Fellow	LGN,NCI
	J.F. Mushinski	Medical Director	LGN,NCI

COOPERATING UNITS *(if any)*
H.C. Morse, III, LVD, NIAID; L. Neckers, LP, NCI

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NCI, NIH, Bethesda, MD 20892

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.)*

Myeloid lineage leukemias represent a high proportion of leukemias in the human population. Some of these leukemias are consistently associated with chromosomal translocation, deletions and gene amplifications in which oncogenes have been implicated. Our group has found a useful model in the murine hemopoietic system for studying these types of leukemias. The overall goal is to look at the transforming effects of various oncogenes on mouse cells in vivo and in vitro with a particular emphasis on their cooperative effects of oncogenes on cells of the myeloid lineage.

The laboratory, because of its experience in constructing and testing retroviruses, uses naturally occurring, as well as genetically engineered retroviruses, as vehicles for introducing oncogenes into cells. We are taking advantage of two modes for leukemia induction 1) one in which oncogenes or potential oncogenes are introduced via replication defective retrovirus vectors and are allowed to transform cells directly, and 2) one in which replication competent viruses, such as Moloney murine leukemia virus, are introduced and spread through mice until they activate an oncogene by insertional mutagenesis. These studies have led to the discovery of two experimental protocols for induction of myeloid tumors in pristane primed mice. In one system using the myc gene containing retrovirus, the tumors that arise are mature macrophage cells whereas in the other system, using Moloney murine leukemia virus, the cells are rather immature myelomonocyte cells. Some newer studies include the construction and biological testing of viruses encoding potential oncogenes such as the transferrin receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08000-17 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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: I. Pastan Chief, Laboratory of Molecular Biology NCI

G. Merlino Staff Fellow LMB, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	5	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.)*

Regions essential for the expression of the EGF receptor gene have been identified by deleting various portions of the promoter. Proteins which can bind to these regions have been identified by their ability to protect DNA from the promoter region from being digested by exonuclease III (exonuclease mapping). An oligonucleotide corresponding to one of the sites has been synthesized and used to purify one of these proteins; this protein is currently being characterized.

A crude cell-free system which carries out transcription of the EGF receptor has been developed. Deletion analysis of the EGF receptor template has identified a small region necessary to support transcription. A crude transcription extract has been subjected to fractionation by heparin agarose and DEAE cellulose chromatography to identify factors necessary for transcription. Methylation of the DNA template appears to have no effect on RNA transcription.

Previously, EGF and phorbol ester (PMA) were shown to promote accumulation of EGF receptor mRNA. Currently possible mechanisms responsible for receptor mRNA accumulation are being evaluated to distinguish between effects of these ligands on initiation of transcription and mRNA stabilization.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08001-16 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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: I. Pastan Chief, Laboratory of Molecular Biology NCI

COOPERATING UNITS *(if any)*

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0	0	0

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

☐ (a1) Minors

☐ (a2) Interviews

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

Work on this project has been temporarily halted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08010-14 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Morphologic Mechanisms of Organelle Function and Transformation in Cultured Cells

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

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

COOPERATING UNITS *(if any)*

Laboratory of Kidney and Electrolyte Metabolism, DIR, NHLBI
Laboratory of Biochemistry and Metabolism, DIR, NIDDK

LAB/BRANCH

Laboratory of Molecular Biology

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Ultrastructural Cytochemistry Section

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NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0		

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☐ (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 of which can be studied using morphologic techniques. We have used morphologic methods to study three general areas: 1) We have employed light microscopic immunocytochemistry to evaluate and select monoclonal antibodies for use as immunotoxin reagents for cancer therapy. A large scale screening chamber has been devised for use with immunofluorescence as a primary selection for surface-reactive monoclonal antibodies to human ovarian cancer cells. Hybridoma clones selected in this way were further evaluated by screening against normal human tissues and ovarian tumors using peroxidase immunocytochemistry in cryostat sections. Using these methods we have found two hybridomas highly reactive with human ovarian tumors: OVB1 and OVB3. We have characterized the tissue distribution and biochemical properties of these antigens in cultured cells, in tumors and in normal tissues. 2) We have studied the phenomenon of multidrug-resistance (MDR) in cultured cells, and have used a monoclonal antibody (MRK-16) to the human mdr1 gene product (P170) as a probe to demonstrate P170 on the surface and in the Golgi stacks of drug-resistant KB cells in culture. We also showed the absence of P170 from coated pits, suggesting that this protein is immobilized in the plasma membrane. We have also used this antibody to examine the distribution of P170 in normal human tissues, and we have evaluated the amounts of P170 in progressively resistant derivatives of KB cells using immuno-fluorescence. 3) In other studies, we have localized p55, a major thyroid hormone binding protein found in the endoplasmic reticulum and nuclear envelope of cultured cells in normal tissues using immunoperoxidase histochemistry. This protein was found in highest amounts in cells that have abundant endoplasmic reticulum. Other cytochemical studies demonstrated a role for clathrin-coated pits in the loss of ADH-responsive water transport in isolated perfused rabbit cortical collecting ducts. We also demonstrated the localization of carbohydrate sites reactive with wheat germ lectin on nuclear pores in isolated nuclei.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08011-13 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	K. M. Yamada	Chief, Membrane Biochemistry Section	LMB, NCI
Other:	M. Obara	Visiting Fellow	LMB, NCI
	M. S. Kang	Biotechnology Training Program Fellow	LMB, NCI
	S. K. Akiyama	Guest Researcher	LMB, NCI
	M. J. Humphries	Guest Researcher	LMB, NCI
	K. Olden	Guest Researcher	LMB, NCI
	J. W. Lash	NRSA Senior Fellow	LMB, NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	3.7	0.7

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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.)*

Fibronectin is a major cell surface and extracellular matrix glycoprotein involved in cell adhesion and migration. Synthetic peptide inhibitors derived from its sequence were examined for effects on a variety of adhesion systems. The pentapeptide Gly-Arg-Gly-Asp-Ser (GRGDS) was the most effective peptide against fibronectin-mediated adhesion, and a related sequence was active for vitronectin. Such peptides had little activity on laminin, collagen, or cell-cell adhesion, indicating specificity. An artificial inverted peptide identified a possible common denominator of function in four of these five systems. GRGDS inhibited experimental metastasis of B16 melanoma cells in mice as measured by pulmonary colonization, and the relative activities of analogues closely matched those for inhibition of cell adhesion in vitro. Peptide treatment also substantially prolonged survival of these animals. Several strategies to increase peptide effectiveness in vitro and in vivo are under investigation. This pentapeptide adhesive recognition sequence also appears to function in Drosophila gastrulation and in vertebrate somite formation; somite precursor cells showed induced cell-cell adhesion. An additional region required for full-affinity binding to the cell surface (50- to 100-fold augmentation) is being defined by recombinant DNA expression studies. Large fibronectin fragments expressed in E. coli as λgt11 fusion proteins retained >80% adhesive activity; eukaryotic post-translational modifications were thus not needed. Deletion mutagenesis experiments show that the second site is at least 20kD away from the GRGDS site. A separate class of novel, cell-type specific binding sites were discovered elsewhere in fibronectin at sites regulated by alternative splicing. A synthetic peptide from one site was only 2.4-fold less active than intact fibronectin. The critical amino acid sequences and the biological functions of these novel regions in cell migration and metastasis will be determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08700-15 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*
PI: B. de Crombrugghe Chief, Gene Regulation Section LMB, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION
Gene Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	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. A tissue-specific transcriptional enhancer has been identified in the first intron of the mouse α_2(I) collagen gene.
2. A functional analysis of deletions in the promoter of the mouse α_2(I) collagen gene indicates there are at least two different segments upstream of the start of transcription that are important for optimal expression of this gene.
3. Transgenic mice were generated in which an α_2(I) collagen promoter-chloramphenicol acetylase chimeric gene has been stably introduced in the germline. These new mouse strains show a tissue specific pattern of expression for the chimeric gene that coincides with that of the endogenous type I collagen genes.
4. Several factors present in nuclear extracts of NIH 3T3 cells have been identified, which bind to defined segments of the α_2(I) collagen promoter. A factor which binds to the CCAAT sequence has been extensively purified. Evidence has been obtained that the factor consists of two components.
5. The hormone tumor growth factor β stimulates transcription from the α_2(I) and α_1(III) collagen promoters in fibroblasts and osteosarcoma cells.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08705-11 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	M. M. Gottesman	Chief, Molecular Cell Genetics Section	LMB, NCI
Other:	S. Goldenberg	Research Biologist	LMB, NCI
	M. Chapman	Research Biologist	LMB, NCI
	R. Fleischmann	Staff Fellow	LMB, NCI
	S. Kumar	Visiting Fellow	LMB, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION

Molecular Cell Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	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.)*

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 the manner in which cyclic AMP regulates cell growth and gene expression. The mechanism of cAMP action on CHO cells has been studied by isolating CHO mutants resistant to growth inhibiton by cAMP. These mutants have defective cAMP dependent protein kinases with either altered regulatory (RI) or catalytic (C) subunits. We have used DNA from cells carrying dominant cAMP-resistant defects to transfer the cAMP-resistance phenotype to sensitive cells. The mechanism of mutation in these cell lines is being studied with cloned RI and C subunit probes. Cloned segments of RI have been sequenced from cosmid libraries prepared from our mutant cell lines. The intent of these studies is to isolate cloned mutant cAMP dependent protein kinase subunits for use as movable genetic elements capable of inactivating the protein kinase system in a variety of differentiated cells and transgenic animals. We have also used the CHO protein kinase mutants to demonstrate that the expression of many promoters linked to the reporter gene encoding chloramphenicol acetyl transferase is positively regulated by cAMP in a manner which depends on cAMP dependent protein kinase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08709-12 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Gene Expression and Differentiation by ADP-ribosylation of Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: G. S. Johnson Research Chemist LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Cell Genetics Section

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NCI, NIH, Bethesda, MD 20892

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

Drugs which inhibit $(ADP\text{-ribose})_n$ synthetase and decrease endogenous ADP-ribosylation of chromosomal proteins cause accumulation of mRNA for glucocorticoid sensitive genes. Considerable variations in the extent of accumulation have been observed during the course of this study. Thus, factors in addition to the total amount of nuclear $(ADP\text{-ribose})_n$ are involved. Glucocorticoid agonists or partial agonists are more effective agonists in cells devoid of $(ADP\text{-ribose})_n$. Interestingly, certain steroids which bind to the receptor but do not normally induce genes are also very good agonists in these cells. We conclude that ADP-ribosylation of some essential protein(s), possibly the steroid receptor, influences expression of steroid-sensitive genes.

Nicotinamide and its synthetic N'-methyl derivative induce maturation of cultured human promyelocytic leukemia HL60 cells. The actions of these compounds are synergistic with retinoic acid, another agent which induces maturation of these cells. N'-methylnicotinamide is converted into N'-methylnicotinamide adenine dinucleotide. This NAD analog may be the active intracellular compound in the cells.

Thermal stress of cultured mouse mammary carcinoma cells results in a loss of basal and glucocorticoid-induced mouse mammary tumor virus (MMTV) RNA within about 60 min. Cycloheximide treatment prior to the temperature shift prevents loss of the RNA. A nuclease which is subject to a rapid turnover may be activated by the heat shock.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08710-11 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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: S. Wickner Research Chemist LMB, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION
Biochemical Genetics Section

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NCI, NIH, Bethesda, MD 20892

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

The molecular mechanisms involved in DNA replication are being studied biochemically. In particular, in vitro replication reactions are being used to study origin specific initiation of replication. I have developed an in vitro DNA replication system that replicates exogenously added plasmid DNA containing the origin of replication of bacteriophage P1. The system consists of a purified P1 replication protein, the product of repA, and a partially purified mixture of E. coli replication proteins prepared from uninfected cells. The system requires the E. coli dnaA initiation protein in addition to the P1 RepA initiation protein. In collaboration with D. Chattoraj (LB, NCI), we have shown by electron microscopic techniques that replication is initiated in the region of the P1 origin of replication and proceeds unidirectionally. Since P1 normally exists as a unit copy plasmid, this system is being used to study the molecular mechanisms involved in the initiation and regulation of a stringently controlled replicon. I have also continued studying the replication of plasmid DNA containing the origin of replication of bacteriophage λ in vitro. This reaction requires two phage functions, the O and P gene products, many host replication proteins and several other host proteins including the heat shock proteins, dnaJ, dnaK, and grpE. Replication also requires a specific DNA site for initiation. I have constructed deletions in vitro extending into this region and tested them for activity in in vitro DNA replication reactions dependent on O and P proteins. The smallest piece of DNA that supports the initiation of replication is 89 bp. It contains two of the four O protein binding sites and the adjoining adenine and thymine rich region that very likely is the site where dnaB protein is transferred to the DNA. In a collaborative electron microscopic study with M. Dodson and H. Echols (University of CA, Berkeley), nucleoprotein structures formed by O, P, dnaB, dnaJ, dnaK, and Ssb at oriλ have been visualized.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08712-12 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Plasma Membrane Proteins in the Regulation of Cell Behavior and Drug Resistance

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

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

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4	1.5

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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.)*

Human KB cells which are resistant to adriamycin (A), vinblastine (V), colchicine (C) and actinomycin D (AcD) have been shown to contain an amplified gene (mdr1) responsible for the drug resistance. A fragment of this gene has been cloned and used to screen a cDNA library and identify cDNAs encoding a 170 kd cell membrane protein also found to be increased in multidrug resistant cells. The 170 kd protein binds vinblastine and related drugs, and this binding is overcome by drugs such as verapamil, quinidine, and diltiazem which overcome multidrug resistance. The cDNA sequence indicates the 170 kd protein should bind ATP; this was directly confirmed by ATP binding studies. Expression of the mdr1 gene was found to be elevated in normal colon, kidney, liver and adrenals and cancers of the colon, kidney and adrenal. Expression was also increased in a few tumors showing acquired drug resistance. The mdr1 cDNA has been cloned into an animal cell expression vector and when inserted into drug sensitive cells makes them drug resistant.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08714-10 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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: S. Gottesman Chief, Biochemical Genetics Section LMB, NCI

COOPERATING UNITS *(if any)*

Laboratory of Genetics, Division of Cancer Biology and Diagnosis

S. Rudikoff LG, NCI

J. Pumphrey LG, NCI

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Biochemical Genetics Section

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NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	5.2	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.)*

We have been studying the role that protein degradation plays in regulating cell growth control, through the study of mutants defective in ATP-dependent protein turnover. E. coli lon mutants are defective in cell division regulation after DNA damage, and we have previously demonstrated that this defect is due to stabilizaton of the highly unstable cell division inhibitor, SulA. lon mutants also overproduce capsular polysaccharide; we have identified an unstable positive regulator of capsule synthesis, RcsA, which is stabilized in lon mutants. The sequence of the rcsA gene shows no striking similarities to other lon substrates. rcsA-lac fusions which we have isolated will allow us to examine the transcriptional and translational control of this gene. Using cells devoid of lon activity, we have biochemically identified a novel two-component, ATP-dependent protease. Using amino acid sequence data from one of the purified components, plasmids carrying the gene are being identified. Using genetic screens, we have also identified and partially mapped a function which is capable of substituting for lon, and presumably codes for another protease with potential regulatory functions in E. coli.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08715-09 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Synthesis and Function of a Transformation-Dependent Secreted Lysosomal Protease

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

PI:	M. M. Gottesman	Chief, Molecular Cell Genetics Section	LMB, NCI
Other:	S. Goldenberg	Research Biologist	LMB, NCI
	M. Chapman	Research Biologist	LMB, NCI
	S. Gal	Biotechnology Fellow	LMB, NCI
	B. Troen	Medical Staff Fellow	LMB, NCI
	S. Kane	Guest Researcher	LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Molecular Cell Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

Cultured mouse fibroblasts which are malignantly transformed or treated with TPA or growth factors such as PDGF synthesize and secrete a 39,000 Mr-phosphoglycoprotein (major excreted protein, MEP) in large amounts. The purified 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 with a lysosomal localization. The secreted form of MEP is the precursor to a lower molecular weight novel thiol protease (cathepsin) with an acid pH optimum capable of hydrolyzing a wide variety of proteins including the extracellular matrix proteins collagen, fibronectin and laminin. The specificity of peptide bond cleavage and the profile of inhibition of MEP is the same as cathepsin L. Sequence analysis indicates that mouse MEP and its human homolog represent precursors to cathepsin L. Overproduction of cloned mouse or human MEP/cathepsin L in a nontransformed cell results in secretion of this lysosomal enzyme. Secreted MEP can bind to the mannose 6-phosphate receptor of many cells and be endocytosed and processed intracellularly. In antigen presenting cells, MEP uptake reduces the efficiency of antigen presentation, thereby interfering with immune response. Transformation, TPA and PDGF stimulate MEP synthesis by increasing levels of MEP specific mRNA transcription. We have cloned a functional MEP gene from the mouse and have identified the 5' flanking region presumed to contain the MEP promoter. We are studying this system as a model of regulation of lysosomal protease synthesis, processing and secretion and how malignantly transformed cells perturb normal host functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08717-09 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Functions, Structure, and Regulation of Receptors for Cell Adhesion Proteins

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

PI:	K. M. Yamada	Chief, Membrane Biochemistry Section	LMB, NCI
Other:	S. Saga	Visiting Fellow	LMB, NCI
	S. K. Akiyama	Guest Researcher	LMB, NCI
	S. S. Yamada	Guest Researcher	LMB, NCI
	J. L. Duband	Guest Researcher	LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.2	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.)*

Cells interact with extracellular matrix molecules by specific receptors. We have characterized membrane binding molecules for fibronectin and collagen. The distribution of fibronectin receptors was disrupted to a diffuse pattern after transformation of avian cells. This alteration could be mimicked by treatment of normal cells with a synthetic peptide that blocks receptor function, and it could be partially reverted by treatment of transformed cells with exogenous cellular fibronectin. This receptor was found to be required for a developmentally-regulated invasive event in which hemopoietic precursor cells transiently gain the ability to invade across basement membranes in vivo and in vitro. A related fibronectin receptor equivalent to the human Ic-IIa complex was discovered on platelets, and it was shown to function in platelet adherence to fibronectin. A key role for carbohydrate processing in the acquisition of fibronectin receptor function is being examined. We have also further characterized a major 47K collagen-binding protein that we found to be a transformation-sensitive, novel heat-shock regulated glycoprotein, whose phosphorylation was increased after transformation. Immunofluorescence and immunoelectron microscopic studies localized it primarily to the endoplasmic reticulum; it was expressed in cell-type specific patterns in vivo. Its binding to collagen was regulated by pH, with maximal release of bound ligand at pH 6.3. This finding permitted its isolation in native form and determination of a unique N-terminal 36-amino sequence. We will further characterize this and other collagen-binding proteins, exploring the role of fibronectin and collagen receptors in cell adhesion, migration, and invasion.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08719-07 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI: Bruce H. Howard Chief, Molecular Genetics Section LMB, NCI

COOPERATING UNITS *(if any)*

LNP, NINCDS

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD

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 primary focus of work in the Molecular Genetics Section continues to be on growth regulation in mammalian cells. The investigation of growth inhibitory activity detected in a DNA-mediated gene transfer system is emphasized within this framework. Molecular cloning of growth inhibitory sequences is being pursued by analysis of a cosmid library derived from WI38 human embryo fibroblast genomic DNA. From this library a single candidate plasmid, which contains a 7SL pseudogene, is being analyzed for both growth suppression activity and function as an origin of replication. Development of two new assays for transient growth regulation has been a major goal over the past year. The first assay employs fluorescence activated cell sorting (FACS) to identify the transfected cell subpopulation in conjunction with appropriate staining techniques to measure cell cycle characteristics of that subpopulation. The second assay involves the use of a new magnetic affinity cell sorting (MACS) method.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08750-07 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	S. Adhya	Chief, Developmental Genetics Section	LMB, NCI
	S. Garges	Microbiologist	LMB, NCI
Other:	J. Kim	Biotechnology Fellow	LMB, NCI
	R. Wartell	IPA Investigator Professor, Georgia Institute of Technology, on Sabbatical Leave	LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Betehsda, MD 20892

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

The cAMP receptor protein (CRP) in the presence of cAMP can modulate the transcription initiation of many operons of E. coli. The protein is inactive in the absence of cAMP; when cAMP is present, the cAMP binds to CRP, causing a conformational change to an active form. We are studying how cAMP causes the conformational change in an effort to determine how a transcriptional regulatory factor can itself be regulated and to determine how an allosteric change in a protein can be accomplished. We have isolated, using mutagen-induced as well as site-specific mutagenesis, several classes of mutations within the crp gene that encodes CRP: crp* mutations that allow CRP to function in the absence of cAMP, crp*-intragenic suppressor mutations that force a crp* mutant to require cAMP, and crp** mutations that have even more cAMP independence than crp* mutants. Our current model for how cAMP induces the allosteric change in CRP is that cAMP binding alters the relative orientation of specific amino acids that are involved in subunit-subunit alignment, domain-domain alignment, and positioning of the DNA-binding F α-helices. Based on the locations within the CRP molecule of the substituted amino acids that cause the change, we have identified regions of CRP that are involved in the cAMP-induced conformational change.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08751-07 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	S. Adhya	Chief, Developmental Genetics Section	LMB, NCI
Other:	R. Haber	Microbiologist	LMB, NCI
	A. Majumdar	Visiting Scientist	LMB, NCI
	J. Tokeson	Guest Researcher	LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

We are studying the mechanisms by which the two promoters of the gal operon of E. coli are regulated. We have previously shown that each of the promoters is negatively regulated by binding of Gal Repressor to two operator elements, one of which (O_E) is located upstream to the promoters and the other (O_I) inside the galE structural gene. O_E and O_I are separated by 114 bp. We have proposed various models by which Gal Repressor inhibits transcription by binding to two distal sites. These models have been tested by various genetic and biochemical experiments: competition binding experiments between Repressor, RNA polymerase and CRP; measurement of binding energies when Repressor binds to O_E and O_I; the effect of changing the angular orientation between the two Repressor contact points; physical measurements of the effect of Repressor binding on DNA structure; changing one or both of the operators into lac operator(s) by site directed mutagenesis and then studying the effect of Gal and Lac Repressors on operator combinations: O_E^G-O_I^G, O_E^G-O_I^L, O_E^L-O_I^G and O_E^L-O_I^L. The results obtained so far suggest that Gal Repressor binding inhibits gal expression not by sterically sterically hindering the binding of RNA polymerase and/or CRP, but by changing the structure of RNA polymerase to an inactive form.

We are also studying the structure of Gal Repressor and the nature of its interaction with the operators by genetic and biochemical analysis, e.g. mutational analysis and defining the contact points by chemical protection studies. The results show that each half of symmetrical operator is occupied by a subunit of dimeric Gal Repressor. Gal Repressor contacts at least two major grooves lying on one face of the dyad symmetry. A third major groove on the opposite face is also affected by Repressor either through direct contact by wrapping Repressor around the helix, or indirectly by Repressor changing the DNA helical structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08752-07 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

PI:	S.-y. Cheng	Research Chemist	LMB, NCI
Other:	I. Pastan	Chief, Laboratory of Molecular Biology	NCI
	C. Parkison	Chemist	LMB, NCI
	T. Obata	Visiting Fellow	LMB, NCI
	T. Fukuda	Visiting Fellow	LMB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	4.1	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.)*

I. Cloning of a cellular thyroid hormone binding protein (p55): A cellular thyroid hormone binding protein was previously purified to homogeneity. This protein was found concentrated on the lumenal face of the endoplasmic reticulum and nuclear envelope. A cDNA encoding p55 was cloned and its sequence was determined. It contains a single open reading frame of 1524 nucleotides which encodes a polypeptide of 491 amino acids and a putative signal sequence of 17 amino acids. The deduced amino acid sequence shows excellent correspondence with the two partial protein sequences. In vitro translation of mRNA prepared by transcription of the cDNA clone with T7 RNA polymerase after cloning into pGEM3 yielded proteins which were immunoprecipitated by monoclonal and polyclonal antibodies against p55. The isolation of the cDNA clone should allow elucidation of the cellular function of p55.

II. Regulation of p55 by 3,3',5-triiodo-L-thyronine (T_3): The effect of T_3 on the stability and synthesis of p55 was evaluated. Rat pituitary tumor GH_3 cells were grown in regular, thyroid hormone-depleted (Td) and Td supplemented with T_3 medium. Immunoprecipitation of ^{35}S-methionine-labeled cellular extracts indicated that p55 is two-fold more abundant in cells grown in Td medium than in cells in regular or Td + T_3 medium. Northern blot analysis indicated that no difference in mRNA level was detected in cells grown in three different conditions. Pulse-chase experiment indicated that p55 is two-fold more stable in cells grown in Td medium. Thus the down regulation of p55 by T_3 occurs at a post-translational level.

III. Purification and characterization of another thyroid hormone binding protein (p58): Analysis of the cellular extracts of A431 cells indicated the presence of another binding protein for T_3. In contrast to p55, this binding component has an apparent MW of 58K. p58 has been purified to homogeneity. Antibodies to p58 are being prepared and its cellular functions will be evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08753-05 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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: I. Pastan	Chief, Laboratory of Molecular Biology	NCI
M.C. Willingham	Chief, UCS	LMB, NCI
D. FitzGerald	Senior Staff Fellow, UCS	LMB, NCI

COOPERATING UNITS *(if any)*

Metabolism Branch, DCBD, NCI
Laboratory of Theoretical Biology, Medicine Branch, DT, NCI
Cetus Corporation, Emeryville, CA

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

Antibodies reacting with human ovarian cancer cells have been coupled to Pseudomonas toxin. Such immunotoxins kill ovarian cancer cells in tissue culture and human ovarian cancer cells growing in the peritoneal cavity of nude mice. Two new monoclonal antibodies reacting with ovarian cancer cells have been isolated: OVB1 and OVB3. OVB3 reacts with a small number of human tissues and OVB3-PE is active in an animal model. OVB3-PE is being prepared for a human trial in patients with ovarian cancer.

The function of the three structural domains of PE has been defined by deletion mapping using a T7 promoter to express the gene in E. coli. Domain I is the cell recognition domain, domain II is the translocating domain and domain III is the ADP ribosylating domain. Domain I has been deleted and replaced by a cDNA for TGFα. The chimeric protein produced kills EGF receptor bearing cells. Phase I studies have begun in collaboration with T. Waldmann to treat adult T-cell leukemia with an antibody to the IL2 receptor (anti-Tac) conjugated to Pseudomonas exotoxin.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08754-04 LMB

PERIOD COVERED
October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Analysis of the Multidrug Resistance Phenotype in Tumor Cells

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

PI: M. M. Gottesman — Chief, Molecular Cell Genetics Section LMB, NCI
I. 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, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	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.)*

Resistance to multiple drugs is a major impediment to the successful chemotherapy of human cancers. To investigate the genetic and biochemical basis for this multidrug resistance (MDR) phenotype, we have developed a model system using the cultured KB cell, a human carcinoma cell line selected independently for resistance to high levels of either colchicine, adriamycin or vinblastine which is cross-resistant to colchicine, adriamycin, vincristine, vinblastine, puromycin and actinomycin D. Expression of MDR correlates with the presence of a 4.5 kb mRNA encoded by the mdr1 gene which was identified and cloned from highly MDR cell lines in which it is amplified. A complete cDNA sequence for the mdr1 gene product has been obtained and the deduced amino acid sequence specifies the membrane-spanning, nucleotide-binding 170,000 dalton P-glycoprotein. Expression of the cloned mdr1 cDNA or the large (>100 kb) mdr1 gene in drug-sensitive cells confers the MDR phenotype. P-glycoprotein, the product of the mdr1 gene, is membrane located and can be labeled with ATP and with a photoaffinity analog of vinblastine. This vinblastine binding is inhibited by agents such as verapamil, which are known to reverse MDR. These data indicate that P-glycoprotein is an energy-dependent drug efflux pump. Expression of mdr1 RNA occurs in normal kidney, liver, colon, and adrenal and in tumors derived from these tissues which are intrinsically resistant to chemotherapy. Acquired drug-resistance in childhood leukemia, neuroblastoma and in pheochromocytoma may be associated with increased mdr1 RNA levels.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08755-01 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Development of Antibodies to P-glycoprotein from Multidrug Resistant Human Cells

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

P.I.	Nancy Richert	Senior Investigator	LMB, NCI
Other:	Ira Pastan	Chief, Laboratory of Molecular Biology	NCI
	M.M. Gottesman	Chief, MCGS	LMB, NCI
	M.C. Willingham	Chief, UCS	LMB, NCI

COOPERATING UNITS *(if any)*

David Liu, Cetus Corporation, Palo Alto, CA 94303

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Ultrastructural Cytochemisty Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

Multidrug resistance (MDR) in human tumor cells is correlated with the presence of a 170,000 dalton membrane glycoprotein called P170 or P-glycoprotein. The complete cDNA sequence of the mdr-1 gene which encodes P170 is known. In the present study, monospecific antibodies to P170 were prepared by immunizing rabbits with 11 synthetic peptides directed against 4 major domains of P170: 1) an N-terminal intracellular domain; 2) an N-terminal extracellular domain; and (3+4) two C-terminal extracellular domains. Thus far two of these peptides have produced high titer antibodies. Antibody P_7 is to domain 1 (residues # 28-35) and P_0 is to domain 3 (residues #741-750). Both antibodies have been affinity purified using peptide-bovine serum albumin conjugates coupled to affigel. Both antibodies specifically precipitate an [^{35}S]methionine labeled 170 kd protein from vinblastine resistant KB cell extracts ($KB\text{-}V_1$) which is not present in the drug-sensitive parent cell line (KB-3-1). P170 was quantitated in seven of the resistant sublines, two revertant sublines, and in two NIH-3T3 lines transfected with mdr-DNA and found to correlate with the level of drug-resistance. P170 is synthesized as a 140 kd precursor which is glycosylated slowly over the next four hrs. The oligosaccharide is asparagine-linked, endoglycosidase H resistant, and contains no sialic acid. The half-life of P170 in $KB\text{-}V_1$ cells is 48-72 hrs, and increasing drug resistance is not due to an altered turnover rate of the protein. P170 was also detected in membrane preparations of normal human kidneys by Western blotting with the P_7 antibody.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08300-15 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

SAAM, Development and Applications for Analogic Systems Realizatior

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
Other Professional Personnel:		
Vernon D. Parker, Ph.D.	IPA, Investigator	LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Ray Boston, Murdoch Univ.,Australia; Dr. Trevor Redgrave, Univ. Western Australia; Dr. Charles Schwartz, Medical Coll. of Virginia,Richmond, VA; Dr. Waldo R. Fisher, Univ. of Fla., Gainesville; Dr. Barbara Howard, Phoenix

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.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.)*

Continuing development of a computer system (SAAM) for the simulation, analysis, and modeling of bio-kinetic systems. In 1986-1987yr we advanced the SAAM/CONSAM extensions to allow for increases in the number of compartments, components and adjustable parameters having previously accomplished the increase in the number of data points which can be used with the simulator. A "Unix (Ultrix 1.5)" version of SAAM29/CONSAM was implemented because of the number of users which have chosen to use this operating system and because of the anticipation of the conversion of the Laboratory of Mathematical Biology to this standard operating system. A 30 compartment version of SAAM and CONSAM has been developed using these new tools and is presently being tested for errors. A version of SAAM29 was developed for the UNIX-PC and tested using the previous library of SAAM problems. Because the UNIX-PC functions as both the computer and terminal, software for a virtual terminal was added to CONSAM. The 30+, C-language, subroutines which makeup the virtual terminal utilize the GSS-CKS C bindings. This implementation necessitated the addition of a graph name characteristic to the CONSAM plot command. CONSAM and the virtual terminal are executed as separate independent processes in which information and control can be passed from CONSAM to the virtual terminal on the plot command.

The metabolism of human IgE was studied in normals, severe atopics and patients with the hyperimmunoglobulin E-recurrent infection (HIE;Job's) syndrome to understand how IgE metabolism is altered in disorders with marked elevations of serum IgE. Following the development of a new compartmental model for IgE metabolism, it was determined that the fractional catabolic rate for IgE is significantly less for atopic patients (mean±SEM=0.20±0.01) and for the HIE patients (0.15±0.02) than for the normal volunteers (0.52±0.06; $P<0.01$) and is inversely related ($r=-0.851$; $P<0.001$) to the serum IgE concentration. Evaluation of these data using the model further lead to a unifying hypothesis of immunoglobulin catabolism.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08303-15 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

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:

Anne Walter, Ph.D., Senior Staff Fellow	LTB, NCI
Ofer Eidelman, Ph.D., Visiting Associate	LTB, NCI
Anu Bali, Ph.D., Visiting Fellow	LTB, NCI
Debi, Sarkar, Ph.D., Visiting Fellow	LTB, NCI

COOPERATING UNITS *(if any)*:
Dr. Kenneth Spring, NHBLI; Dr. Michel Ollivon, CNRS, France, Dr. Abraham Loyter, Hebrew University, Israel.

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Membrane Structure & Function Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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.)*

The research goals in the Membrane Structure and Function Section are directed towards an understanding of mechanisms of membrane fusion mediated by viral spike glycoproteins. We are specifically studying the mode of action of the G protein of Vesicular Stomatitis Virus (VSV), the HN and F proteins of Sendai Virus, and the HA protein of influenza virus. Specific topics include: i) development of fluorescent methods to study kinetics and extent of adhesion and fusion using intact and reconstituted virions, and liposomes and cells as targets; ii) development of methods to analyze reconstitution of viral spike glycoproteins; iii) functional reconstitution of viral spike glycoproteins into lipid vesicles iv) studies of mechanism based on an allosteric model: the role of ligand binding, conformational changes and cooperativity of viral spike glycoproteins in mediating membrane fusion v.) studies of the effects of modifications of viral spike glycoproteins by pH temperature, enzymes, and chemicals on their fusogenic activities vi) studies of the relationship between virus-induced membrane destabilization (permeability changes) and fusion vii.) Studies of viral entry into the cell by endocytosis using fluorescent techniques. viii.) Application of image processing using video-enhanced fluorescent microscopy controlled by a computor to analyse viral entry pathways. ix) Examination of the disposition of the fusion protein after the fusion event; x) Identification of possible fusion intermediates; xi) Development of methods to study fusion activity of mutants of viral proteins using cloned viral membrane protein sequences expressed in transfected cells; xii) Structural studies of viral proteins; xiii) Development of methods for using reconstituted viral envelopes as vehicles for specific delivery of materials into cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08320-12 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:		
Saul Jacchieri, Ph.D.	Visiting Fellow	LTB, NCI
David Covell, Ph.D.	Expert	LTB, NCI
Robert Guy, Ph.D.	Sr. Staff Fellow	LTB, NCI
Kai-Li Ting, Ph.D.	Computer Programmer	LTB, NCI

COOPERATING UNITS *(if any)*

Dr. J. Ferretti, Laboratory of Chemistry, NIHLB; Dr. F. Wang, National Bureau of Standards, Gaithersburg, Md.

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.6	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.)*

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, especially proline, aromatic and polar groups.

NMR investigations of Substance P, a neuropeptide, indicates no dominant conformations in water, but in methanol it is highly ordered with a large number of 2-Dimensional NMR NOE cross-peaks. With these data, molecular models consistent with the experimental data have been developed. Subsequently additional data obtained have assisted in indicating which of the alternative conformations are most probable.

Experimental studies of a 13 amino acid fragment of Ribonuclease A indicated it to be a stable helix at low temperatures. The existence of such a small relatively stable structure has been surprising. We have been looking in detail at possible molecular interactions to try to understand the remarkable stability of such a small peptide as a helix in water.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08335-11 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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.	Chief, Theoretical Immunology Section,	LTB, NCI
Other Professional Personnel:		
Christopher Black, Ph.D.	Visiting Associate	LTB, NCI
Anne Lewis, B.S.	Biologist	LTB, NCI
Bobak Mozayeni, M.S.	Howard Hughes Fellow	LTB, NCI
Mary Jane Talley, B.S.	Biologist	LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Theoretical Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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. Current studies are directed toward HIV-infected cells.

(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 have been extended to antibody-bearing liposomes.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08341-09 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Studies of Lipid-Protein and Protein-Protein Interactions in HIV

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

John N. Weinstein, M.D., Ph.D.	Chief, Theoretical Immunology Section	LTB, NCI

Other Professional Personnel:

Bobak Mozayeni, M.S.	Howard Hughes Fellow	LTB, NCI
H. Robert Guy, Ph.D.	Senior Staff Fellow	LTB, NCI
Robert Jernigan, Ph.D.	Theoretical Physical Chemist	LTB, NCI
Kai-Li Ting, Ph.D.	Computer Programmer	LTB, NCI

COOPERATING UNITS *(if any)*

Dr. T. Innerarity and Dr. R. Pitas, University of California at San Francisco; Dr. Richard Klausner, LBM, NIAMDD; Dr. R. Schwartz, LI, NIAID; Dr. G. Shearer, IB, NCI. Drs. J. Segrest & Arantharamaiah, U. Alabama, Birmingham

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Theoretical Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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 structure by an essentially irreversible and quasistoichiometric process. In the case of HDL, the major apoprotein, A-I, recombines with dimyristoyl phosphatidyl choline vesicles 40:1 lipid-protein to form discs approximately 100 Å in diameter and 32 Å in thickness, with protein on the rim. These structural results were obtained by a combination of neutron scattering, electron microscopy, column chromatography, and fluorescence techniques.

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 this 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.

Statistical and more general mechanical algorithms (HAL, HALP, HALCO) were devised for evaluating amphipathic helical structures and more general structure-function relationships in proteins and peptides. This is being used to define issues of structure and immunogenicity with respect to HLA antigens and to the envelope polyprotein of HIV. Lipid membrane systems and human cell isolates are being used experimentally to investigate the interaction between characterized synthetic antigenic peptides and T-cells in the recognition process. The results may have application to the design of vaccines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08359-06 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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.	Chief, Theoretical Immunology Section	LTB, NCI
Other Professional Personnel:		
Christopher D.V. Black, Ph.D.	Visiting Associate	LTB, NCI
David G. Covell, Ph.D	Expert	LTB, NCI
Renee Eger, B.S	Guest Researcher	LTB, NCI
Anne Lewis, B.S.	Biologist	LTB, NCI
Mary J. Talley, B.S.	Biologist	LTB, NCI

COOPERATING UNITS *(if any)* Dr. A. Keenan, Dr. S.M. Larson, LHM, 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; Dr. J. Mulshine, NCI-NMOB, DCT.

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Theoretical Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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. The approach was extended to include endoscopic techniques for reaching lymph node groups not accessible by subcutaneous injection. 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 and cutaneous T-cell lymphoma (CTCL). Similar protocols have been approved for breast carcinoma, Hodgkin's disease, small cell lung carcinoma, and non-small cell lung carcinoma. Our studies of CTCL have produced the most efficient antigen-specific imaging yet achieved in humans by any techniques.

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 #Z01CB08368-2 Selective Cytotoxicity in the Lymphatics). Our longer term aim is to understand the pharmacology of monoclonal antibodies and other ligands in order to develop criteria for rational molecular design of biological antitumor agents.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08363-05 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

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

H. Robert Guy, Ph.D.	Senior Staff Fellow	LTB, NCI
Other Professional Personnal:		
Robert Jernigan, Ph.D.	Theoretical Physical Chemist	LTB, NCI
David Covell, Ph.D.	Expert	LTB, NCI
James Ferretti, Ph.D.	Physical Chemist	H IR CH
Candice Pert, Ph.D.	Pharmacologist	M BPB

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	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.)*

The primary goal of this project is to develop methods to predict structures of proteins from their sequences and available experimental data and to use these methods to develop structural models of specific membrane proteins. Previously developed models of the action potential sodium channel and the voltage dependent anion channel (VDAC) of mitochondrial membrane were improved and refined and methods were expanded to include peptides that act as T-cell antigens and that interact with HIV receptors.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08365-05 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Prediction of T-cell Antigenic Sites from the Primary Sequence

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

James L. Cornette, Ph.D.	IPA Investigator	LTB, NCI

Other Professional Personnel

Hanah Margalit, Ph.D.	Visiting Fellow	LTB, NCI
John L. Spouge, M.D., Ph.D.	Visiting Associate	LTB, NCI

COOPERATING UNITS *(if any)*

Jay A. Berzofsky, Metabolism Branch, DCBD, NCI;
Charles DeLisi, Director for Health & Environmental Research, U.S. Dept. of Energy

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.9	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have developed an algorithm to predict protein antigenic sites recognized by T lymphocytes, based on the finding that a majority of immunodominant T-cell sites tend to form amphipathic α-helices. We have conducted a thorough examination of hydrophobicity scales and computational procedures useful in detecting potential amphipathic helices. We also have developed a powerful statistical procedure based on Monte Carlo methods that evaluates physical-chemical properties proposed to be characteristic of T-cell antigenic sites. The computer program implementing the procedure compares any proposed property as expressed in the known T-cell sites with its expression in comparable randomly chosen sites. Other characteristics of known T-cell sites with potential predictive value are being sought. The methods developed are not unique to T-cell antigenic sites, but are useful in identifying physical-chemical characteristics of many biomolecular features (antibody binding sites, enzyme active sites, DNA promoter regions, for example). [See also report Z01 CB 04020-09 MET of Jay A. Berzofsky, who initiated many of the questions and with whom this work is closely coordinated.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08366-04 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

COOPERATING UNITS *(if any)*

Dr. L. Liotta, LP, DCBD; Dr. S.M. Larson, NM, CC; Dr. B. Bunow, LAS, DCRT

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Theoretical Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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. 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. (5) Most interesting, antibodies with low affinity may be preferable to those with high affinity for some therapeutic applications.

We plan to test predictions of the model using micrometastases of human melanoma in nude mice. 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

Z01 CB 08367-04 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

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

Other Professional Personnel:

John N. Weinstein, M.D., Ph.D.,	Senior Investigator	LTB, NCI
Anne Lewis, M.S.	Biologist	LTB, NCI
Mary Jane Talley, B.S.	Biologist	LTB, NCI

COOPERATING UNITS *(if any)*

Drs. R.J. Parker and S.M. Sieber., OD, DCE, NCI; Dr. O.A. Gansow and R.W. Atcher., COP, DCT, NCI; Drs. R.A. Kroczek and E.M. Shevach., LI, NIAID

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Theoretical Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	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 08359-03 LTB). This finding prompted us to attempt specific therapy using monoclonal antibody conjugates.

An immunotoxin made from the A-chain of ricin coupled to an anti-mouse MHC antibody has proved to be highly toxic for lymphoid cells and similar results have been found with ricin A-chain conjugated to monoclonal antibodies against subsets of mouse lymphocytes in vitro. We have also been able to augment the effects of these toxins by the action of certain drugs which are known to affect cell biological processes. These studies have led to a new hypothesis for the cell biological pathway (the "neutral bypass") by which toxin molecules enter the cytoplasm from antibody conjugates to kill a cell.

A further type of immunoconjugate for specific cell killing in vivo consists of a radioactive compound chelated to an antibody. We have demonstrated selective ablation of lymph node B lymphocytes in mice injected subcutaneously with an anti-murine B cell antibody labeled with the alpha particle emitter ^{212}Bismuth. The relative potency of this conjugate for B cells in vivo was 10-fold higher than for T cells taken from the same nodes.

In order to assess the effects of antibodies and antibody conjugates in vivo, we have established two models of lymph node T cell activation. In one, the stimulus is the plant lectin concanavalin A administered into the footpad; the other stimulus is allogeneic cells. Both of these stimuli induce T cells to express receptors for IL-2. The concanavalin A model is susceptible to the inhibitory effects of cyclosporin A whereas the allogeneic model is not.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08369-03 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Image Processing Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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

B

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

The object of this part of the program is to make available to the general biomedical community the various microscope control and image acquisition /analysis procedures developed within the section. The plan is based on the quantitative microscopist having available to him as a minimum system a PC/AT (or a fully compatible MS-DOS machine) with a minimum of 512 Kb of memory and a standard 20 megabyte hard disk. All interfacing with the microscope will be accomplished via commercially available interface cards which have the appropriate analog and digital signal I/O. Image acquisition hardware will also be of the commercial type. We plan to place in the public domain a series of source programs, written in C (for maximum portability in the PC world) which will allow incrementally more varied and complex functions to be performed on images acquired under computer microscopic control. The procedures which the user can employ will depend upon how much additional resources he has available beyond the above noted minimum. Functionally speaking, the programs for control, will for example, apply equally well to inverted microscopy (opening up tissue cultures to controlled image acquisition). Liberal provision is being made in the software for control of additional stepping motor functions which could be user defined (e.g. condenser focus, flow of liquids into and out of a chamber, etc). It is planned to include the possibility for interactive image processing, employing a PC compatible high resolution display, with some gray level capability. This and other options are available as a function of user interest and the level of resources available on his PC.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08370-04 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

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:

David Covell, Ph.D. — Expert — LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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.)*

Compact conformations are generated on a lattice by restricting the extent of the chain as it is being generated, in each direction. This procedure enormously reduces the number of conformations, especially when the lattice is densely populated. The intention is to generate most compact conformations and to assess their quality in a general way, based upon what has been observed in globular protein crystal structures. This effectively leads to a "fuzzy" look at protein conformations, with the major features but not all the details. We will use effective residue-residue interaction energies statistically derived from protein X-ray structures. The way these were obtained was: 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. These energies will be used to assess the qualities of lattice generated conformations. If the procedure yields appropriate conformations, then they can be refined subsequently with energy calculations that include all atoms.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08371-04 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

Akinori Sarai, Ph.D., Visiting Associate LTB, NCI
Bruce Shapiro, Ph.D., Computer Specialist IPS, LTB, NCI

COOPERATING UNITS *(if any)*: Dr. Ruth Nussinov, Institute of Molecular Medicine, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; Dr. Jacob Mazur, Program Resources, Inc., Frederick, MD

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	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.)*

Conformations of DNA span a wide range, from the usual Watson-Crick B-form helix to the left handed Z-form. Recently it has become clear that there is a broad spectrum of conformations possible. Specifically there are sequence dependent variations in the vicinity of the B-form. It is interesting to consider whether or not these variations are large enough to affect recognition processes. We have investigated solvent effects on the DNA conformations and have found that some specific sequences exhibit smooth bends and some others give sharp bends. Experimentally these bent DNA's exhibit anomolously slow migration in gel electrophoresis and would yield a higher apparent molecular weight. We obtain good fit to a set of experiments on the sequences $(VA_4T_4X)_i$, $(V_2A_3T_3X_2)_i$, $(VT_4A_4X)_i$, and $(VA_3T_3X)_i$, where V and X are G or C. The models we have constructed for these sequence yield a broad super-helix of radius 120 A for i = 25, a slightly smaller radius super-helix, a very tightly coilecd super-helix, and a nearly straight rod. The moment of inertia about the smallest principal axis of each of these conformations appears to be closely related to the apparent molecular weight.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08372-04 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

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

Akinori Sarai, Ph.D.,	Visiting Associate	LTB, NCI
Other Professional Personnel:		
Robert Jernigan, Ph.D.,	Theoretical Physical Chemist	LTB, NCI

COOPERATING UNITS *(if any)*

Yoshinori Takeda, Ph.D., Program Resources Inc., Frederick, Md.	LTB, NCI

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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 binding of Cro repressor to the synthetic operator DNA has been studied by systematic base substitutions. From this data we deduce specific interactions between Cro and the operator DNA and estimate the energetic contribution of each interaction to the specific binding. Such studies clearly show that the recognition of specific DNA sequences by Cro repressor is mediated by a combination of bi-dentate H-bonds between amino acid side chains and base pairs, and hydrophobic interactions with thymine's methyl groups as exposed within the DNA major groove. Losses of such H-bond or hydrophobic interactions reduce the binding free energy by 0.9 to 3.1 Kcal/mol or 0.8 to 1.6 Kcal/mol, respectively. The free energy changes are principally additive for the specific binding, but not additive for nonspecific binding. These interactions described here are not only the specificity determinants in the sequence recognition, but also provide a large part of the binding free energy for the specific interaction of Cro repressor with DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08374-03 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Membrane Fusion: Structure, Topology, and Dynamics of Tight and Gap Junctions

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

Pedro Pinto da Silva, Ph.D., Chief, Membrane Biology Section, LTB, NCI

Other Professional Personnel:

Kazushi Fujimoto, Ph.D, Visiting Fellow LTB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Membrane Biology Section

INSTITUTE AND LOCATION: NCI, FCRF, Frederick, MD 21701

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 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 studied the dynamics of in vitro proliferation of tight junction strands in rat small intestine epithelia. The structure of the strands is reanalyzed in our preparations and in published micrographs. Our aim is to advance further a general model for the structure of the tight junction that explains present morphological data and accounts for permeability characteristics of diverse epithelia. The work is both experimental and analytical. To this end we induce the massive in vitro assembly of junctional strands by incubation of excised tissue (rat prostate, rat small intestine, toad bladder) in a variety of buffers at 37°C. The tissues are then freeze-fractured, replicated and examined. Detailed morphological analysis includes the examination of stereo-pairs and comparison of the strands of tissues exposed to conditions that may lead to alteration of the strand itself (lipid perturbers, solvents, chelating agents). Results from the extensive literature on junctions are also being examined in detail.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08375-03 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

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

Pedro Pinto da Silva, Ph.D. Chief, Membrane Biology Section, LTB, NCI

Other Professional Personnel:

Maria L.F. Barbosa, Ph.D., Senior Staff Fellow, LTB, NCI
Catarina. Forsman, Ph.D., Visiting Fellow, LTB, NCI

COOPERATING UNITS *(if any)*

Dr. J. Chevalier, Dr. D. Appai, & Dr. J. Bariety, Department of Renal and Vascular Pathology, Broussais Hospital (INSERM/unit 28), Paris, France.

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Membrane Biology Section

INSTITUTE AND LOCATION: NCI, FCRF, Frederick, MD 21701

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.)*

Fracture-permeation is a new technique developed in our laboratory to probe the compactness of the cytomatrices in glutaraldehyde-fixed cells. In fracture-permeation, macromolecules of known size are used to assess the mesh of a chemically-fixed matrix. Fracture-permeation of rat cardiac muscle revealed details of the organization of mitochondria, in particular variations in the compactness of the mitochondrial matrix. In other experiments we used fracture-permeation with cationized ferritin to assess the intermolecular spacing in the extracellular matrices of rat kidney glomeruli: the basement membrane and the mesalgyal matrix. We have also devised a variant of the above permeation technique where glutaraldehyde-fixed tissue was minced instead of freeze-fractured. This alternative method duplicated the results of the fracture-permeation technique establishing the distribution of intermolecular spaces in sarcomeres of striated muscle and the structure of the mitochondrial matrix.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08376-03 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Fracture-Label: Cytochemistry of Freeze-Fracture Cells

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

Pedro Pinto da Silva, Ph.D. Chief, Membrane Biology Section, LTB, NCI

Other Professional Personnel:

Maria L.F. Barbosa, Ph.D., Senior Staff Fellow, LTB, NCI
Catarina. Forsman, Ph.D., Visiting Fellow, LTB, NCI

COOPERATING UNITS *(if any)* Dept. of Renal and Vascular Pathology, Broussais Hosp. (INSERM/ unit 28), Paris, France (J. Chevalier, D. Appai, J. Bariety); Institute of General Pathology, Univ. of Rome School of Med., Rome, Italy (M.R. Torrisi, A. Pavan); Dept. of Anatomy, Univ. of Montreal School of Med., Montreal, Canada (F.W.K. Kan)

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Membrane Biology Section

INSTITUTE AND LOCATION: NCI, FCRF, Frederick, MD 21701

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.)*

We used preparative freeze-fracture to crush glutaraldehyde fixed tissues after impregnation in cryoprotective solution. The fragments were thawed and labeled with wheat germ agglutinin permeated with concanavalin A. They were also fracture-permeated with native ferritin and with cationized ferritin (see Project Number Z01 CB 08375). In autonomic ganglia, primary sensory ganglia and peripheral nerves, fracture-label provides access to all plasma and intracellular membranes as well as extracellular matrices. Con A, WGA and cationized ferritin intensely labels the basal membrane. Furthermore, con A and WGA appears to be localized to synaptic vesicles and the synaptic complex.

We worked also with rat glomeruli where freeze-fracture was used as a means to get generalized access to the cell surfaces. This new approach by-passes successive permeability barriers (endothelial cells, basement membranes) that had to be passed in previous cytochemical studies of the glomerulus. In human lymphocytes we used monoclonal anti-T3 and anti-T4 antibodies to study the partition of the transmembrane proteins which contain these antigens. During freeze- fracture, the T4 antigen partitions completely with the protoplasmic half of the membrane, gave an indication of its association with components of the membrane skeleton or of the cytoskeleton.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08377-03 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: The Role of Myoglobin in Oxygen Transport

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. John Jacquez, Department of Physiology & Biostatistics, University of Michigan, School of Medicine; Dr. Paul Ponganis, Scripps Institute of Oceanography, Physiological Research Laboratories, La Jolla, California

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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.)*

We have examined the role of myoglobin to facilitate oxygen diffusion to active mitochondria in skeletal muscle by constructing computer simulation experiments. Steady state mitochondrial oxygen consumption under different conditions of supply PO_2's in a system with and without myoglobin were examined for a one-dimensional slab of tissue. Oxygen consumption by mitochondria was saturable with the mitochondria located in bands at uniform intervals throughout the tissue. Under these conditions, myoglobin provides a measurable increase in oxygen transport for supply PO_2's below 10 torr and diffusion lengths expected for skeletal muscle fibers. We conclude that only under circumstances where hypoxia lowers P_{O_2} below 10 torr does myoglobin begin to provide a measurable increase in oxygen delivery to mitochondria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08378-03 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Label-Fracture

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

Pedro Pinto da Silva, Ph.D., Chief, Membrane Biology Section, LTB, NCI

Other Professional Personnel:

Maria L.F. Barbosa Senior Staff Fellow LTB, NCI

Catarina Forsman Visiting Fellow LTB, NCI

COOPERATING UNITS *(if any)* Dept. of Renal and Vascular Pathology, Broussais Hosp. (INSERM/ unit 28), Paris, France (J. Chevalier, D. Appai, J. Bariety); Institute of General Pathology, Univ. of Rome School of Med., Rome, taly (M.R. Torrisi, A. Pavan); Dept. of Anatomy, Univ. of Montreal School of Med., Montreal, Canada (F.W.K. Kan)

LAB/BRANCH

Laboratory of Mathematical Biology

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.)*

The development and application of label-fracture cytochemistry was pursued. In label-fracture cells fixed or unfixed are labeled and then freeze-fractured. Label-fracture permits the observation in a single, coincident image the distribution of surface receptors and antigens superimposed on conventional Pt/C casts of freeze-fractured membranes. The resolution (5nm attainable) approaches the molecular level. Normal human lymphocytes were label-fractured with monoclonal anti-T3 and anti-T4 antibodies before and after chemical fixation by glutaraldehyde. In this work the experiments are designed to map T3 and T4 antigens on the surface, their relation to intramembrane particles revealed by freeze-fracture and their dynamics during capping. We show that while T3 and T4 glycoproteins are associated to IMP domains they do not constitute a structural subset recognizable as a specific cluster of IMPs after capping.

In other work we are currently studying the lectin-binding sites in neuronal membranes. We used synaptosomal membrane preparations from rat brain and showed binding sites of concanavalin A and WGA-ovomucoid-gold on the surface associated mainly to IMP domains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08379-03 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Multicompartmental Analysis of Calcium Metabolism

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. Alfred Yergey, Lab. Theoretical & Phys. Biology, NICHD

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

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.)*

Abnormal calcium metabolism in adolescent children and postmenopausal women can have devastating consequences. The objective of this study is to elucidate the kinetics of calcum metabolism in normal children and to evaluate disease related changes in calcium metabolism in children and adults. Stable calcium isotopes were administered to children and women of childbearing age and serial samples were obtained for two to four days. Two stable isotopic tracers were used in these studies; one given orally and one given intravenously. The use of two tracers allows direct measurement of several important parameters of calcium metabolism, principally the fraction of calcium absorbed orally and the endogenous fecal excretion. Thermal ionization isotope ratio mass spectrometry was used to measure tracer enrichments in serum, urine, feces and food. The data was used to develop a multicompartmental model of calcium metabolism that better characterized calcium metabolic fluxes between regions of the body.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08380-03 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Structure of Animal Viruses and Cells by Computational Analysis

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

Jacob V. Maizel, Jr., Ph.D.	Chief, Laboratory of Mathematical Biology,	NCI

Other Professional Personnel:

Devjani Chatterjee, Ph.D.	Visiting Associate	LTB, NCI
John Owens	Computer Specialist	LTB, NCI
Ruth Nussinov, Ph.D.	Consultant	LTB, NCI
Lewis Lipkin, M.D.	Chief, Image Processing Section	LTB, NCI

COOPERATING UNITS *(if any)*

Dr. George Vande Woude, Basic Research Program, Litton Bionetics, Inc, Frederick, Maryland

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI/FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	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.)*

Picornaviruses cause diseases typified by polio, colds, hepatitis, and foot-and-mouth diseases. Sequences of these viruses have been examined for relationships among them and to other known and hypothetical proteins. Secondary structures of the RNAs have been found to vary in correlation with pathological and sequence differences.

Adenoviruses are studied with a goal to understanding early events in virus replication wherein the cell's metabolism is subverted to viral functions, and late events during which assembly and morphogenesis occurs. Early viral proteins, whose existence was known from biochemical studies, have been analyzed by comparing their sequences to cellular proteins of known function.

Techniques of biochemistry, virology, electron microscopy and computer analysis are used to study sequences of picornaviruses, adenoviruses, human immunodeficiency viruses and genes. Analyses of proteins and nucleic acids have been developed and implemented. Graphic representations revealing homology, and reverse complementarity are coupled with numerical methods to aid the prediction of secondary structure, splicing, promoters, and recombination in nucleic acid molecules. Programs are developed locally and elsewhere for application on Cray XMP, VAX and graphic workstations to perform sequence analysis and structure predictions. Methods to assess the significance of predictions use Monte Carlo simulations, evolutionary comparisons and biochemical data. Protein secondary structure is being predicted from amino acid sequences. New sequences are compared with computerized databases to detect relationships with known proteins.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08381-04 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Computer Aided Two-Dimensional Electrophoretic Gel Analysis (GELLAB)

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

Peter Lemkin, Ph.D.	Computer Specialist	IPS, LTB, NCI

Other Professional Personnel:

Lewis L. Lipkin, M.D.	Chief, Image Processing Section	LTB, NCI
Morton Schultz	Senior Engineer	IPS, LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Eric Lester, Univ. of Tenn. Medical School; Dr. Peter Sondregger, Univ. Zurich Medical School.

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Image Processing Section

INSTITUTE AND LOCATION

Frederick Cancer Research Facility, Frederick, MD 21701

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 for the analysis of a set of 2D electrophretic gels. It incorporates sophisticaed subsystems for image acquisition and processing, data base manipulation and graphics as well as statistical analysis. It has been applied to a variety of experimental systems in which quantitative and qualitative changes in one or more proteins among hundreds or thousands of unaltered proteins is the basic analytic problem. Keeping track of changes detected using the statistical methods is also a major attribute of the system. It has been applied to helping analyze a set of gels from adult human leukemias as well as axonal proteins synthesized during axonal regeneration. The objective of creating an exportable version of GELLAB (one that will run on reasonably powerful workstation microcomputers — affordable by a laboratory) is being actively pursued.

Much of the time this past year was spent upgrading software and hardware from the TOPS10 envirnment to UNIX - moving our part of the laboratory from Rockville to Frederick/FCRF and in bringing up various elements on the FCRF/LTB-ASCL network.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08382-04 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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)*

Bruce Shapiro, Ph.D.,	Computer Specialist	IPS, LTB, NCI

Other Professional Personnel:

Lewis L. Lipkin, M.D.	Chief, Image Processing Section	LTB, NCI
Peter Lemkin, Ph.D.,	Computer Specialist	IPS, LTB, NCI
Morton Schultz	Senior Engineer	IPS, LTB, NCI
Jacob V. Maizel, Jr., Ph.D.	Chief, Laboratory of Mathematical Biology	NCI

COOPERATING UNITS *(if any)*: Dr. Ruth Nussinov, Institute of Molecular Medicine, Sackler Faculy of Medicine Tel Aviv University, Tel Aviv, Israel; Dr. Jacob Mazur, Program Resources, Inc. Frederick, MD.

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Image Processing Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors B

☐ (a2) Interviews

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

Work on nucleic acid structures has continued to evolve with a variety of collaborations and directions. Among these include DNA structural variations from straight B-DNA. Extensions have been made to the system that detects structural features in DNA and the use of a 2-dimensional version of the system for comparing multiple sequences. Futher studies have ensued related to curved B-DNA in attempting to relate predicted curved sequences to their experimental gel moblility (with Jernigan, Sarai and Nussinov). Energetic calculations are progressing using the Cray which will eventually allow more accurate rules to evolve then the current models suggest for curved DNA.

The DNA structure analysis program has also been enhanced to include rules for DNA melting. Using this and mutation data for procaryotic promoters research was done on how DNA local energy characterics are related to promoter activity.

The one-dimensional structural analysis program mentioned above has also been paritally translated to C to run on the SUN's and/or VAXES. Also, this program now includes the abiltity to generate 3-D coordinates for B-DNA based upon the Calladine/Dickerson rules.

Work is also progressing on a new similarity alogorithm that will both pictorally an quantitatively indicate when RNA secondary structures are similar with also the possibility of developing consensus structures.

Work is accelerating on the development of a nucleic acid expert system which would be directed to both intelligent queries by research and non-ad hoc structuring of relationships that exist between the various software/hardware complexes available at the Frederick Cancer Research Facility.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08384-01 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

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

James L. Cornette, Ph.D. IPA Investigator LTB, NCI

COOPERATING UNITS *(if any)*

Charles DeLisi, Director for Health & Environmental Research, U.S. Dept. of Energy

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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.)*

In steps towards the major goal of sequencing the human genome, several investigators are developing partial maps of chromosomal DNA. Libraries consisting of clones of overlapping DNA fragments exist in several laboratories. The goal is to reconstruct the order of the fragments as they appear in the original DNA, thus obtaining sequences of overlapping fragments that cover large segments of the DNA. The overlapping fragments may be originally obtained by partial digestion of the DNA by a single enzyme or total digestion by two or more enzymes used individually. A critical requirement is that the fragments be of a size appropriate to cloning in some bacterial system. The second step is to identify as many pairs of overlapping fragments as possible; theoretically, if the library covers the chromosomal DNA and all intersecting pairs can be identified, a single sequence of overlapping fragments reaching from one end of the DNA to the other would be obtained. Identification of overlapping pairs may be made by observing a common pattern of restriction fragment lengths when each fragment of the pair is digested with a certain digest. Alternatively, the same subset of a panel of probes (complementary DNA fragments) may bind specified parts of each fragment of the pair. Using a randomly generated sequence as a model DNA, we are evaluating several of these procedures to determine which of them should lead to the highest percentage coverage of the original chromosome, with the greatest accuracy of ordering, and with the fewest number of experimental steps.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08385-01 LTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

DNA Conformations in Control Regions

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

Robert L. Jernigan, Ph.D.,	Theoretical Physical Chemist	LTB, NCI
Hanah Margalit, Ph.D.,	Visiting Fellow	LTB, NCI
Bruce A. Shapiro, Ph.D.,	Computer Specialist	LTB, NCI
John Owens, Ph.D.,	Microbiologist	LTB, NCI

COOPERATING UNITS *(if any)*

Dr. Ruth Nussinov, Institute of Molecular Medicine, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

LAB/BRANCH

Laboratory of Mathematical Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	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.)*

Extracting details of the mechanism of promoter function in transcription has been widely studied. We have chosen to look at one of the simplest aspects of this process, namely, the strength of strand pairing in the promoter region. Specifically, the stability of the DNA double helix was determined in the vicinity of the promoter by computing the free energy for strand separation as a function of dinucleotide free energy values taken from the calorimetric measurements of Breslauer et al. The stability of 224 prokaryotic promoter regions was studied within a window of +/- 250 nucleotides on either side of the mRNA start sites. We found that for this set of promoters the -10 region was significantly the least stable. We also compared the free energies of 121 promoter mutations within the -35 and -10 regions with the free energies of their corresponding wild type sequences and found a correlation between the free energy change and the mutation type in the -10 region. 80% of the mutations in this region with increased/decreased promoter activity were observed to be less/more stable than the wild types.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08386-01 LTB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Analyses of the cDNA Clones for β 1-4 Galactosyltrasferase.

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

Pradman K. Qasba, Ph.D. Research Chemist LTB, NCI

Other Professional Personnel:

Anil Puri, Ph.D. Visiting Fellow LTB, NCI

COOPERATING UNITS *(if any)*: Dr. M. Braun, Fredrick Cancer Research Center, Frederick Maryland

LAB/BRANCH: Laboratory of Mathematical Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

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.)*

A specific glycosyltransferase is required for the synthesis of oligosaccharide moieties of glycoproteins and glycolipids, which are referred to as glycoconjugates. Galactsyltransferases (gal-transf), a subgroup of the transferases, constitute a family of enzymes which transfer galactose from UDP-gal to the non-reducing residues of oligosaccharides of various glycoconjugates as well as to monosaccharides. We have initiated the molecular cloning approach to understand the modulation of the gal-transf activity, essential for generating specific cell-surface antigenic determinants. In our previous studies on the gene structural analyses of alpha-lactalbumin, a modifier protein of gal-transf, we showed that the domain of alpha-lactalbumin that interacts with gal-transf is coded entirely by a separate exon. We have now cloned and sequenced cDNA coding for bovine GlcNAc 1—4 gal-transf. Analysis of several sequence-related cDNA clones showed: 1) There are atleast two chromosomal sequences for 1—4 gal-transf; 2) There are two major classes of mRNAs which share common nucleotide sequences encoding the proteins with the same carboxy-terminal end of 120 residues and share same 3'noncoding sequence. One class of clones encode 1—4 gal-transf protein sequence; 3) The enzyme must be synthesized as a proprotein and secreated as cleaved product which is enzymaticaly active; 4) Sereis of poly(A) sites present in the gene sequence are utilized to generate mRNAs of different sizes which vary in length at the 3'non-coding region; 5) The cDNA clones which have different nucleotide sequence at the 5'end compared to the clones coding for the 1—4 gal-transf, do not encode any protein sequence in any of the three open reading frames suggesting that they may represent unspliced precursor mRNAs; 6) The nucleotide sequence analysis of several related cDNA clones suggest that a complex alternative processing of the precursor mRNA occurs to generate gal-transf mRNAs.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00853-34 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Surgical Pathology

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

PI: M.J. Merino Chief, Surgical Pathology Section LP NCI
OTHER: (see next page)

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Surgical Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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 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. The frozen section and surgical pathology processing area was constructed adjacent to the new operating rooms and has been in use since April, 1983. This new facility has greatly enhanced processing of specimens and communication of diagnostic findings with attending physicians. It is equipped with intercom and television viewing screens in each operating room to facilitate communication.

The staff is actively engaged in a variety of projects involving clinicopathological correlation and pathologic characterization of diseases studied at the Clinical Center. Up-to-date immunocytochemical techniques have been applied to the study of tumors and other non-neoplastic diseases. The use of immunohistochemical staining has greatly facilitated more precise diagnosis in selected cases and with the increasing number of monoclonal antibodies available this technique should have even greater value in diagnostic and research pathology. A major renovation of the histology laboratory is in the planning stage. This will include space allocated for performance of special stains and immunocytochemistry.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09145-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Neuropathology

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

PI: D.A. Katz Neuropathologist OCD NINCDS

COOPERATING UNITS *(if any)*

Surgical Pathology and Postmortem Section, LP, NCI
Ultrastructural Pathology Section, LP, NCI

LAB/BRANCH

Office of the Clinical Director, NINCDS

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

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

As described previously, subspecialty expertise in diagnostic neuropathology is provided to the Laboratory of Pathology, NCI, and to all other institutes, via the Office of the Clinical Director, NINCDS. The neuropathology service is integrated with the Surgical Pathology and Postmortem Section and with the Ultrastructural Pathology Section. Within the Laboratory of Pathology, both diagnostic (patient care) service and teaching (of pathology residents) are provided. The service also functions in a collaborative manner to provide neuropathological expertise in a wide range of clinicopathologic investigations.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09154-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Prognostic Significance of Thymidine Labelling Index in Breast Cancer

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

PI:	M.J. Merino	Chief, Surgical Pathology Section	LP NCI
OTHER:	S. Kennedy	Visiting Associate	LP NCI
	S. Swaine	Senior Staff Fellow	MB NCI
	M. Lippman	Head, Medical Breast Cancer Section	MB NCI

COOPERATING UNITS *(if any)*

Medicine Branch

LAB/BRANCH

Laboratory of Pathology

SECTION

Surgical Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

The main purpose of the study will be to determine whether the thymidine labelling index could provide any significant information regarding prognosis in breast carcinoma, and to what extent the TLI could identify those patients that could have an early relapse. Until now, histologic type of the tumor and steroid receptor status have played an important role in the planning of adjuvant therapy, but we believe that the proliferation characteristics of tumor cells may also be a critical determinant since nuclear DNA contents in populations of breast cancer cells have been proven to have prognostic significance.

The thymidine labelling technique relies on the uptake of titrated thymidine by replicating cells which is then detectable by autoradiography. We will investigate the thymidine labelling index range for different histologic types of breast cancer, tumors of patients that have received radiation and/or chemotherapy; the changes in TLI of residual normal breast tissue and the effect of hormonal manipulation in TLI.

The rapid processing of autoradiographs allows cell kinetic data for clinical evaluation in three days, which may be a useful adjunctive diagnostic tool on selected cases.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00852-34 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	D. Solomon	Pathologist	LP NCI
	Y. Ye	Visiting Fellow	LP NCI
	T.A. Wood	Biologist	LP NCI
	L. Galito	Biologist	LP NCI
	A.M. Wilder	Biologist	LP NCI
	E. Sanders	Bio. Lab. Tech.	LP NCI
	K. Condron	Microbiologist	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Cytopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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 also routinely applies immunocytochemistry techniques to affirm and/or enhance cytological diagnostic efficacy. In addition, the section collaborates in various clinical research projects utilizing special techniques including special staining.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00897-04 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Cytological Diagnosis of Lymphomas by Immunocytochemistry

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

PI:	D. Solomon	Pathologist	LP NCI
OTHER:	E.S. Jaffe	Chief, Hematopathology Section	LP NCI
	Y. Ye	Visiting Fellow	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Cytopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.25	.25

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 cytological diagnosis of malignant lymphoma can be extremely difficult because the cytological features of the malignant cells in small cell and mixed small and large cell lymphomas may be indistinguishable from those of reactive lymphoid cells. We have examined the usefulness of the avidin biotin immunoperoxidase technique and a battery of antibodies to T and B cell markers to the diagnosis of lymphoma in cytological specimens. We conclude that immunocytochemistry is very useful in the cytological diagnosis of non-Hodgkin's lymphoma. Further, it is possible to diagnose the vast majority of lymphomas using only the immunoglobulin light chain markers κ and λ and the T-cell markers Leu-1, Leu-2, and Leu-3.

We are extending the utilization of lymphoid markers to fine needle aspiration specimens of lymph nodes. Fine needle aspiration may obviate the need for repeat biopsies in patients with recurrent lymphoma.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09128-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Cytologic Diagnosis of Carcinoma Cells in Effusions Using Monoclonal Antibodies

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

PI:	D. Solomon	Pathologist	LP NCI
OTHER:	J. Simpson	Expert	LTIB NCI
	Y. Ye	Visiting Fellow	LP NCI
	J. Schlom	Chief, Laboratory of Tumor Immunology and Biology	LTIB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Cytopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.25	.25

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 cytologic diagnosis of metastatic adenocarcinoma in effusions can be very difficult. Not only can malignant cells have very bland cytologic features but reactive mesothelial cells can assume a very atypical appearance. Using the avidin-biotin immunoperoxidase technique on cytospin preparations of pleural and peritoneal effusions, we investigated the reactivity of purified monoclonal antibody B72.3 with benign and malignant effusions. Initial effusion specimens studied were carefully selected to include only cytologically malignant effusions from patients with a history of adenocarcinoma and cytologically benign effusions from patients with no history of adenocarcinoma. Of the 78 malignant effusions studied, 90% of cases demonstrated cells with specific positive staining with B72.3. There was no detectable staining of cells in reactive effusions. We conclude that immunostaining with B72.3 is useful in the cytologic diagnosis of metastatic adenocarcinoma in effusions.

We are now continuing our investigations using other monoclonal pancarcinoma antibodies. We are currently evaluating the efficacy of immunocytochemistry to discriminate between primary peritoneal mesothelioma and metastatic ovarian carcinoma.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09153-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Cytophenotypic Analysis of Tumor Suspensions and TIL Cultures in Immunotherapy

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

PI:	D. Solomon	Pathologist	LP NCI
OTHER:	Y. Yee	Visiting Fellow	LP NCI
	S. Topalian	Fellow	SB NCI
	S. Rosenberg	Chief	SB NCI
	K. Condron	Microbiologist	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Cytopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

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.)*

Clinical trials employing the adoptive transfer of expanded tumor infiltrating lymphocytes to patients with metastatic disease are currently underway under the direction of the Surgery Branch, NCI. Our collaborative effort in this project involves immunocytochemical analysis of tumor cell suspensions to identify (1) the percentage and phenotypic expression of subsets of tumor infiltrating lymphocytes present in the tumor and (2) tumor markers, if any, which are expressed by the tumor cells. Once the tumor infiltraing lymphocyte cultures have been expanded and are to be harvested for patient therapy, we analyze the material using routine cytologic preparations and immunocytochemistry to ensure the cultures are free of tumor cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00545-09 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

NCI, NIH, Bethesda, MD 20892

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.

This project is now complete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00874-05 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

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, the so-called Askin tumor, which in reality is a form of peripheral neuroepithelioma. 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.

This project is now finished.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09125-04 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	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 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 and the closely related chest wall tumor described by Askin et al., the so-called Askin tumor. The break point on chromosome 22 is close to a known oncogene, c-sis. No amplification or rearrangement of c-sis has been detected. In the case of peripheral neuroepithelioma, c-sis is not amplified, but c-myc is. Unlike classic neuroblastoma, N-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.

This project is complete.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09137-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Ewing's Sarcoma: Differentiation 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.O. Cavazzana	Guest Researcher	LP NCI
	S.J. Mims	Biologist	LP NCI
	J.A. Jefferson	Biologist	LP NCI

COOPERATING UNITS *(if any)*

Dr. Samuel Navarro, U.S.-Spain Cooperative Agreement

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2 1/2	1 1/2	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 histogenesis of Ewing's sarcoma remains enigmatic, despite much work to elucidate its origins. We have assumed that Ewing's sarcoma, in its usual state of differentiation, lacks any specific features of known childhood tumors. Certain lines of evidence from other studies, such as the presence of a reciprocal (11:22) chromosomal translocation in Ewing's sarcoma and peripheral neuroepithelioma, and similar patterns of reactivity with panels of monoclonal antibodies, have suggested a possible common histogenesis for these otherwise dissimilar tumors. Since neural tumors in general are known to respond to differentiating agents such as dibutyryl cyclic AMP, nerve growth factor, and retinoic acid by developing features of differentiated neural tissues such as neurites and increased numbers of dense core granules, we have treated a series of Ewing's sarcoma tumor cell lines in vitro with these agents under a variety of conditions, alone and in conjunction with one another.

To date, the initial results strongly suggest that at least those tumors which are successfully grown in vitro are intrinsically capable of neural differentiation in response to treatment with these agents. Four of four lines so studied (and reported previously to lack any spontaneous evidence of neural differentiation, even after year of growth in vitro) responded by producing long, slender processes in culture. Ultrastructural examination of these processes revealed dense core granules. Immunocytochemistry with antisera to neuron-specific enolase, an antigen found in neural tissue, was negative prior to treatment but positive afterwards in all four lines. These initial results are being confirmed with other techniques, including catecholamine fluorescence, neurotransmitter enzyme profiles, extracellular matrix synthesis studies, and patterns of monoclonal antibody reactivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09138-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

In situ Hybridization Studies of N-myc Expression by Neuroblastoma

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

PI:	P. Cohen	Clinical Associate	PB NCI
OTHER:	R. Seeger	Physician in Chief, Pediatric Hem.-Oncol., Univ. of Southern California	
	M. Israel	Sr. Attending Physician	PB NCI
	T.J. Triche	Chief, Ultrastructural Pathology Section	LP NCI

COOPERATING UNITS *(if any)*

Department of Pediatric Hematology-Oncology, USC, Los Angeles, CA

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2 1/2	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.)*

Neuroblastoma, alone among extra-CNS childhood tumors, has been shown to express a unique proto-oncogene, N-myc. Further, elevated expression and/or amplification of this oncogene has been correlated with adverse clinical course; no stage I or II patients express the gene abnormally, while over 50% of stage III and IV patients do. Current work indicates these patients fare especially poorly. Nonetheless, no work to date has attempted to correlate the expression of N-myc by individual tumor cells with stage and outcome. Bulk techniques employed to date cannot distinguish a small population of N-myc expressor tumor cells admixed with non-expressors, yet such patients may prove to have a prognosis equally adverse as those with high levels of N-myc expression. This might be the case with the N-myc negative stage III and IV patients reported to date.

The present study will examine N-myc expression as DNA copies, RNA transcripts and N-myc protein as detected by in situ hybridization with radiolabelled DNA frgments of the N-myc gene, transcribed in vitro and hybridized to frozen sections of approximately 80 tumors provided by one of us (RS). Likewise, N-myc protein will be detected immunocytochemically by antibodies specific for oligopeptide regions of the N-myc protein product. The incidence of positive tumor cells and their morphology will be assessed in each case, and upon completion of the study, the identity, stage, and status of each patient will be correlated with N-myc expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09139-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Monoclonal Antibodies to Ewing's Sacoma

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
	S. Arakawa	Fogarty Fellow	LP NCI
	C.P. Reynolds	Tissue Transplantation Unit	NMC NCR

COOPERATING UNITS *(if any)*

Tissue Transplantation Unit, NMC, NCR

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

This is a new project, just commencing. Extensive studies of Ewing's sarcoma and related round cell tumors of childhood have served to distinguish most from one another on the basis of positive reactivity with one or more antibodies. No antibody specific for Ewing's sarcoma, or restricted to a few tumors including Ewing's, has been identified to date. It is the aim of the present study to produce a battery of monoclonal antibodies against selected, well-characterized Ewing's sarcoma lines established in this laboratory. They will then be studied and compared in their reactivity with other monoclonal antibodies previously reported. Any antibody found to be strongly reactive with Ewing's sarcoma cells will be further characterized, with the eventual intention to use same as a potential diagnostic, imaging, and therapeutic tool.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09140-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

A New High Molecular Weight Extracellular Matrix Protein

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:	S. Scarpa	Visiting Fellow	LP NCI
	P.U. Reddy	Visiting Fellow	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	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 the course of studying extracellular matrix synthesis by various childhood tumors, we have encountered a previously unidentified, high molecular weight (500,000 D) protein secreted into the conditioned medium of several tumor cell lines. This protein is secreted in connection with laminin, type IV collagen, and fibronectin, but is immunologically distinct therefrom. It is also non-reactive with antibodies to basal lamina proteoglycan. It fails to label with radiolabelled sodium sulfate or glycosyl precursors, which further distinguishes it from conventional proteoglycans. Selective enzymatic degradation studies indicate that the protein is trypsin and pepsin sensitive, but collagenase and GAG degrading enzyme insensitive. Ultrastructural studies of rotary shadowed, purified molecules reveal a single, unbranched chain of over 700 nm length. The molecule co-purifies with laminin and type IV collagen under normal circumstances. Current efforts are aimed at devising preparative purification techniques which will allow purification of quantities sufficient to initiate the generation of monoclonal antibodies and biologic function studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09160-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

N-myc Expression in Small Round Cell Tumors of Childhood

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

COOPERATING UNITS *(if any)*

C.P. Reynolds, D.J. Slamon, and R.E. Seeger, UCLA

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

N-myc oncogene expression is an important factor in neuroblastoma prognosis and biologic behavior. N-myc has been sporadically reported in rare other childhood tumors. Further antibody detection methods have recently become available. We have used a rabbit polyclonal antibody to detect the N-myc protein in tissue sections of the common tumors of childhood. We have further correlated these results with conventional Northern, Southern, and Western analysis of examples of each of these tumors. We find no evidence of N-myc amplification, expression, or protein accumulation in any of these tumors save neuroblastoma.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09162-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Nude Mouse Growth, Cytogenetics and Oncogene Expression in Rhabdomyosarcoma

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

PI:	M. Tsokos	Expert	LP NCI
OTHER:	G. Kouraklis	Visiting Fellow	LP NCI
	T.J. Triche	Chief, Ultrastructural Pathology Section	LP NCI

COOPERATING UNITS *(if any)*

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SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	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.)*

There is cumulative clinical evidence that alveolar (A) rhabdomyosarcoma (RMS) carries a more aggressive biologic behavior than embryonal (E) RMS. On the other hand, the histologic distinction of one from another is not always clear, especially when an overt alveolar pattern is absent. The present project aims at: (1) establishing a model to test biologic aggressiveness of A vs E RMS and (2) defining parameters of biologic aggressiveness in these tumors.

(1) Six human RMS cell lines were transplanted into nude mice. Two cell lines were classified as E RMS, two as A RMS and two as solid alveolar (SA) RMS. Tumor growth was measured weekly for a total of 5 weeks. Animals bearing tumors were sacrificed weekly after 3 weeks, and the tumor tissue was processed for routine histology and electron microscopy.

(2) Chromosome analysis of the cell lines was carried out on samples which had been passaged within 24 hours (logarithmic growth phase). Patterns of proto-oncogene (c-myc and N-myc) expression in the above RMS cell lines were examined by Northern blot analysis of total cellular RNA.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00523-08 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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:	J. Cashel	Biologist	LP NCI

COOPERATING UNITS *(if any)*

D. Smith, Associate Professor, Virginia Polytechnic Institute, Blacksburg, VA;
J. Smith, Ortho Diagnostics

LAB/BRANCH

Laboratory of Pathology

SECTION

Biochemical Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	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.)*

Carbohydrate chains released by trifluoroacetolysis of 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00525-08 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

COOPERATING UNITS *(if any)*

L. Jarrett, Professor, Department of Pathology, University of Pennsylvania; Dr. J. Mato, Department of Metabolism, Nutrition, and Hormones, Institute for Biomedical Investigation, Madrid, Spain

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SECTION

Biochemical Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.1	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.)*

Separation of reduced and permethylated oligosaccharides by gas chromatography can be facilitated by the use of a fused silica capillary column 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.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00556-05 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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. Duk	Visiting Fellow	LP NCI

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

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.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00879-04 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Nucleotide Sequencing of Hybridoma Antibodies of V_H-GAC Family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboretory, 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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SECTION

Biochemical Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

Antibodies of the J606 murine V_H family, GAC (group A-streptococcal carbohydrate) (GAC-related V_H genes), have been seen to derive in normal mice from at least $3V^H$ gene segments (of a locus whose complexity is 10-12). Two of these are GAC-related, one inulin related. In CBA/N mice, which do not respond to either GAC or inulin by conventional immunization methods, antibodies of the J606 V_H family origin were found to constitute about 5% of their B cell V_H pre-immune repertoire. The extent of expression of the members of the J606 V_H family has been examined by nucleotide sequencing of hybridoma derived V_H J606 genes from their purified mRNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09155-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Analysis of Complex Carbohydrates by Affinity Chromatography

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:	M. Duk	Visiting Fellow	LP NCI
	W. Wang	Visiting Fellow	LP NCI
	J. Fernandez	Bio Lab Tech	LP NCI

COOPERATING UNITS *(if any)*

A. Lundblad, Head Physician, University of Lund, Sweden; J. Dakour, Predoctoral Student, University of Lund, Sweden

LAB/BRANCH

Laboratory of Pathology

SECTION

Biochemical Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	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.)*

Monoclonal antibodies coupled to solid supports provide a suitable matrix for rapid, specific chromatographic separation of oligosaccharides. Thus, antibodies that define or recognize carbohydrate determinants such as blood group-related or cancer-associated antigens can be used to identify and purify their targets. New approaches are being sought to use antibodies of relatively low affinity to analyze a spectrum of biologically interesting carbohydrates.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00559-05 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 & Metastases Section	LP NCI
OTHER:	U. Wewer	Visiting Fellow	LP NCI
	C.N. Rao	Visiting Associate	LP NCI
	I.M.K. Margulies	Biologist	LP NCI

COOPERATING UNITS *(if any)*

Laboratory of Developmental Biology and Anomalies, NIDR

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Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

The overall goal of the research has been to identify and characterize biochemical mechanisms involved in the interaction of metastatic tumor cells with the extracellular matrix. A major objective has been to analyze the events which take place at the interface between the tumor cell and the basement membrane during attachment.

Accomplishments

1. Laminin, a glycoprotein of basement membranes, was shown to play a major role in metastatic tumor cell attachment.
2. The cell surface laminin receptor was purified and partially sequenced.
3. Monoclonal antibodies and polyclonal antibodies were prepared against the human breast cancer laminin receptor. These antibodies have provided new information about the distribution and function of the receptor. The antibodies were also used to screen a cDNA expression library to isolate a putative cDNA clone from the human gene coding for the receptor.
4. The binding domain on the receptor and the ligand were identified. Additional functional domains were located on the ligand and a model of the attachment interface was developed.
5. A fragment of the laminin ligand was produced which blocks the receptor and markedly inhibits or abolishes hematogenous metastases in a nontoxic fashion in animal models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00891-04 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Stimulated Motility in Tumor Cells

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

PI:	E. Schiffmann	Research Chemist	LP NCI
	L. Liotta	Chief, Tumor Invasion & Metastases Section	LP NCI
OTHER:	M. Stracke	Biotechnology Fellow	LP NCI
	R. Guirguis	Guest Researcher	LP NCI
	I.M.K. Margulies	Biologist	LP NCI

COOPERATING UNITS *(if any)*

Robert Bassin, Chief, Cellular and Molecular Physiology Section, LTIB, NCI; Joel Moss, LCM, NHLBI; B.F. Sloane, Department of Pharmacology, Wayne State Univ., Detroit, Michigan; Brian Liu, UCLA, Los Angeles, California; D. Salomon, DCBD, NCI

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Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

B

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

Since locomotion is essential for metastasis of tumor cells, we are studying the biochemical nature of this process. Human melanoma and breast cancer cells produce in culture proteins (~ 55 KDa) that stimulate motility in the producer cells - autocrine motility factors (AMF). These have been purified in procedures involving sequential gel filtration, hydrophobic interaction and ion exchange HPLC. Studies on the mechanism of action of AMF have identified a pertussis-sensitive G protein pathway as a requirement for motility and exclude the adenylate cyclase system from having a direct role in cell motility. Requirements for extracellular Ca^{++} and for lipoxygenase metabolites of arachidonic acid were suggested in studies with murine tumor cell lines. Investigations of early morphological events in tumor cell locomotion have indicated that pseudopod formation may be necessary for initiating motility. Pseudopod formation appears to be independent of attachment mechanisms. Pseudopodia are enriched in receptors for matrix components and cytoskeletal proteins, suggesting a sensory role for them in cell locomotion. In exploratory studies on clinical applications of cell motility, we have found that urine samples from patients with urinary tract cancers contain motility factors whose potency is well correlated with the aggressiveness of the tumors. There may well be a family of motility factors with a common active site. This point and the role of motility factors in metastasis would be clarified when we have achieved one of our principal objectives: cloning the gene for AMF.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00892-04 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	P.S. Steeg	Biotechnology Fellow	LP NCI
OTHER:	M.E. Sobel	Senior Investigator	LP NCI
	G. Bevilacqua	Guest Researcher	LP NCI
	L.A. Liotta	Chief, Tumor Invasion & Metastases Section	LP NCI

COOPERATING UNITS *(if any)*

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Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	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 molecular biology of tumor metastasis has been investigated. A cDNA clone, pNM23, has been identified which recognizes specific RNAs present to a greater degree in low metastatic K1735 murine melanoma cell lines than in related, highly metastatic K1735 melanoma cell lines. The pNM23 cDNA clone has been tested in three additional animal experimental metastases systems: rat nitrosomethylurea-induced mammary carcinomas, murine mammary tumor virus-induced mammary carcinomas and rat embryo fibroblasts transfected with ras + adenovirus Ela. In each case, NM23 RNA levels were inversely correlated with metastatic potential. Preliminary experiments suggest that NM23 RNA levels are also differentially expressed in human breast cancer. Approximately 750 bp of the NM23 gene have been isolated and are being characterized, with the eventual goal of transfecting the full length NM23 gene into highly metastatic cells. Computer analysis of the 3' 600 bp of the NM23 cDNA indicate that it is a novel gene. The data identify a new gene which is associated with the metastatic process. Further, the NM23 gene demonstrates that tumor metastasis is not only associated with the acquisition of invasive and other traits, but may also involve the loss of other cellular functions. Experiments underway will determine the diagnostic and therapeutic potential of this finding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00893-04 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	L.A. Liotta	Chief, Tumor Invasion and Metastases Section	LP NCI
	H. Krutzsch	Expert	LP NCI
	R.J. Muschel	Senior Investigator	LP NCI

COOPERATING UNITS *(if any)*

R. Pozzatti (Fellow), LMV, NCI; S. Garbisa, Padova Institute of Histology, Italy

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Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

Activated ras oncogene transfection into suitable recipient cells has been shown to induce the metastatic phenotype (Thorgeirsson et al., Mol. Cell Biol. 5: 259-262, 1985). With R. Pozzatti and G. Khoury, we extended this work to ras^H transformed rat embryo cells. These cells were also highly metastatic when transformed either by ras^H or by ras^H linked to an enhancer. However, when the cooperating oncogene Ela was used in conjunction with ras^H, metastasis was only rarely seen. We have used this model system to study the correlation of basement membrane collagenolysis with metastatic propensity. The c-Ha-ras oncogene alone, or combined with v-myc, transfected into early passage rat embryo fibroblasts, induce these cells to secrete high levels of type IV collagenolytic metalloproteinase and to concomitantly exhibit a high incidence of spontaneous metastases in nude mice. Cotransfection of c-Ha-ras plus the adenovirus type 2 Ela gene yields cells which are highly tumorigenic but nonmetastatic and fail to produce type IV collagenase. This effect is due to a suppression of collagenase elaboration, not increased production of a collagenase inhibitor, and not decreased production of a collagenase activator. The characteristics of the collagenase are identical to tumor type IV collagenase described previously. The nonmetastatic cells which failed to produce type IV collagenase retain the ability to secrete high levels of plasminogen activator. Transfection with the proto-oncogenic forms of Ha-ras or mos, or spontaneous transformation of NIH 3T3 cells or chemical transformation of BALB 3T3 cells yields cells which fail to produce collagenase, are tumorigenic, but totally nonmetastatic. These data support a biochemical linkage of type IV collagenase expression with the metastatic phenotype in this rodent system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08266-07 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	L.A. Liotta	Chief, Tumor Invasion & Metastases Section	LP NCI
OTHER:	C.N. Rao	Visiting Associate	LP NCI
	G. Taraboletti	Visiting Fellow	LP NCI
	I.M.K. Margulies	Biologist	

COOPERATING UNITS *(if any)*

D. Roberts, Senior Staff Fellow, NIDDKD; K. Tryggvasson, Oulu, Finland; D. Eppstein, Syntex, Inc.

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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. Type IV collagen is a rope-like structure (360 nm) with a globular domain at the carboxyterminal end and a disulphide-rich amino terminal end. A major binding site for laminin is identified at about 100 nm away from the globular end of type IV collagen. The binding domain on laminin for its receptor has now been isolated using protease treatment of laminin. A monoclonal antibody is shown to recognize this domain and block the binding of laminin to the receptor. A complete model has been developed for the orientation of the cell surface laminin receptor, laminin itself, and type IV collagen in the basement membrane.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09127-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Effect of TPA on Type IV Collagenolytic Activity in Normal and Neoplastic Cells

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

PI:	M. Ballin	Visiting Fellow	LP NCI
OTHER:	U.P. Thorgeirsson	Expert	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

Diversified effects of the tumor promoter, TPA, on type IV collagenolytic activity, was observed in fibroblasts at different stages of malignant transformation. Normal human diploid lung fibroblasts were not affected by TPA, but immortalized aneuploid mouse fibroblasts, NIH/3T3, expressed up to 400% increase in type IV collagenolytic activity, and a fibrosarcoma cell line, HT1080 expressed up to 50% increase in the presence of TPA. The collagenolytic activity of the normal fibroblasts was not dependent on the stage of confluency.

These results suggest that certain cellular changes, possibly related to the initiation step in the carcinogenic process, are necessary for the induction of type IV collagenolytic activity by the tumor promoter TPA.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09130-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Laminin Receptor in Breast Tissue, Benign and Malignant Tumors

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

PI:	G.J. Bryant	Expert	LP NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion & Metastases Section	LP NCI
	C.N. Rao	Visiting Associate	LP NCI

COOPERATING UNITS *(if any)*

A. Schwartz, George Washington University Hospital, Washington, D.C.;
R.R. Brentani, Ludwig Institute for Cancer Research, Sao Paulo, Brazil;

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.8	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.)*

Laminin, a major glycoprotein of basement membranes, exhibits saturable binding to the surface of certain neoplastic and normal cells. Examination of various cell types (i.e. human pancreatic carcinoma, melanoma and bladder carcinoma) via live cell binding techniques indicate the number of receptors to be 50-110,000 per cell depending on cell type. In our laboratory, the laminin receptor has been isolated from human breast carcinoma cells and tissue and mouse melanoma cells. The receptor and subsequent ligand binding have been implicated to play an important role in tumor cell attachment, one of the steps in tumor invasion and metastasis. The proposed study is designed to measure and correlate laminin receptor binding capacity of breast tissues with clinical information. Laminin receptor binding capacity markedly differs between benign, malignant and normal human breast tissue. Preliminary studies indicate a 50-fold increase in specific laminin binding activity in malignant versus benign breast tissues. These findings suggest a site for possible intervention in the sequence of tumor invasion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09131-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Cloning of Connective Tissue Matrix Molecules

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:	C.N. Rao	Visiting Associate	LP NCI
	A. Mackay	Visiting Fellow	LP NCI
	U. Wewer	Visiting Fellow	LP NCI
	M. Agelli	Breast Cancer Task Force Fellow	LP NCI
	A.P. Claysmith	Biologist	LP NCI
	F. Highsmith	Stay-in-School	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

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NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.5	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 interaction of the tumor cell with its extracellular matrix may play an important role in determining its metastatic and invasive properties. To better understand the protein components that make up the extracellular matrix, their regulation and how they interact with the tumor cell, we have undertaken to construct, isolate, and characterize molecular clones of laminin receptor and of several different collagens. Laminin receptor is a cell surface protein to which laminin (a major component of basement membrane) specifically binds with high affinity. We previously isolated a human laminin receptor cDNA which encoded the carboxy-terminal half of the protein and showed that laminin receptor mRNA in the tumor cell is a rate-limiting control step in the biosynthesis of the receptor, and hence in the regulation of cellular attachment to basement membranes via laminin. During the past year, we have extended our sequence analysis of the laminin receptor gene. In particular, we have obtained more amino terminal sequences. We have discovered that there is more than one laminin receptor gene which may encode more than one protein expressed in the cell. We have also cloned a murine laminin receptor cDNA and have found that murine cells, like their human counterparts, have multiple laminin receptor genes. Studies of the regulation of the laminin receptor gene indicate that differentiated cells express less laminin receptor mRNA than do related, undifferentiated precursor cells. This is consistent with the general observation that aggressive undifferentiated tumors which metastasize express more laminin receptor.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09156-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Cloning of Human Type IV Collagenolytic Gene(s)

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

PI:	M. Ballin	Visiting Fellow	LP NCI
OTHER:	U.P. Thorgeirsson	Expert	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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 standerd unreduced type. Do not exceed the space provided.)*

Two approaches were taken for cloning the gene for type IV collagenase from a tumor cell cDNA library. A human melanoma (A2058) γgtll library (custom made for Dr. Liotta by Clontech Co.) was first screened with a polyclonal antibody against type IV collagenase. The collagenase was purified from culture supernatant of the A2058 cell line which was used to make the cDNA library. The second approach was to screen the melanoma library with a mammalian collagenase cDNA clone (supplied by C. Brinkerhoff). After the first screening with the antibody, 18 clones were isolated, of which four were plaque purified after 4-5 rounds of screening. All four clones produced clear plaques, which indicates an insert-bearing phage, but failed to demonstrate an insert by restriction analysis; the restriction pattern was identical to that of a wild type γgtll phage DNA. Likewise, negative results were obtained when the same melanoma cDNA library was screened with the mammalian collagenase clone. Six clones which were strongly positive through four rounds of screening turned out to produce non-recombinant blue plaques without an insert as judged by restriction analysis of phage DNA. Since there may be problems with the melanoma library, we will start the cloning process all over again using another tumor cell cDNA library.

Presently, we are beginning to focus our attention on the role of endothelial cells in metastasis. Therefore, we are in the process of screening a human endothelial cDNA library, purchased from the Clontech Co. Using the mammalian collagenase cDNA clone under low stringent conditions, we expect to pull out mammalian collagenase and other related genes, expressed by endothelial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09157-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Interaction between Endothelial Cells and Tumor Cells During Vascular Invasion

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

PI:	U.P. Thorgeirsson	Expert	LP NCI
	M. Ballin	Visiting Fellow	LP NCI
	J. Hartzler	Physical Science Technician	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

Over the next year a new project studying the effect of tumor endothelial cell interaction on vascular invasion, will be initiated. Quantitation of tumor cell collagenase gene expression will be used at first as a marker for invasive potential. Secreted factors that either inhibit or stimulate the tumor cell collagenase will be looked for in conditioned media from capillary, venous and arterial endothelial cells. The effect of attachment between endothelial and tumor cells on collagenase expression will be studied in co-cultures of the two cell types. Tumor cell collagenase expression in the co-cultures will be quantitated by using in situ hybridization technique, followed by positive identification of the endothelial cells through immunostaining for factor VIII antigen. If collagenase stimulating factors expressed by endothelial cells, either as secreted or cell surface proteins will be identified, we will proceed to isolate and purify such factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09158-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

The Effect of Increased v-H-ras Expression on Metastatic Potential

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

PI:	U.P. Thorgeirsson	Expert	LP NCI
OTHER:	M. Ballin	Visiting Fellow	LP NCI
	J. Hartzler	Physical Science Technician	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

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.)*

Activated ras oncogenes have been shown to confer the metastatic phenotype upon different non-neoplastic and benign neoplastic cell types. We have evaluated the effect of amplified expression of v-H-ras in an NIH/3T3 line, 433, which contains the ras under a transcriptional control of the glucocorticoid-sensitive mammary tumor virus long terminal repeat. Thus, treatment with a glucocorticoid, such as dexamethasone for 6 days, results in a 20-fold increase in P21 synthesis, and simultaneously suppresses the proteolytic activity of the 433 cells. The untreated 433 cells produced experimental metastases but not spontaneous metastases in nude mice. However, after dexamethasone treatment, the lung colonizing capacity was decreased two- to three-fold.

These data show that amplification of expression of the ras gene under the control of a glucocorticoid promoter does not augment the metastatic capacity of the ras transfected cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09159-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Thrombospondin and its Receptors: Role in Cell Adhesion and Motility

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

PI:	G. Taraboletti	Visiting Fellow	LP NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion & Metastases Section	LP NCI

COOPERATING UNITS *(if any)*

D. Roberts, Laboratory of Structural Biology, NIDDKD, NIH

LAB/BRANCH

Laboratory of Pathology

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Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

Thrombospondin induces the migration of human melanoma and carcinoma cells. Using a modified Boyden chamber assay, tumor cells migrated to a gradient of either soluble thrombospondin (chemotaxis) or substratum bound thrombospondin (haptotaxis). A series of human melanoma and carcinoma cells exhibited different levels of response when compared for their relative motility stimulation by thrombospondin haptotaxis versus chemotaxis. Human A2058 melanoma cells which exhibit a strong haptotaxic and chemotaxic response to thrombospondin were used to study the structural domains of thrombospondin required for the response. Monoclonal antibody C6.7 which binds to the COOH terminal region of thrombospondin inhibited haptotaxis in a dose dependent optimal manner. C6.7 had no significant effect on thrombospondin chemotaxis. In contrast, monoclonal antibody A2.5 heparin and fucoidan which bind to the NH_2 terminal heparin binding domain of thrombospondin, inhibited thrombospondin chemotaxis but not haptotaxis. Monoclonal antibody A6.1 directed against the internal core region of thrombospondin had no significant effect on hepatotaxis or chemotaxis. The 140 kDa fragment of thrombospondin lacking the heparin binding amino terminal region, retained the property to fully mediate haptotaxis but not chemotaxis. When the COOH region of the 140 kDa fragment, containing the C6.7 binding site, was cleaved off, the resulting 120 kDa fragment (which retains the RGDA sequence) failed to induce haptotaxis. Separate structural domains of thrombospondin are therefore required for tumor cell haptotaxis versus chemotaxis. This may have implications during hematogenous cancer metastases formation.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09161-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Laminin Receptor and its Role in the Function of Natural Killer Cells

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

PI:	G.J. Bryant	Expert	LP NCI
OTHER:	L.A. Liotta	Chief, Tumor Invasion & Metastases Section	LP NCI

COOPERATING UNITS *(if any)*

J. Hiserodt, Pittsburgh Cancer Institute, Pittsburgh, PA

LAB/BRANCH

Laboratory of Pathology

SECTION

Tumor Invasion and Metastases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.8	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.)*

Natural killer (NK) cells, a subpopulation of large granular lymphocytes (LGL) are involved in the spontaneous lysing of tumor or virally infected target cells. The killing action requires binding of the killer cells to the target cells. The role of the laminin receptor in this process of specific binding is being examined in our laboratories. Using murine NK cells, the presence of laminin-like molecule and a receptor for laminin binding has been established (Kd 10^{-11} M, approximately 990,000 receptors/cell).

Further studies will attempt to define and compare the receptor on NK cells versus the already established laminin receptor located on many tumor cells; the interactions between these two receptors during cell lysis will be explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00550-07 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 professionel personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.S. Jaffe	Chief, Hematopathology Section	LP NCI
OTHER:	J. Cossman	Senior Investigator	LP NCI
	D.L. Longo	Senior Investigator	MB NCI
	L.M. Neckers	Research Chemist	LP NCI
	M.A. Bookman	Senior Investigator	MB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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 in addition can be identified as belonging to specific developmental and functional subpopulations. This data is then correlated with clinical and pathologic data. Morphologic features are analyzed to achieve improved classification of lymphoproliferative lesions.

This information is utilized to develop improved classifications of disease and to distinguish new clinicopathologic entities. It also will be used as a basis for potential immunotherapy or adjunctive immunotherapy in a program of autologous bone marrow transplantation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00552-07 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Basis of the Diagnosis of Human Lymphoproliferative Disease

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

PI:	J. Cossman	Senior Investigator	LP NCI
OTHER:	M. Raffeld	Senior Staff Fellow	LP NCI
	J. Sundeen	Biotechnology Fellow	LP NCI
	P. Cohen	Biotechnology Fellow	LP NCI
	M. Uppenkamp	Guest Researcher	LP NCI
	R. Coupland	Guest Researcher	LP NCI
	R. Andrade	Visiting Fellow	LP NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI; Medicine Branch, NCI; Metabolism Branch, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5	.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 spece provided.)*

We have undertaken investigations to characterize the molecular genetic basis of the pathogenesis of lymphoproliferative disorders and Hodgkin's disease.

By DNA hybridization analysis, our investigations have demonstrated the existence of "benign clonal lymphadenopathy" and the evolution of malignant transformation to high grade lymphoma. The significance of this discovery, from a diagnostic standpoint, is that monoclonality is not necessarily tantamount to malignancy. We have further applied genomic blot hybridizations to analyze the use of specific variable genes of the T cell receptor gamma locus. Notably, gamma variable genes are not selected during a wide variety of human immune responses and immunodeficiency disorders.

The defective mutants we have developed of the early T cell line, CEM, demonstrate a single critical step on T cell development surrounding the regulation of T cell receptor alpha gene transcription.

To determine the origins of Hodgkin's disease, we have molecularly cloned the rearranged immunoglobulin heavy and T cell receptor beta genes from a Hodgkin's cell line. By sequence analysis, we can now investigate whether Hodgkin's cells have undergone chromosomal rearrangements as a key step in their malignant transformation. Our molecular genetic investigations of rearranged genes in Hodgkin's disease has now been expanded to include several cell lines and primary cases. This approach will enable us to uncover common molecular pathologies in Hodgkin's disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00850-05 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Clonal Evolution of Lymphoid Neoplasms

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

PI:	J. Cossman	Senior Investigator	LP NCI
OTHER:	M. Raffeld	Senior Staff Fellow	LP NCI
	M. Uppenkamp	Guest Researcher	LP NCI

COOPERATING UNITS *(if any)*

Metabolism Branch, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

By means of Southern blot analysis, we have discovered that recurrence in B cell lymphoma, in particular follicular lymphoma, is derived from occult disease which is resistant to therapy. Multiple samples from patients obtained as much as ten years apart demonstrate that primary neoplasm and recurrences were derived from a common stem cell in all patients. No biclonal neoplasms were seen. Clonal evolution was frequent (42% of cases) and was characterized by secondary genetic events affecting rearranged immunoglobulin genes. Despite this, the rearranged bcl-2 locus in follicular lymphoma, a consequence of t(14;18) translocation, remained stable.

To further analyze minimal residual disease, we have developed a method for detecting rare follicular lymphoma cells which elude detection by conventional Southern blot analysis. This method may prove useful for prediction of recurrence following clinical remission in follicular lymphoma.

We have developed cell lines from the T cell leukemia of a patient with atexia telangiectasia. A chromosomal translocation t(14;18) is being analyzed by pulse-field electrophoresis to identify involvement of the T-alpha gene at band 14q11. Our leukemia cell lines from ataxia telangiectasia provide a model of clonal evolution of T cell neoplasms as a consequence of breakage of chromosomal fragment sites.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 00855-05 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Pathologic Features of HTLV-I Associated Diseases

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

PI:	E.S. Jaffe	Chief, Hematopathology Section	LP NCI
OTHER:	W.A. Blattner	Senior Investigator	EEB NCI
	R.C. Gallo	Senior Investigator	LTCB NCI
	P. Levine	Senior Investigator	EEB NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.03	0.02

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

A

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

Pathologic material from patients identified to be seropositive for HTLV-I 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.

In selected populations where HTLV-I is endemic, such as Jamaica, prospective studies of all newly diagnosed lymphoma patients are conducted. Such studies are useful in identifying the clinicopathologic spectrum of HTLV-I associated diseases. Prospective studies of all lymphomas in similar geographic regions with differing incidences of adult T cell leukemia/lymphomas are studies to discern factors which make an impact on the incidence of HTLV-I and HTLV-I associated diseases.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 00881-06 LP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Lymphocyte Activation and Proliferation

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

PI:	L.M. Neckers	Research Chemist	LP NCI
OTHER:	J.B. Trepel	Biologist	NMOB NCI
	R. Nordan	Staff Fellow	LG NCI
	O. Colamonici	Visiting Fellow	LP NCI
	S. Loke	Guest Researcher	LP NCI

COOPERATING UNITS *(if any)*

Laboratory of Genetics, NCI
Naval Medical Oncology Branch, NCI

LAB/BRANCH
Laboratory of Pathology

SECTION
Hematopathology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	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.)*

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. We have demonstrated that G_1 arrest occurs in both normal and malignant cells when transferrin receptors are blocked, even if cells are expressing high levels of growth factor receptor messenger RNA and c-myc and c-myb messenger RNA. Furthermore, either blockade of calcium channels or addition of cAMP to cells results in G_1 arrest and loss of transferrin receptor mRNA. The effect of cAMP can be detected at the level of transcription.

We have begun to study the role of nuclear proteins in the transition of T cells from G_0 to S phase. In an initial study, a c-myc antisense oligomer completely blocked the appearance of c-myc protein in mitogen treated T cells, yet these cells went on to express IL-2 receptors, transferrin receptors and DNA polymerase α protein. Yet they failed to synthesize DNA. We plan to continue to use antisense constructs to various nuclear proteins to study their role(s) in cell growth and activation. Initially, we are examining the requirements for several nuclear proteins in DNA polymerase α activity. To date, we have determined that two such proteins, c-myc and K_i-67, are required for polymerase α activity in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09146-02 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Biology of Transferrin Receptor Expression

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

PI:	L.M. Neckers	Research Chemist	LP NCI
OTHER:	J. Trepel	Biologist	NMOB NCI
	L. Wolff	Senior Investigator	LG NCI

COOPERATING UNITS *(if any)*

Laboratory of Genetics, NCI
Navy Medical Oncology Branch, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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.)*

Although it is clear that transferrin receptors are regulated in part by intracellular iron, it is also clear that other regulating mechanisms are involved. Since probes to the transferrin receptor gene have recently become available, we are utilizing molecular biology techniques to study the regulation of transferrin receptor expression at the molecular level. One part of this project involves the study of mouse plasmacytoma mRNA. We have found that there are two transferrin receptor mRNAs in these cells. One message lacks the untranslated part of the full-length mRNA, while retaining the coding sequences for the protein.

Using this model system, we plan to study the ability of iron to regulate TfR mRNA levels at the level of the message. We will determine if the untranslated part of the message is necessary for iron regulation.

Another part of the project involves the regulation of TfR gene transcription in HL-60 cells by cAMP. We have shown that cAMP shuts off transcription of both c-myc and TfR within 30' - 2 hrs after addition. We will investigate the mechanism of this shut-off and determine if the c-myc gene has any effect on transcription of the TfR gene.

We have produced a TfR construct which contains only the coding region of the cDNA under LTR control. We have successfully transfected 3T3 cells with this construct and have demonstrated the existence of human TfR on their surface by immunoprecipitation and FACS analysis. We will use these cells to study the regulation of the virally controlled TfR sequence.

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09147-02 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Defective TfR in HTLV-I Infected Human T cells

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

PI:	L.M. Neckers	Research Chemist	LP NCI
OTHER:	C.A. Vidal-Soto	Guest Researcher	LP NCI
	O.R., Colamonici	Visiting Fellow	LP NCI
	S. Matsushiya	Fogarty Fellow	COP NCI
	H. Mitsuya	Fogarty Fellow	COP NCI
	S. Broder	Senior Investigator	COP NCI

COOPERATING UNITS *(if any)*

Clinical Oncology Program, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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 found that HTLV-I infected T cells express 10-20 fold more TfR on their surface, but that this is due to a redistribution of the receptor from cytoplasm to surface and not to an increased receptor synthesis. Further, the TfR in these cells is poorly internalized and poorly phosphorylated by phorbol ester. The receptor cannot deliver iron to these cells, which nevertheless require iron for proliferation. Antibodies which prevent iron binding to the TfR still inhibit the growth of these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09149-02 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Differentiation of Immature T Cell Neoplasms by Interleukin-2

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

PI:	L.M. Neckers	Research Chemist	LP NCI
OTHER:	O.R. Colamonici	Visiting Fellow	LP NCI
	J.B. Trepel	Biologist	NMOB NCI
	D.G. Poplack	Senior Investigator	PB NCI
	I. Kirsh	Senior Investigator	NMOB NCI

COOPERATING UNITS *(if any)*

NMOB, NCI; PB, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	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 studied 45 cases of T cell and myeloid leukemia trying to further characterize them. In 15 cases, these cells respond to treatment with IL-2, in that they develop the ability to kill tumor cells. Their phenotype changes to resemble that of a more mature T cell. Using these cases, we have studied the role of the T cell receptor in generation of cytotoxic activity. In one case, IL-2 induced a proliferation of cells utilizing TCR γδ. We demonstrated that this TCR was functional in that anti-CD3 stimulated an elevation of intracellular [Ca^{2+}] and augmented MHC-unrestricted cytolysis. Trigerring of the TCR γδ in this case leads directly to release of cytolytic granule enzymes.

In studying the role of IL-2 in this process, we observed that the changes we recorded following IL-2 were transduced solely via the P75 IL-2 binding protein and not the P55 (TAC) protein. Based on these findings, we have begun a clinical protocol, in collaboration with DRS. Poplack and Kirsh, to study the effectiveness of rIL-2 in vivo as a treatment for immature T cell malignancies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09150-02 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Induction of Monocytic Differentiation by Sphingomyelinase

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

PI:	O.R. Colamonici	Visiting Fellow	LP NCI
OTHER:	L.M. Neckers	Research Chemist	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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 unraduced type. Do not exceed the space provided.)*

We have demonstrated that the enzyme sphingomyelinase is able to induce differentiation of the promyelocytic cell line HL-60. This new pathway for monocytic differentiation may explain the ability of TPA to differentiate HL-60 cells. Differentiation is mediated by the breakdown product of sphingomyelin:phosphorylcholine. Preliminary evidence suggests that the phorbol ester, TPA, differentiates HL-60 cells via stimulation of sphingomyelinase and not via C-kinase.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09151-02 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Immunopathology of LAK-IL2 Treated Tumors

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:	P. Cohen	Biotech. Fellow, Hematopath. Section	LP NCI
	M. Lotze	Senior Investigator, Surgery Branch	C DCT COP
	S. Rosenberg	Chief, Surgery Branch	C DCT COP
	R. Steis	Acting Chief, Clinical Res. Branch	BRMP FCRF
	J. Clark	Senior Investigator	BRMP FCRF

COOPERATING UNITS *(if any)*

Surgery Branch, DCT, NCI
BRMP, FCRF

LAB/BRANCH

Laboratory of Pathology

SECTION

Hematopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

A

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

Lymphocytes activated with IL2 both in vivo and in vitro are being used for the experimental treatment of human malignancies. The mechanism of action of this therapy is not clearly established, and it is not known whether those lymphokine-activated killer cells (LAK) infused actually migrate and infiltrate tumors, or whether other effector cells mediate the observed tumor regression. In order to better understand the pathophysiology of LAK-IL2 treatment, tumor biopsy specimens are obtained before, during, and after conclusion of LAK-IL2 treatment. Specimens are studied with an immunohistochemical technique for a battery of tumor markers as well as markers capable of identifying T cells, B cells, histiocytes, NK cells, and other effector cells. These data are then correlated with conventional, clinical and pathologic data to determine whether the immunopathology observed can predict clinical response.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09144-03 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Identification of Proteins Binding to c-myc Regulatory Sequences

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

PI:	D.L. Levens	Senior Staff Fellow	LP NCI
OTHER:	J. Quinn	Visiting Fellow	LP NCI
	M. Takimoto	Visiting Fellow	LP NCI
	M. Avigan	Medical Staff Fellow	LP NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	3.4	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 have been applying a technique developed by us (Levens, D. and Howley, P., Mol. Cell. Biol. 5: 2307-2316, 1985) for the rapid identification of sequence specific DNA binding proteins to examine transcriptional regulatory factors binding to the c-myc oncogene. By combining this technique with a sensitive exonuclease footprinting assay (Wu, C., Nature 317: 84-87, 1985), DNA-protein complexes can be formed, rapidly separated from the vast majority of protein and competitor nucleic acids, centrifugally concentrated and "footprinted".

This approach has led to the identification of multiple factors binding to a 2.3 kb region upstream from the c-myc promoters P1 and P2. The pattern of proteins binding, reflects the physiological state and particular cell source of the proteins. One of the factors examined has also been shown in our laboratory to bind to the enhancer of the gibbon ape leukemia virus and correlates well with the activity of that enhancer. This protein has been enriched and identified from nuclear extracts with a single step of two cycles. The protein is a 38-40 kd peptide. We are currently devising a purification scheme for this and other c-myc regulatory proteins. The role of these factors in c-myc regulation will be studied by in vitro transcription where we hope to reconstitute some of the properties of the physiological regulation of c-myc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03657-13 D

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

P.I.: S.I. Katz, Branch Chief, Dermatology Branch, DCBD, NCI

OTHER: S. Shimada, Guest Researcher, Dermatology Branch, DCBD, NCI
W. Caughman, Senior Staff Fellow, Dermatology Branch, DCBD, NCI
A. Gaspari, Medical Staff Fellow, Dermatology Branch, DCBD, NCI
C. Hauser, Guest Researcher, Dermatology Branch, DCBD, NCI
T. Furue, Visiting Fellow, Dermatology Branch, DCBD, NCI

COOPERATING UNITS *(if any)*

Dermatology Branch, USUHS, Bethesda
Immunology Branch, DCBD, NCI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	5	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 major area of study of this laboratory is the role of the epidermis as an immunological organ. We have found that epidermal Langerhans cells are derived from precursors in the bone marrow and play an essential role in many of the immunological reactions affecting the skin. We have also identified an epidermal Interleukin 1-like cytokine which may serve as a second signal in the generation of T cell responses as well as a proinflammatory agent affecting other cells - especially neutrophils. We have demonstrated that when epidermal Langerhans cells are cultured for 2-3 days they become very potent antigen presenting cells compared to freshly prepared Langerhans cells. We have therefore utilized cultured Langerhans cells for the generation of primary immune responses in resting unsensitized T cells. We have demonstrated that when cultured cells are modified with hapten, they can generate primary immune responses. The sensitized T cells thus generated respond preferentially to the same hapten in vitro. We have also concentrated our efforts on the characterization of murine dendritic thy 1 positive epidermal cells. We have utilized highly enriched populations of freshly prepared cells and identified their T cell nature by demonstrating that they express -like T cell receptor along with the associated T3 components. As these cells are also present in nude mice they may represent an extra thymic source of T cells in nude as well as normal mice. The other major focus of this laboratory has been the study of the function of class II MHC bearing keratinocytes which appear in humans and mice during cell-mediated reactions in the skin. We have demonstrated that these cells can 1) present peptide fragments to T cell hybridomas, 2) serve as targets for class II specific cytotoxic T lymphocytes, and 3) induce secondary alloreactive T cell responses.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 03667-03 D

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecules Defined by Autoantibody - Mediated Skin Diseases

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

P.I.: John R. Stanley, M.D., Dermatology Branch, DCBD, NCI

OTHER: Russell Eyre, M.D., Medical Staff Fellow, Dermatology Branch, DCBD, NCI
Stephan Muller, M.D., Visiting Fellow, Dermatology Branch, DCBD, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Dermatology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	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 general and long-term goal of my laboratory is to study autoantibody-mediated skin diseases in order to further our understanding not only of the pathophysiology of these diseases but also of the structure and function of normal epidermis and epidermal basement membrane zone. Specifically, antibodies in these diseases define molecules in the normal epidermis. We have characterized the antigens defined by three of these diseases: bullous pemphigoid (BP), pemphigus vulgaris (PV), and pemphigus foliaceus (PF). We have defined the cells which synthesize these antigens, as well as the antigen defined by the autoantibodies found in patients with epidermolysis bullosa acquisita (EBA). We have used the binding of antibodies to specific molecules to make diagnoses of BP, EBA, PV or PF in various complicated cases of these diseases. We have also used antibodies to BP antigen, as well as to other basement membrane components, to rapidly diagnose various types of epidermolysis bullosa, often within the first few days after birth. We have demonstrated that autoantibodies from patients with fogo selvagem, a form of pemphigus endemic to Brazil, have similar specificities to autoantibodies from patients with sporadic North American PF. We have demonstrated that PF antibodies bind a protein found in desmosomes, and define a calcium-sensitive epitope on a desmosomal protein complex. Thus, PF is an autoimmune disease in which the antibody target is the desmosome. We have demonstrated that antibodies from all PF patients bind a unique complex of proteins that is distinct from the complex bound by PV antibodies. Using a lambda gtll expression library, we have isolated a cDNA clone that synthesizes part of the BP antigen. We are just beginning to characterize this clone.

PHS 6040 (Rev. 1/84) GPO 914-910

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03666-09 D

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

PI: Thomas J. Lawley, M.D., Dermatology Branch, DCBD, NCI

OTHER: Robert Swerlick, M.D., Medical Staff Fellow, Dermatology Branch, DCBD, NCI
Yasuo Kubota, M.D., Visiting Fellow, Dermatology Branch, DCBD, NCI
Carol McNeely, M.D., Medical Staff Fellow, Dermatology Branch, DCBD, NCI
Edith Garcia-Gonzalez, Visiting Fellow, Dermatology Branch, DCBD, NCI
Joseph Cason, Technician, Dermatology Branch, DCBD, NCI

COOPERATING UNITS *(if any)*

LCI, NIAID

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

Soluble mediators of inflammation such as the complement derived anaphylatoxins play important roles in a variety of immunologically mediated human systemic and cutaneous diseases. We have purified human C5a and C5a des Arg and studied their in vivo and in vitro reactivity. Their in vivo role was assessed by the first in-depth analyses of the cutaneous reactivity of these complement fragments in man. We have also documented the ability of C5a and C3a to modulate cell surface receptors for immunoglobulin and complement on the surface of leukocytes. We have assessed the contribution of polymorphonuclear neutrophils (PMN's) and mast cells to C5a induced cutaneous inflammation by studying the cutaneous reactivity of patients with bone marrow failure who lack PMN's and by selectively depleting the mast cells in the skin of volunteers prior to skin testing with C5a. Increasing evidence indicates that human endothelial cells, under certain circumstances, can be induced to become immunologically competent. We have isolated human umbilical vein and dermal microvascular 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. In addition we have induced human endothelial cells to differentiate in vitro. Under specific culture conditions the endothelial cells undergo angiogenesis forming small tubular structures that possess lumens and resemble blood vessels. We have also developed an assay to detect antiendothelial cell antibodies in huma sera and have detected these antibodies in patients with certain forms of vasculitis.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03659-13 D

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

P.I.: G.L. Peck, Senior Investigator, Dermatology Branch, DCBD, NCI

OTHER: J.J.DiGiovanna, Senior Staff Fellow, Dermatology Branch, DCBD, NCI
I. Tokar, Registered Nurse, Dermatology Branch, DCBD, NCI
K. Kraemer, Senior Investigator, Cell Genetics Br., NCI

COOPERATING UNITS *(if any)*

Cancer Prevention Studies Branch, DCPC, NCI, NIH, Bethesda, Maryland 20892

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.9	

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.)*

Oral isotretinoin was effective in the prevention of skin cancer and in the treatment of a variety of disorders of keratinization (lamellar ichthyosis, Darier's disease, pityriasis rubra pilaris), and cystic acne. Oral etretinate was more effective and less toxic than isotretinoin in the treatment of the disorders of keratinization. Psychological testing revealed decreased anxiety and depression in cystic acne patients after treatment with isotretinoin. One chronic toxicity, "retinoid hyperostosis," characterized by anterior spinal ligament calcification and osteophyte formation of vertebrae, has been observed in 80% of patients treated with long-term, high-dose isotretinoin. Sixty percent of acne patients with moderate doses of isotretinoin for 9 months also developed vertebral osteophytes. Predominantly extraspinal tendon and ligament calcification occurred commonly after chronic therapy for psoriasis and disorders of keratinization with etretinate, an aromatic retinoid. The usual sites of involvement were the ankles, pelvis and knees.

Seven patients with xeroderma pigmentosum were entered into a cancer chemoprevention study using isotretinoin: Preliminary results indicate significant partial inhibition of new tumor formation during therapy and a marked increase in new tumor formation after therapy. The need for continuous maintenance therapy for chemoprevention of skin cancer with isotretinoin may vary with the underlying etiology (genetic or environmental of the patient's tumors.

PHS 6040 (Rev. 1/84) GPO 814-910

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03630-17 D

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

P.I.: G.L.Peck, Senior Investigator, Dermatology Branch, DCBD, NCI

OTHER: J.J. DiGiovanna, Senior Staff Fellow, Dermatology Branch, DCBD, NCI
T. Mehrel, Visiting Fellow, Laboratory for Cellular Carinogenesis and Tumor Promotion, DCE, NCI

COOPERATING UNITS *(if any)*

Laboratory for Cellular Carcinogenesis and Tumor Promotion, DCE, NCI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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.)*

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 normal human skin, newborn mouse 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.

Using molecular hybridization probes specific for transcripts of individual keratin genes, keratin gene expression in skin cancer and cutaneous disorders of keratinization indicate the following. In contrast to normal epidermis where the expression of proliferation-associated keratin genes is suppressed after cells migrate from the basement membrane, hyperproliferative disorders of the epidermis exhibit inappropriate expression of proliferation-associated keratin genes in the more superficial layers of the epidermis. In addition, skin cancers fail to express differentiation-associated keratins, indicative of their undifferentiated state.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03638-18 D

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

PI: J. H. Robbins, M.D., Dermatology Branch, DCBD, NCI

COOPERATING UNITS *(if any)*

Biostatistics Branch, DCCP, NCI

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	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.)*

Studies in this laboratory are designed to elucidate the role of DNA repair processes in carcinogenesis and in normal and abnormal aging. Most studies have been conducted with cells from patients with xeroderma pigmentosum 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 primary neuronal, muscular, and retinal degenerations are also being studied. These diseases include ataxia telangiectasia, Alzheimer disease, Parkinson disease, Huntington disease, Duchenne muscular dystrophy, retinitis pigmentosa, Friedreich ataxia, and Cockayne syndrome. These studies are designed to elucidate the pathogenesis of these disorders. We assess the biological effectiveness of DNA repair by 1) in vitro assays of cell survival after treatment of the cells with DNA-damaging agents; 2) analysis of chromosome and chromatid aberrations in cells treated with DNA-damaging agents; and 3) transfection studies using irradiated shuttle vector plasmids. We search for DNA damage by 1) extracting the DNA and having it subjected to analysis by capillary-gas-chromatography mass-spectrometry; 2) studying unscheduled DNA synthesis induced in cultured cells by chemical carcinogens; and 3) determining the alkaline elution profile of DNA from cultured cells after their treatment with DNA-damaging agents.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03656-14 D

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

P.I.: 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, NIDDK

LAB/BRANCH

Dermatology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	5.5	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The biosynthesis, structure and function of the principal differentiation products of human and mouse epidermis are being studied. Keratin subunits polymerize in vitro into native-type intermediate filaments. Details of their structure are being investigated by use of: solid state NMR on isotopically-labeled filaments; transmission and scanning transmission electron microscopy of intact filaments or subfilamentous forms; optical diffraction and image analysis procedures; and limited proteolysis experiments to ascertain alignment of constituent subunits. Model structures generated by these methods are being computationally tested for compatibility with other physico-chemical data and amino acid sequence information on individual subunits. cDNA clones to keratins 1 and 10 are being used to characterize the number, organization and complexity of their genes isolated from cosmid libraries. The expression of the human genes is being studied by the production of transgenic mice produced from various constructs of these genes. cDNA clones are being used to isolate and characterize the genes for mouse and human filaggrin, which appear to be initially expressed as a very large polyprotein precursor. cDNA clones encoding a major cell envelop protein have been isolated and are being sequenced. Constructs of keratins, filaggrin and the cell envelop protein clones are being assembled with pGEM vectors for use in insitu hybridization experiments in order to study the expression of these proteins in epidermal keratinizing disorders.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04002-18 MET

PERIOD COVERED

October 1, 1986 through September 30, 1987

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 Dysfunctions

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

PI:	Thomas A. Waldmann	Branch Chief	MET, NCI
	Michael Davey	Medical Staff Fellow	MET, NCI
	Robert Kozak	Guest Researcher	MET, NCI
	Mitsura Tsudo	Visiting Fellow	MET, NCI
	William Kaulfersch	Visiting Fellow	MET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	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.)*

Resting T cells do not express high-affinity IL-2 receptors, but receptors are rapidly expressed on T cells after activation with antigen or mitogen. There are two classes of IL-2 receptors that differ in their affinity for IL-2, including one with a very high affinity and the other with a much lower affinity. In a major development over the past year, a novel 75-kDa non-Tac IL-2 binding peptide was identified. Cell lines bearing either the 55-kDa Tac or the p75 peptide alone manifested low-affinity IL-2 binding, whereas cell lines bearing both peptides manifested both high- and low-affinity receptors. Fusion of cell membranes from low-affinity IL-2 binding cells bearing the Tac peptide alone with membranes from a cell line bearing the p75 peptide alone generated hybrid membranes bearing high-affinity receptors. These observations suggested a multichain model for the high-affinity IL-2 receptor in which both the p55 Tac and the p75 IL-2 binding peptides are associated in a receptor complex. Essentially all T-cell functions require that the T cell be activated and express both the 55-kDa Tac peptide as well as the novel 75-kDa IL-2 binding peptide. The pattern with lymphokine-activated killer (LAK) cells and natural killer (NK) cells is quite different. Over the past year, it was shown that such LAK and NK precursor cells, as well as different types of leukemias of large granular lymphocytes with NK activity, express the p75 but not the 55-kDa IL-2 binding peptide.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04004-25 MET

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Amino Acids and Growth Factors in Cell Activation

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

PI:	James M. Phang	Section Head	MET, NCI
	A. James Mixson	Medical Staff Fellow	MET, NCI
	Patricia Cortazar	Guest Researcher	MET, NCI

COOPERATING UNITS *(if any)*

Grace C. Yeh, CPB, DCT, NCI

David Valle, M.D., Johns Hopkins Hospital School of Medicine, Baltimore, MD

LAB/BRANCH

Metabolism Branch

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The findings during this past year strongly support our hypothesis that pyrroline-5-carboxylate (P5C) is a novel mediator of intercellular communication and transmembrane signaling. First, we showed that P5C stimulates the turnover of membrane phosphoinositides. With the P5C stimulation of PRPP production in intact cells as an endpoint, we found a pattern of synergism with specific growth factors and sensitivity to inhibitors consistent with the interpretation that P5C produced its effect by activating protein kinase C at a point proximal to the production of diacylglycerol. Related and perhaps linked to this mechanism for transmembrane signaling, a novel uptake mechanism for P5C was identified and characterized. This mechanism is specific, carrier mediated, energy dependent, but sodium independent. Importantly, P5C is converted to proline concomitant to cellular entry. This group translocation directly couples the uptake of P5C to the transfer of redox within or at the plasma membrane. Finally, the functional association of P5C translocation to P5C reductase, the enzyme that mediates redox transfers, has been corroborated with the direct demonstration of the enzyme's association with plasma membranes. Using sucrose density gradients, we showed that P5C reductase, previously considered to be cytosolic, is, in fact, associated with cellular organelles. One component is associated with plasma membranes and a second with mitochondria. The membrane component has 7-fold higher affinity for NADPH than the mitochondrial component.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04015-16 MET

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Development and Function of Humoral and Cellular Immune Mechanism

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

PI:	R. Michael Blaese	Section Chief	MET, NCI
	Andrew V. Muchmore	Senior Investigator	MET, NCI
	Donald Kohn	Medical Staff Fellow	MET, NCI
	Thomas Fleisher	Senior Investigator	CPD, CC
	Robert Moen	Senior Staff Fellow	LMH, NHLBI
	French Anderson	Chief	LMH, NHLBI
	Alan Palestine	Senior Investigator	NEI
	Michael Wood	Howard Hughes Scholar	MET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	6	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.)*

A principal project is directed toward gene therapy of the immune deficiency disease caused by the absence of adenosine deaminase (ADA). We have established T-cell lines from ADA-deficient patients and shown that these cell lines are sensitive to deoxyadenosine and are ADA deficient, just as the cells from these patients are in vivo. A murine retrovirus vector gene transfer system has been modified to contain the gene for human ADA. Our data show that such a gene vector system can successfully transfer a functioning human ADA gene into these defective cells with a high efficiency and that such "gene-treated" cells are reconstituted to normal function. We have continued to study Wiskott-Aldrich syndrome (WAS) patients and have developed a new test to detect carriers of this X-linked disease. The test is based on the observation that, although all female somatic cells contain two X chromosomes, only one X chromosome is active in each cell. Using restriction fragment length polymorphisms to distinguish the two X chromosomes and methylation-sensitive restriction endonucleases to determine which of the X chromosomes was inactivated, we have shown that carrier females of the WAS gene have a predictable unbalanced pattern of X inactivation involving their T lymphocytes, B lymphocytes, and granulocytes. This unbalanced pattern of X inactivation is not seen in normal females and presumably reflects selection against the expression in the carrier females of the X chromosome bearing the mutant WAS gene. Our studies developing and defining the new immunosuppressive drug succinylacetone (SA) have continued. We have found that this agent is perhaps the most potent T- and B-cell immunosuppressive compound available, totally blocking allograft rejection and graft-versus-host disease (GVHD) in F1 animals and preventing GVHD during total allogeneic bone marrow transplantation while allowing normal engraftment and reconstitution to occur. SA is also a very potent inhibitor of both primary and secondary antibody responses. Experimental trials of SA use in autoimmune disease have shown that it is remarkably effective in preventing a type of uveitis that usually results in blindness in experimental animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04016-14 MET

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Action of Insulin-like Growth Factors ('B')

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

PI:	S. Peter Nissley	Senior Investigator	MET, NCI
	Marie Gelato	Medical Staff Fellow	MET, NCI
	Wieland Kiess	Guest Researcher	MET, NCI
	Matthew M. Rechler	Senior Investigator	NIADDK
	Wayne Anderson	Senior Investigator	LTIB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolism Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
DCBD, NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3	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 spece provided.)*

We previously identified in fetal rat serum a binding species capable of specifically binding ^{125}I-IGF-II that is considerably larger than the 150- and 40-kDa carrier proteins. We now have immunologic and affinity crosslinking data to show that this binding species is the type II IGF receptor. Rat serum was gel filtered on a Sephadex G-200 column (0.05 M NH_4HCO_3, pH 8), and ^{125}I-IGF-II binding was measured in individual column fractions. ^{125}I-IGF-II binding activity was found in the void volume (Vo) in addition to the carrier protein regions. Competitive binding studies using ^{125}I-IGF-II and binding activity from the G-200 Vo showed the characteristics of the type II receptor: IGF-II was more potent than IGF-I, and insulin did not compete. Importantly, a specific anti-type II receptor IgG that recognizes neither the 40- and 150-kDa serum IGF carrier proteins nor the type I IGF receptor completely blocked ^{125}I-IGF-II binding. ^{125}I-IGF-I did not bind to the Vo fractions, demonstrating absence of the type I IGF receptor. Independent support for identification as the type II IGF receptor came from affinity crosslinking experiments using disuccinimidylsuberate. Crosslinking of ^{125}I-IGF-II to the G-200 Vo material demonstrated a specific band at 210 kDa without reduction and 240 kDa with reduction of disulfide bonds. The size was confirmed by Western blotting of G-200 Vo material with the anti-type II receptor IgG which revealed a band slightly smaller (10 kDa) than the type II receptor from rat placental membranes. Immunoquantitation by Western blotting using pure type II receptor from rat placental membranes as standard showed a developmental pattern. In fetal rat serum (19 days gestation) and in sera from 3- and 10-day-old rats, 1-5 g/ml receptor protein was measured. The levels of type II receptor declined dramatically between age 20 days and 40 days, but receptor was still measurable at age 12 months. We conclude that the type II IGF receptor is found in rat serum and is developmentally regulated. The circulating receptor's tissue source and role are not defined; it is possible that the circulating receptor modulates IGF action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04017-09 MET

PERIOD COVERED

October 1, 1986 through September 30, 1987

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
	David K. Wagner	Medical Staff Fellow	MET, NCI
	Luisa Marcon	Visiting Fellow	MET, NCI
	Luigi Notarangelo	Guest Researcher	MET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch

SECTION

Immunophysiology

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Studies were performed to examine the maturation and regulation of the human immune response in normal individuals and in patients with congenital and acquired immune deficiency states associated with a high frequency of cancer. The interaction of T lymphocyte-derived lymphokine, interleukin-2 (IL-2), with its cell membrane receptor (IL-2R) plays a major role in the establishment and maintenance of the immune response. Soluble Tac protein from the human IL-2R is secreted by activated normal lymphocytes/monocytes and by leukemic cells in vitro. Soluble Tac protein is 10 kDa smaller than the Tac protein on cell surfaces and specifically binds IL-2 with low affinity (20 nM). Soluble Tac protein was measurable in the serum of all normal individuals. Elevated serum Tac protein levels were found at the time of diagnosis in several human retroviral-related disorders, including the HTLV-I-associated adult T-cell leukemia (ATL), hairy cell leukemia (HCL), and the HIV-related acquired immunodeficiency syndrome (AIDS). In patients with ATL and HCL, favorable responses to therapy were associated with reductions in serum levels of Tac protein. In patients with non-Hodgkin's lymphoma, serum Tac proteins levels at the time of diagnosis were the best predictor of survival. The administration of recombinant IL-2 to cancer patients in vivo caused a 1000-fold elevation in serum levels of Tac protein. Measurement of Tac protein in serum is useful in the diagnosis and management of certain cancer patients, as well as in monitoring the state of immunologic activation in humans in vivo.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04018-11 MET

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Immunoregulatory Glycoproteins Purification and Characterization

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

PI:	Andrew V. Muchmore	Senior Investigator	MET, NCI
	Anne Sherblom	IPA	Univ. Maine

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolism Branch, DCBD, NCI

SECTION

INSTITUTE AND LOCATION

DCBD, NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our laboratory has concentrated on the purification and characterization of a unique 85-kilodalton immunosuppressive glycoprotein termed uromodulin, which was originally isolated from human pregnancy urine. This material blocks antigen-specific proliferative assays that are dependent upon T cell proliferation at concentrations of 10^{-10} M. Uromodulin is also a specific inhibitor of interleukin-1 (IL-1) and blocks both thymocyte and IL-1-dependent T cell lines at concentrations of 10^{-10} M. Interestingly, uromodulin is a specific and high-affinity ligand for both recombinant IL-1 alpha and IL-1 beta, and it appears that this is the mechanism by which uromodulin is able to regulate the activity of IL-1. We have expanded these studies and recently have been able to sequence over 200 amino acids of native uromodulin. Using this sequence data, we isolated a full-length message for uromodulin. This message has been inserted into several different vectors and is in the process of being expressed in several transient expression systems. We have also been characterizing the mechanism of binding of IL-1 to uromodulin and find that IL-1 actually recognizes carbohydrate sequences expressed by uromodulin. A number of studies have been utilized, including digestion with endoglucoaminidase F, pronase, and isolation of released fragments by HPLC and high-performance thin layer chromatography to further characterize the carbohydrate sequence responsible for binding to IL-1. Clinical studies have also been instituted. We have developed a number of ELISA assays based on several monoclonal antibodies generated in our laboratory, and evidence suggests that uromodulin is elevated in a number of clinical conditions that are associated with elevations of IL-1. These observations have been generalized, and we now know that interleukin-2 and tumor necrosis factor also exhibit carbohydrate binding specificity.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04020-10 MET

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Antigen-Specific T-Cell Activation and Genetic Control of Immune Responses

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

PI:	Jay A. Berzofsky, M.D., Ph.D.	Senior Investigator	MET, NCI
	Kemp B. Cease, M.D.	Medical Staff Fellow	MET, NCI
	Shoichi Ozaki, M.D., Ph.D.	Fogarty Visiting Fellow	MET, NCI
	Masaharu Kojima, Ph.D.	Fogarty Visiting Fellow	MET, NCI
	Sara Brett, Ph.D.	Guest Researcher	MET, NCI
	Akihiko Kurata, M.D., Ph.D.	Guest Researcher	MET, NCI
	Hidemi Takahashi, M.D., Ph.D.	Fogarty Visiting Fellow	MET, NCI

continued next page

COOPERATING UNITS *(if any)*

See next page

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NIH, NCI, DCBD, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.5	8.5	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.)*

Antigen recognition by T lymphocytes is a complex process involving processing of the antigen into fragments by another cell and presentation of these to T cells in association with a major histocompatibility (MHC) molecule on the surface of that other cell. Therefore, only a few segments of a protein antigen will be seen by T cells, in contrast to antibodies. For vaccine development, it will therefore be important to locate such immunodominant antigenic sites. Fortunately, T cells have one advantage over antibodies in that T cells crossreact with short synthetic peptides of the protein much more effectively than do most antibodies. We have used synthetic peptides to characterize in detail the antigenic sites of a model protein antigen, myoglobin, recognized by murine T cells and T-cell clones established in this lab. These studies indicated that such sites tended to be amphipathic helices, i.e., helices that have hydrophilic and hydrophobic residues separated on opposite sides. We have developed a computer algorithm which locates potential amphipathic helical segments of proteins and requires knowledge only of the amino acid sequence. This algorithm identifies 18 of 23 known immunodominant T cell sites on 12 proteins ($p < 0.001$). We applied this to two proteins of importance for vaccine design, the circumsporozoite protein (CSP) of malaria , and the HIV (AIDS virus) envelope. In both of these we predicted major T-cell antigenic sites, synthesized the corresponding peptides, and showed that these elicited T-cell immunity in mice. In the malaria case, we coupled the T-cell site to a known target site of neutralizing antibodies to construct a totally synthetic immunogen capable of eliciting antibodies in strains of mice that could not respond to the antibody site alone. These approaches should be useful in the rational design and construction of synthetic and recombinant fragment vaccines. Also, we used avidin blocking of presentation of biotinylated peptide to specific T-cell clones to demonstrate the presence of peptide on the cell surface where it was suspected but had not previously been demonstrable in most cases with antibodies to the antigen. Finally, we demonstrated that B lymphocytes very efficiently take up, process, and present antigens to T cells via their surface immunoglobulin, but that this immunoglobulin sterically hinders processing of part of the antigen and so leads to selective processing and presentation to only a subset of antigen-specific T cells. This may provide a mechanism to explain how immune response genes, which control T-cell specificity, can also indirectly control the specificity of antibodies produced, by selective T help of only those B cells producing the right peptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 04023-01 MET

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms of Lymphoid Development and Transformation

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

PI:	Ajay Bakhshi	Senior Investigator	MET, NCI
	John J. Wright	Medical Staff Fellow	MET, NCI
	Suzanne Zorn	Guest Researcher	MET, NCI
	Christine Hua	Fogarty Visiting Fellow	MET, NCI
	Hon-Sum Ko	Fogarty Visiting Fellow	MET, NCI
	Robert Coupland	Guest Researcher	LP, NCI
	Jeffrey Cossman	Senior Investigator	LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolism Branch

SECTION

INSTITUTE AND LOCATION
DCBD, NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	7	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

To understand normal and abnormal B lymphocyte development, we have undertaken studies to identify and characterize genes important in these processes. We characterized the t(14;18)(q32;q21) chromosomal translocation present in human follicular lymphoma and identified a candidate transforming gene at 18q21. This gene, termed BCL2, is presumably also important in normal pre-B-cell development. We have analyzed both germline substrates and derivative products of the t(14;18) translocation to define the mechanism of this exchange. To elucidate the structural consequences of this recombination, we sequenced normal and translocated BCL2 cDNAs. The normal BCL2 gene uses six potential polyadenylation signals in exon 3 and two different 5' exons (exons 1 and 2) with their respective promoters. The t(14;18) translocation results in the deregulated expression of chimeric BCL2/IgH mRNA transcripts with somatic mutations in the BCL2 protein coding region. We have developed antibodies to the BCL2-predicted protein that should help define the function of the normal and translocated BCL2 protein. We have extended the approach of identifying new putative proto-oncogenes by cloning chromosomal breakpoints to the analysis of Hodgkin's disease and have cloned potential breakpoints within the immunoglobulin heavy chain (14q32) and T-cell receptor beta chain (7q35) loci. Finally, we screened an expression lambda gt11 library with an antibody that recognizes a 120-kDa lymphocyte activation antigen and identified one positive clone. Further analysis of these transforming and activation genes should provide important insights into B-cell transformation/differentiation.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05003-22 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	D. Segal	Senior Investigator	IB, NCI
	R. Yetter	Guest Researcher	NIAID
	H. Morse	Laboratory Chief	NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	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 cellular basis for in vitro generation of mouse MHC-nonrestricted, activated killer cells against tumor cells has been further established by cell reconstitution tests. Results confirm and extend previous observations that the generation of activated killer cells in response to the nonspecific inducing agent polyinosinic acid requires accessory cells, T-helper cells ($L3T4^+$ or $Lyt2^+$), and cytotoxic precursor cells. This knowledge has been used 1) to generate thymic veto cells which induce selective tolerance, a possible mechanism for establishing tolerance to self, and 2) to identify and circumvent depressed in vitro generation of activated killer cells by splenocytes from mice with acquired immunodeficiency disease induced by murine leukemia viruses.

Normal human PBL have been activated in vitro with recombinant IL-2 and retargeted against human ovarian carcinoma cells by heteroconjugated monoclonal antibodies which crosslink immune cells via selected activation sites with the tumor cells. Retargeted effector cells are cytotoxic in vitro for ovarian carcinoma cells and block intraperitoneal growth of the tumor cells in nude mice when the mice are treated 4-6 days after tumor growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05018-17 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Target Cell 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:	C. Yue	Medical Staff Fellow	IB, NCI
	W. Munger	Investigator	IB, NCI
	T. Soares	Microbiologist	IB, NCI
	C. W. Reynolds	Investigator	BTB, FCRF, NCI
	H. Young	Expert	BTB, FCRF, NCI
	A. Kuta	Biotechnology Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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 functional and biochemical properties of cytoplasmic granules of rat large granular lymphocyte tumors have been further delineated. Antibodies against the two BLT esterases purified to homogeneity were used to show that these proteins are serologically not cross-reactive, confirming previous enzymatic inhibition data. The possible interactions of these enzymes in cytolysin function was examied by mixing the pure LGL granule cytolysin and the pure major BLT esterase and testing for lytic activity. On tumor target cells (but not red cells), a synergistic effect was observed which could increase the cytolysin potency as much as ten-fold. Target preincubation with the enzyme did not cause this effect, nor was it seen with the minor BLT esterase. While these results suggest that the major enzyme can potentially increase the lytic efficiency of cytotoxic lymphocytes, the mechanism of this effect is still unclear. The ability of granules of cytotoxic lymphocytes to release DNA from target nuclei was studied in order to explain the rapid DNA degradation seen when cytotoxic lymphocytes attack target cells. Purified LGL tumor granules cause nuclear DNA release along with lysis of tumor cells, while the purified cytolysin causes lysis with no DNA breakdown. Two additional granule components cause DNA release from detergent permeabilized cells, but these appear to be minor granule proteins. Our results suggest that target cell DNA breakdown can be accounted for by the granule exocytosis mechanism. In order to begin a molecular biology approach to LGL granule proteins, a cDNA library in lambda gt10 was constructed from rat LGL tumor cell mRNA. The purified cytolysin protein was subjected to cyanogen bromide digestion and the resulting peptides separated by HPLC. Sequences of 25 amino acid residues of two of these were obtained by Edman degradation. Based on these sequences, synthetic oligonucleotide probes were synthesized in order to probe the cDNA library for the cytolysin clone.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05021-16 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Senior Investigator	IB, NCI
	C. H. Chester	Guest Researcher	IB, NCI
	Y. Sharabi	Visiting Fellow	IB, NCI
	N. Shinohara	Visiting Scientist	IB, NCI
	M. Sykes	Visiting Associate	IB, NCI

COOPERATING UNITS *(if any)*

S. L. Epstein, Senior Staff Fellow, National Center for Drugs and Biologics, FDA

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	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.)*

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; 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; and 5) Mixed allogeneic and xenogeneic chimeras, in which irradiated animals are reconstituted with mixtures of T-cell depleted donor and host marrow, are produced and the mechanism of tolerance and of immune responsiveness in these animals is studied in vivo and in vitro.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05023-16 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Transplantation Antigens of Swine

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

PI:	D. H. Sachs	Chief, Transplantation Biology Section	IB, NCI
Others:	S. A. Rosenberg	Chief, Surgery Branch	SB, NCI
	E. O. Kortz	Medical Staff Fellow	IB, NCI
	R. E. Gress	Medical Officer	IB, NCI
	K. Pratt	Senior Staff Fellow	IB, NCI
	D. S. Singer	Senior Investigator	IB, NCI
	T. Suzuki	Visiting Fellow	IB, NCI
	F. Hirsch	Guest Researcher	IB, NCI

COOPERATING UNITS *(if any)*

NIH Animal Center, Poolesville, Maryland
J. K. Lunney, Research Chemist, USDA Animal Parasitology Institute, Beltsville, MD

LAB/BRANCH

Immunology Branch

SECTION

Transplantation Biology 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 stenderd unreduced type. Do not exceed the space provided.)*

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 human transplantation; 2) Assessment of the immunologic parameters involved in tolerance to allografts in this species; 3) Detection and characterization of intra-MHC recombinants: three 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; 4) 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; 5) Production and characterization of monoclonal antibodies reactive with subsets of pig lymphocytes: antibodies corresponding to many of the OKT series in man have been identified (including T4, T8, and T11). The effects of these antibodies on in vitro and in vivo transplantation immunity are being assessed, and they are also being used to assess mechanism of tolerance; and 6) Analysis of MHC genes: Southern blot analyses using cDNA probes from human class II genes have been performed, and indicate that genes corresponding to each of the major human class II loci are present in the pig genome. In addition, a genomic library in the EMBL-3 phage vector has been constructed and screened with these probes. Class II genes from the pig herds have been isolated and are being characterized and used in in vitro and in vivo transfection studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05033-16 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION
Immunotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors D

☐ (a2) Interviews

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 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05035-15 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Function of B Lymphocyte Fcγ Receptors

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

PI:	H. B. Dickler	Senior Investigator	IB, NCI
Others:	G. Lazlo	Visting Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	0.9

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 Fcγ receptors. Previous findings indicate that the Fcγ receptors of B lymphocytes interact with: a) the lymphocyte cytoskeleton, b) Ia antigens, c) LyM antigens, d) surface IgM, and e) surface IgD. Each of these interactions is distinct, specific, and non-random. Studies utilizing antigen-antibody complexes indicate that B lymphocyte Fcγ receptors cross-linked by their physiologic ligand down-regulate B lymphocyte differentiation without affecting proliferation. Resting but not activated B lymphocytes are susceptible to this negative regulation. Occupancy of B lymphocyte surface IgM by a separate ligand is necessary for inhibition to occur, suggesting that the previously described interaction between these two membrane receptors may be involved in generating the negative signal. Studies using monoclonal anti-FcγR antibody (2.4G2) in a variety of forms including native, chemically cross-linked into homodimers or heterodimers with anti-δ or $F(ab')_2$ anti-μ antibodies, and on a Sepharose matrix indicate that the monoclonal antibody only generates the negative regulatory signal if effective cross-linking of the receptor is obtained. Various B lymphocyte populations have been evaluated for susceptibility to Fcγ receptor mediated down-regulation. LyB5 negative B cells are susceptible but antigen-primed B cells are not. B cells from autoimmune MRL/l mice are susceptible but not those from autoimmune NZB mice. Lack of responsiveness to Fcγ receptor downregulation may play a pathogenic role in NZB autoimmune mice.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05036-15 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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:
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.)*

Several hybridomas reactive with nuclease and/or anti-idiotype have been produced and characterized. 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. Examination of the kinetics of inhibition of nuclease with combinations of monoclonal antibodies is being used to determine mechanism of inhibition of catalytic activity of this enzyme. Site-directed mutagenesis of the nuclease gene has provided numerous point mutants of nuclease which are being studied for changes in immune reactivity. Synthetic peptides of nuclease are being examined to determine the relative importance of specific sites for interacting with T cells and antibodies.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05050-13 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

The Manipulation of Immune Processes With Heterocrosslinked Antibodies

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:	D. P. Snider	Visiting Fellow	IB, NCI
	Q. Jia-Hua	Guest Researcher	IB, NCI
	J. Wunderlich	Senior Investigator	IB, NCI
	M. Garrido	Visiting Fellow	IB, NCI
	T. Hecht	Associate Professor	Univ. MD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.5	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.)*

1. Human T cells and K cells targeted with anti-T3 and anti-FcR, heterocrosslinked to anti-tumor antibodies eradicate established human ovarian cancer cells in nude mice.

2. Heterocrosslinked antibodies containing an antibody against a soluble antigen linked to an antibody against a cell surface determinant on an antigen presenting cell (APC), greatly enhance the efficiency of antigen presentation. Enhanced presentation has been seen when antigen was targeted to Fc receptors, surface immunoglobulin, MHC class I and MHC class II molecules on the APC. Targeted antigen presentation is antigen specific and I-A restricted.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05062-12 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	Members of the Immunology Branch (see text)		IB, NCI

COOPERATING UNITS *(if any)* R. Schwartz, Immunol Lab, NIAID; R. Klausner, NICHHD; S. I. Katz, Chief, Dermatology Branch, NCI; A. Schultz, CSL, DCRT; L. Barden, CSL, DCRT; J. D. Shanley, VA Med Ctr, U. of CT; D. DeLuca, U. of SC; L. E. Hood, Cal Tech; T. Springer, Dana Farber Cancer Inst.; H. Young, BRMP, NCI/Frederick.

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	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.)*

Using rapid flow microfluorometry (FMF) for analysis and sorting of cells, aspects of the following projects have been supported this year: (1) studies of the cellular basis for graft rejection and development of tolerance, (2) studies of the pathogenesis of graft-vs-host disease, (3) analysis of the relationship between memory T cells and their expression of adhesion molecules, (4) investigation of the immune system localized in the skin, (5) investigation of murine MHC class I molecules controlled by genes in the Qa-2 region of chromosome 17, and (6) development of an automated computer aid for multiparameter data processing.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05064-11 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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. Gress	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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

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.)*

The influence of class I MHC gene products on selection of the L3T4$^+$ T helper cell repertoire was investigated. It was found that the generation of immune responses specific for K^{bm6} class determinants involved L3T4$^+$ T helper cells specific for a composite MHC determinant composed of I-A^b + K^{bm6} determinants. Most interestingly, it was found that the selection of L3T4$^+$ T helper cells specific for IA^b + K^{bm6} composite determinants required intra-thymic expression of I-A^b + K^b composite MHC determinants. In other words the selection of Ia-restricted Th cells specific for mutant class I determinants required expression by intrathymic cellular elements of self-class II and self class I MHC determinants. This is the first example of class I and class II gene complementation involved in the selection of the T cell repertoire.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05067-12 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of 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. Makgoba	Fogarty Fellow	IB, NCI
	M. Sanders	Medical Staff Fellow	IB, NCI
	G. Ginther-Luce	Chemist	IB, NCI

COOPERATING UNITS *(if any)*
Timothy A. Springer, Ph.D., Department of Membrane Immunochemistry, Dana Farber Cancer Institute, Boston, MA 02115

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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

Our work in the last two years has led to the realization that antigen-independent adhesion is a critical early event in T lymphocyte interactions with other cells, and we hypothesized that there are two molecular pathways by which such interactions occur: T cell CD2 (T11, E-rosette receptor) interacting with its ligand LFA-3 and T cell LFA-1 interacting with an unknown ligand. This year's work has confirmed and extended those concepts. Biochemical studies of purified CD2 and LFA-3 have confirmed the binding of each to the other -- corroborating our inference based on functional studies that LFA-3 is the ligand for CD2. A particularly interesting example of adhesion mediated by these two pathways is the spontaneous rosetting of Reed-Sternberg cells with human peripheral blood T cells. Furthermore, LFA-3 has been shown to be the erythrocyte ligand which mediates autologous rosetting. The concept of two pathways of adhesion has been extended to indicate its relevance to T-cell mediated cytotoxicity (CML) as well as conjugate formation. Studies of CML as well as adhesion demonstrate functionally that ICAM-1 is the principal ligand for LFA-1 in T cell interaction with some targets, but suggest that other as yet unidentified ligands are involved in interactions with other targets. Because of the functional importance of these adhesion molecules, we have carefully investigated their expression on peripheral blood T cells. Our studies demonstrate that expression of LFA-3, CD2, and LFA-1 is increased on a major subset of peripheral blood T cells with the functional properties of memory cells and raise the possibility that such enhanced expression of these functionally important molecules may contribute to the enhanced responsiveness of memory T cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05069-11 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	A. Finnegan	Senior Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION
Immunotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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 stenderd unraduced type. Do not exceed the space provided.)*

A series of T cell clones which recognized the same antigenic molecule in association with a given $E_\alpha E_\beta$ class II molecule were studied for their susceptibility to inhibition by a panel of 15 different anti I-E monoclonal antibodies specific for determinants on the same I-E molecule. The results demonstrated that different T cell clones appear to recognize antigen in association with distinct determinants or conformations on the same I-E molecule. The responses of T cell clones to the mitogen Mycoplasma arthritidis supernatant (MAS) were also analyzed. It was determined that approximately 15% of the clones tested responded to MAS when presented in the context of I-E bearing antigen presenting cells, and that this reactivity was independent of the primary specificity or MHC restriciton of those clones to conventional antigens.

When the ability of different Ia expressing populations to present to cloned T cells was evaluated, all of the clones tested were able to respond to adherent cell-containing irradiated stimulating cells or to mitomycin treated purified resting B cell populations. In contrast, only a subpopulation of cloned T cells was responsive to LPS activated B cell blasts, in spite of the enhanced Ia expression by these B cell blasts, indicating that the signals required for T cell activation may vary among T cell clones, and that distinct Ia bearing antigen presenting cells may be competent for presentation to different T cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05086-09 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	Senior Staff Fellow	IB, NCI
	J. Berzofsky	Senior Investigator	MB, NCI

COOPERATING UNITS *(if any)*
Dr. John A. Smith, Dept. of Pathology, Harvard Medical School, Boston, MA 02114

LAB/BRANCH
Immunology Branch

SECTION
Immunotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	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.)*

The function of accessory cells in primary and secondary in vitro antibody responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L is under the control of immune response (Ir) genes which map to I-A. 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 clones were restricted to recognizing NASE in the context of either $A_\alpha A_\beta$ or $E_\alpha E_\beta$ products. The antigen fine specificity of cloned NASE-specific T cells was also probed through the use of mutant NASE molecules and synthetic peptides corresponding to segments of NASE. A consistent correlation was found between the fine specificity of a given clone and its MHC restriction specificity. $A^b_\alpha A^b_\beta$ restricted clones were selectively responsive to peptide 91-110; $E^k_\alpha E^k_\beta$ restricted clones were responsive to peptide 81-100.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05088-09 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	F. Hakim	Guest Worker	IB, NCI
	C. S. Via	Medical Staff Fellow	IB, NCI
	M. Fukuzawa	Visiting Fellow	IB, NCI
	S. Sharrow	Chemist	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	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 T cells result in a loss in the ability of the F_1 mice to generate T-cell mediated immune responses in vitro to graft-versus-host immune deficiency (GVHID). Recognition of host class II MHC antigens by donor cells is required to initiate GVHID. Recognition of host class I MHC antigens may or may not induce GVHID, depending on the class I determinants required. Recognition of class II only abrogates $L3T4^+$ T helper cell responses but not $Lyt2^+$ T helper cell responses; recognition of class I and II results in loss of both $L3T4^+$ and $Lyt2^+$ T helper cell responses. Induction of GVHID by class I and II recognition requires both $L3T4^+$ and $Lyt2^+$ cells; induction of GVHID by class II only recognition requires only $L3T4^+$ parental T cells.

Inoculation of parental T cells into F_1 mice can also result in different immune abnormalities, depending on the donor and host strains used. Injection of C57BL/6 cells into $B6D2F_1$ mice resulted in extensive immune suppression, hypogammaglobulinema, and susceptibility to infection. Injection of DBA/2 cells into $B6D2F_1$ mice resulted in selective suppression of $L3T4^+$ T helper cell function, hypergammaglobulinema, and autoantibody production with SLE-like symptoms. The differences in these two forms of SLE were attributed to a defect in DBA/2 anti-F_1 CTL precursor frequency.

The parent-into-F_1 GVH reaction also results in severe defects in bone marrow stem cell function, as well as in a defect in the self MHC restriction ability of the F_1 thymus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05099-07 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synergistic Effects of Murine Cytomegalovirus and Graft-versus-host Reaction

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. M. Segal	Senior Investigator	IB, NCI
	J. Titus	Chemist	IB, NCI
	C. Via	Medical Staff Fellow	IB, NCI
	S. Sharrow	Chemist	IB, NCI

COOPERATING UNITS *(if any)*
J. D. Shanley, V.A. Hospital, Newington, CT

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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.)*

Injection of F_1 hybrid mice with either murine cytomegalovirus (MCMV) or parental spleen cells (graft-versus-host reaction - GVHR) results 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. Furthermore, injection of F_1 mice with parental lymphocytes that recognize only class I MHC determinants does not result in immune suppression. However, a combination of class I recognition and MCMV infection results in profound immune suppression. Infection of host mice with MCMV prior to induction of GVH resulted in augmented immune suppression, whereas infection of donor mice with MCMV before induction of GVH resulted in reduced immune suppression. The combination of MCMV and GVHR also resulted in interstitial pneumonitis, whereas either insult alone had no detectable pathogenic effect on the lungs. These studies permit the investigation of the immunosuppression of MCMV infection and the possibility consequences of CMV infection coupled with a GVHR.

Mice were treated in vivo in such a way as to render them deficient in $L3T4^+$ or both $L3T4^+$ and $Lyt2^+$ T cells, and then infected with MCMV. Mice infected with MCMV after L3T4 depletion survived, whereas mice infected after depletion of both $L3T4^+$ and $Lyt2^+$ cells died. This observation may be relevant for determining the T cell subsets important for protection against CMV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05100-07 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

COOPERATING UNITS *(if any)*

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Immunology Branch

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NCI, NIH, Bethesda, Maryland 20892

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

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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.)*

We have served as consultants for other investigators involved in such studies but no work has been performed in this laboratory.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05101-07 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	E. Gugel	Biological Lab Tech	IB, NCI
	E. Long	Investigator	NIAID

COOPERATING UNITS *(if any)*
R. DeMars, U. of Wisconsin, Madison, WI; M. Sanchez-Perez, Universidad Complutense de Madrid, Madrid, Spain

LAB/BRANCH
Immunology Branch

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

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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.)*

Using T cell recognition (primarily cell-mediated lysis) we have continued to probe the complexities of T cell recognition of HLA alloantigens. We have pursued the novel approach of using cytotoxic T lymphocyte (CTL) clones as the selective agent to enrich for ICR-191 mutagenized lymphoblastoid cell lines (LCL) which have lost their capacity to be lysed by that clone. Using that approach, 24 mutant LCL have been derived which are not lysed by the "selecting" DPw2-allospecific CTL clone. Serological analysis confirms the loss of DPw2 and Nothern blot analysis demonstrates loss of DPα or DPβ mRNA in many of the mutants. These mutants have subsequently been used as informative probes to demonstrate the requirement for DPα and DPβ recognition by other putatively "DPw2-specific" CTL clones with more complex specificity. The specificity of these clones suggests that they may recognize peptide fragments of another HLA gene(s) in a DP-restricted fashion.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05103-06 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Structure and Function of Cytotoxic Helper 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
	W. Munger	Staff Fellow	IB, NCI
	J. Bluestone	Laboratory Leader	IB, NCI
	C. Yue	Medical Staff Fellow	IB, NCI
	R. Hodes	Senior Investigator	IB, NCI
	M. Taplits	Medical Staff Fellow	IB, NCI
	R. Quinones	Senior Staff Fellow	IB, NCI

COOPERATING UNITS *(if any)*

R. Gress	Senior Investigator	IB, NCI
R. R. Dourmarshkin		

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Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

The role of granule serine proteases in CTL lytic function was probed by treatment of intact CTL with PMSF in combination with agents which raise the pH of acidic intracellular compartments, and then compared the residual granule BLT esterase activity with the residual lytic activity. PMSF alone caused losses of about 50% in lytic activity and 90% in BLT esterase, while PMSF and ammonium chloride caused losses of about 70% in lytic activity and 99% in BLT esterase. These results suggest that serine esterases in low pH compartments are unlikely to be directly involved in the lytic function. One of the major CTL granule proteases, a BLT esterase, has been useful as a granule marker for measuring exocytosis. With mouse CTL, the enyzme is secreted after stimulation by target cell antigen or immobilized antibodies against the T cell receptor comlex, and with human CTL, immobilized anti-T3 stimulate. In both these systems, anti LFA-1 antibodies block secretion, showing this can act directly on effector cells rather than necessarily blocking a target cell binding. Using in vivo generated CTL, BLT esterse was found in granules by the same approaches used for cloned CTL. Secretion of this enzyme was triggered specifically by tumor target cells and preceded cytotoxicity. Cytolysin activity was found in dense granules of these CTL, but was about 100 x lower than found in cloned CTL. Cloned helper T cells also contain granules with high levels of BLT esterase which reacts with antibodies to the dimeric "major" LGL serine protease. Using BLT esterase release as a marker, granule exocytosis from these cells has been triggered by antigen after processing by class 2 matched presenting cells, by Con A, by PMA and calcium ionophore, and by immobilized anti-T cell receptor antibodies. In the latter situation, secretion can be measured within one hour, and secretion of the lysosomal enzyme β-N-acetyl hexosaminidase is also detected. EM studies of these helper cells have revealed cytoplasmic granules containing striking and unusual lamellar figures undergoing exocytosis. Using β-N-acetyl hexosaminidase release as an assay, granule exocytosis was not observed from normal T cells stimulated by anti-T3 coated beads.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05104-06 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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, leboratory, and institute affiliation)*
PI: G. M. Shearer Senior Investigator IB, NCI

COOPERATING UNITS *(if any)*

T. H. Hansen, Department of Genetics, Washington University, St. Louis, MO

LAB/BRANCH
Immunology Branch

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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

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☐ (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.

No progress was made in this project during the past year. Due to time constraints, other projects have taken priority over this project. Although we continue to provide Dr. Hansen with cell lines from this project, we are not currently working on this project.

PHS 6040 (Rev. 1/84) GPO 914-919

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05106-06 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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. Abe	Visiting Fellow	IB, NCI
	J. Bluestone	Senior Investigator	IB, NCI
	D. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*
Department of Pathology, University of Utah, Salt Lake City, UT

LAB/BRANCH
Immunology Branch

SECTION
Immunotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	1.0	

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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.)*

The cytotoxic T cell repertoire specific for class I allogeneic and xenogeneic determinants was studied. Through the use of radiation bone marrow chimeras, it was demonstrated that responsiveness to K^b mutant determinants was the outcome of unique interactions between both T cell genotype and maturation environment.

Through the use of a transgenic mouse model, in which porcine class I genes had been introduced into the germ line of murine cells, it was demonstrated that normal murine T cells expressed a cytotoxic T cell repertoire specific for xenogeneic class I determinants expressed on mouse cells. This repertoire was cross reactive with the alloreactive cytotoxic T cell repertoire.

The nature of polymorphism and allelism were re-evaluated in the minor lymphocyte stimulating (Mls) system. Mls determinants are defined by the ability to stimulate primary proliferative T cell responses between major histocompatibility complex (MHC) identical cells. Originally, Mls was described as a single locus system involving at least four polymorphic alleles. Proliferating T cell clones were generated which were specific for Mls^a, Mls^c, or Mls^d. These, in combination with primary proliferative T cell responses, were then employed to analyze the relationship between Mls^a, Mls^c, and Mls^d determinants. It was found that Mls^d cells appear to express the sum of Mls^a and Mls^c determinants. In addition, formal genetic analyses were carried out to identify the relationship between the genes encoding Mls^a, Mls^c, and Mls^d. It was found that Mls^a and Mls^c are encoded by non-allelic and in fact unlinked genes. Moreover, an Mls^d strain expresses independently the products of unlinked Mls^a-like and Mls^c-like genes. Thus, in contrast to previous understanding, the Mls system is composed of the products of at least two unlinked loci, with no evidence for structural polymorphism at either locus at the present time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05108-05 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	M. Taplits	Medical Staff Fellow	IB, NCI
	A. Finnegan	Senior Staff Fellow	IB, NCI
	K. Hathcock	Chemist	IB, NCI
	D. Segal	Senior Investigator	IB, NCI
	R. Guy	Visiting Fellow	IB, NCI

COOPERATING UNITS *(if any)*
Department of Immunology, University of Tokyo, Tokyo, Japan

LAB/BRANCH
Immunology Branch

SECTION
Immunotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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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.)*

It had previously been demonstrated that B cell antibody responses can be activated by helper T cells through two distinct pathways. Individual cloned T cell populations are in fact capable of mediating both MHC-restricted and non-MHC-restricted pathways of B cell activation. Recent studies have demonstrated the ability of such cloned helper populations to function in the MHC restricted activation of B cells through an IL-2 dependent pathway. B cell responses mediated by antigen specific T helper cells are regulated by T suppressor cells through two distinct MHC restricted pathways. It was subsequently demonstrated that cloned lines of Lyt 1^+ 2^- $L3T4^+$ antigen specific and MHC restricted suppressor cells could also mediate suppressor effector function in these T dependent antibody responses.

Autoreactive T cell clones, specific for syngeneic I-A or I-E products were shown to function as T helper cells through two distinct pathways: One pathway was polyclonal and MHC unrestricted at the level of T helper-B cell interaction and the other was MHC restricted and dependent upon antigen specific triggering of responding B cells. MHC restricted, antigen-nonspecific suppressor populations which function to regulate responses by carrier specific T helper cells also function to regulate the responses mediated by autoreactive T helper cells. Activation of B cells by antigen specific and autoreactive T helper cells therefore appears to share susceptibility to similar regulatory influences.

The role of T cells in regulating the fine specificity of B cell antibody responses was studied by examining the T15 idiotype dominant response to phosphocholine (PC) and the CRI_A dominant response to Ars. It was found that cloned populations of carrier specific and MHC restricted T helper cells were capable of supporting T15 or CRI_A idiotype dominant responses in B cells of appropriate haplotype. These findings demonstrated that no absolute requirement exists for the participation of idiotype specific T_{H2} cells in the generation of optimally idiotype dominant responses in this experimental system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05110-05 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	H. B. Dickler	Senior Investigator	IB, NCI
Others:	C. S. Via	Medical Staff Fellow	IB, NCI
	R. C. Gallo	Chief	LTCB, NCI
	R. Yarchoan	Senior Investigator	COP, NCI
	S. Broder	Director	COP, NCI
	J. Berzofsky	Senior Investigator	MB, NCI

COOPERATING UNITS *(if any)*

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Immunology Branch

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NCI, NIH, Bethesda, Maryland 20892

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

A

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

Peripheral blood leukocytes (PBL) from HTLV-III antibody (+) and (-) donors were tested for proliferative (^{3}H) and cell-mediated lympholysis (CML) to influenza virus (S+X) and to HLA allogeneic cells (ALLO). Among antibody (+) donors, approximately 50% failed to respond to S+X, whereas all responded to ALLO, except patients in the critical stages of AIDS, who responded to neither type of immunogen.

Using PBL from antibody (-) donors we demonstrated by cell fractionation techniques that S+X T cell responses are obliged to use $CD4^+$ T cells, whereas ALLO responses can be generated by either $CD4^+$ or $CD4^-$ T cells. Thus, the selective loss of S+X responses probably reflects the loss of $CD4^+$ cells during AIDS development.

Using the above approach, we have tested T cell functions in AIDS patients undergoing therapy with the thymidine analog AZT. Some patients undergoing AZT exhibited a restoration of T cell immune function, but this restoration of function has gradually decreased with time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05111-05 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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:	S. McCarthy	Senior 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 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

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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 begun analyzing the factors influencing the regulation of the MHC class II-specific CTL repertoire in vivo. We have used a neonatal injection protocol to study the requirements for induction of antigen-specific tolerance to non-self class II determinants. Our initial experiments have demonstrated that class II allospecific CTL can be effectively tolerized by our protocol and represents (to our knowledge) the first successful tolerization of this T cell population. We can now compare and contrast the recognition and signalling requirements of $L3T4^+$ and $Lyt2^+$ class II-specific immature CTL populations during neonatal tolerance induction, and relate those findings to what we have already established regarding the recognition and signalling requirements for activation of mature class II-specific CTL populations.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05112-05 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Recognition Structures on T Cells

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

PI:	J. A. Bluestone	Senior Investigator	IB, NCI
Others:	D. H. Sachs	Chief, Transplantation Biology Section	IB, NCI
	R. Cron	Howard Hughes Medical Institute Research Fellow	IB, NCI

COOPERATING UNITS *(if any)*
Larry Samelson and Richard Klausner, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development

LAB/BRANCH
Immunology Branch

SECTION
Transplantation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

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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.)*

The recognition structures of T cells have been examined using anti-receptor antibodies and anti-T cell surface antigen specific antibodies prepared against cytotoxic T cell clones. Monoclonal antibodies produced from mice and hamsters immunized against the CTL clones have been identified which react with the T cell receptor and other cell surface antigens. One mAb has been generated that specifically activates CTL clones by binding in a clonotypic fashion to the T cell receptor. This mAb, 83-7-2, precipitates a 90 kd heterodimeric glycoprotein similar to that observed for other anti-receptor antibodies. The activation of CTL by the mAb resulted in lysis of Fc-receptor targets that did not express the nominal alloantigen recognized by the clone. In addition, a series of other monoclonal antibodies which recognize an Ly6-linked molecule on the surface of the cytotoxic T cell clones has been generated. These monoclonal antibodies appear to subdivide class I specific CTL into two distinct populations and one such mAb, 143-4-2 can activate CTL clones and a subset of $Lyt2^+$ T cells. Further studies will be designed to analyze the monoclonal antibodies which have been developed and to better define the structural determinants expressed on cytotoxic T cells which are involved in T cell activation. Most recently, a mAb reacting with the murine equivalent of the T cell receptor associated T3-ε protein has been developed. This monoclonal antibody immunoprecipates the whole T cell receptor complex under mild denaturing conditions. It has been used to identify a novel T cell receptor on the surface of fetal thymocytes, peripheral T cells, and adult thymocytes. This T cell receptor complex includes a newly identified T cell receptor gene, the gamma, delta receptor. Current studies are underway to examine the role of T cells expressing the gamma, delta in T cell development and tolerence induction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05114-04 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	R. Ehrlich	Visiting Fellow	IB, NCI
	H. Golding	Visiting Associate	IB, NCI
	M. Hinners	Chemist	IB, NCI

COOPERATING UNITS *(if any)*

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Immunology Branch

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NCI, NIH, Bethesda, Maryland 20892

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

The aim of this work is to determine the DNA sequence organization of class I genes contained in the swine major histocompatibility complex (SLA). We have established that this gene family in the miniature swine contains only seven members, making it amenable for a comprehensive analysis. To date, we have isolated six of these genes. DNA sequence analysis is complete for three of the genes and nearly complete for two more. It has been established that the sequence organization of the class I SLA genes is similar to that of other class I genes. Within the family, it is possible to define at least two sub-families, based on their sequences. Homologies between the SLA genes range from 85% between the two genes within a sub-family, to 60% between sub-families. Complex alterations are observed, consistent with the interpretation that some mechanism of gene conversion may operate to generate polymorphism in this family. Physical mapping of the genes has established a tight linkage of the family, consistent with genetic data.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05115-04 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

PI:	D. S. Singer	Senior Investigator	IB, NCI
Others:	R. Ehrlich	Visiting Fellow	IB, NCI
	J. Maguire	Biotechnology Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

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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.)*

The aim of this work is to investigate the mechanisms controlling the expression of a multi-gene family, namely the class I MHC genes. The miniature swine has been chosen as an experimental model because there are only 7 members of the family, of which 6 have been isolated. Five have been extensively structurally characterized. To address the question of the molecular regulation of the expression of the class I MHC genes, two approaches have been taken: 1) analysis of in vivo patterns of expression of each of the genes in a variety of tissues both in the miniature swine and in transgenic mice containing only a single swine gene, and 2) characterization of regulatory elements associated with these genes. Three categories of MHC genes have been identified this way: 1) A set of closely related genes each of which are expressed in L cells and in nearly all swine somatic tissues, although at different levels. At least one of these genes is also expressed in a transgenic mouse with the same tissue distribution as in the swine. These genes encode products which are expressed on the cell surface and are able to bind a monoclonal antibody which recognizes a common determinant, also found on classical transplantation antigens. 2) A distantly related gene which is expressed both in L cells and in vivo but whose pattern of expression is distinct from that of transplantation antigens. 3) A set of genes which is expressed neither in L cells nor in vivo.

Regulatory sequences within one of the transplantation antigen genes have been identified by generating a series of 5' end deletion mutants. The transcriptional promoter has been functionally identified as well as an interferon-responsive element. In addition, novel positive and negative regulatory sequence elements have been identified. It has been further shown that these elements function through the binding of transacting factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05116-04 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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, leboratory, and institute affiliation)*

PI:	R. E. Gress	Senior Investigator	IB, NCI
Others:	R. R. Quinones	Senior Staff Fellow	IB, NCI
	R. Moses	Biotechnology Fellow	IB, NCI
	H. Nakamura	Visiting Fellow	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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 standerd unreduced type. Do not exceed the space provided.)*

Efforts are directed towards the prevention or control of graft-versus-host disease in human allogeneic bone marrow transplantation. Since graft-versus-host disease is mediated by alloreactive T cells in the inoculated marrow, reagents and techniques have been developed to remove these T cells from the marrow inoculum. Murine monoclonal antibodies specific for antigens expressed on human T cells have been developed and utilized for complement-mediated lysis of T cells in marrow. By a new limiting dilution assay, residual T cells in marrow following depletion are at a level less than 0.01% of the total cell population. A bank of such T cell depleted, characterized marrows has been generated for use in the therapy of human malignancy. The potential of such T cell depleted marrow to successfully hematologically and immunologically reconstitute a host has been examined in murine models. The role of T cells in the infused marrow and the susceptibility of host rejecting cells to radiation and monoclonal antibodies administered in vivo are also under study. Within the T cell population, the fine specificity of human CTL has been demonstrated to be sufficient to distinguish among alpha 1 and alpha 2 domain changes of class I major histocompatibility complex molecules. Such human cytotoxic T cells have been further studied with respect to cell surface molecules utilized in their interactions with target cells as the basis for therapeutic interventions with the intent of preventing tissue damage mediated by such alloreactive cytotoxic T cells. It has been shown that inhibition by a monoclonal antibody with specificity for CD18 occurs in the absence of target cells thereby raising the possibility that this inhibition is independent of cell-cell adhesion. Further studies have identified the site of this inhibition as involving cell surface molecule interaction or the T cell receptor associated G protein. This in vitro generated information has been applied to bone marrow transplantation models in monkey and swine. Utilizing T cell depleted marrow, extended solid organ allograft survival (without exogoneous immunosuppression), but not long term tolerance induction, can be achieved in primates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05117-04 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	Senior Investigator	IB, NCI
Others:	J. A. Lewis	Howard Hughes Medical Fellow	IB, NCI

COOPERATING UNITS *(if any)*
S. G. Nathenson and S. Geier, Dept. Microbiology & Immunology, Albert Einstein Col. of Med., Bronx, NY; David Margulies, Laboratory of Immunology, NAIAD; T. V. Rajan and Terry Potter, Dept/Genetics, Albert Einstein Col. of Med., Bronx, NY

LAB/BRANCH
Immunology Branch

SECTION
Transplantation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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 spece provided.)*

Current efforts have been devoted to examining the nature of the allo-determinants recognized by cloned T cell populations. To examine this question, H-2 structural mutants have been isolated from a somatic cell line by mutagenesis and immuno-selection 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. Analysis of the in vitro-derived mutants has shown that new determinants are created by the mAb immunoselection procedure which can be recognized by cytotoxic T cells. The new allodeterminants expressed on the in vitro-derived CTL can function as transplantation antigens in vivo and appear linked to a single amino acid substitution. 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-2L^d and H-2D^d genes. The findings suggested that unlike mAbs which can recognize individual epitopes on different domains, a majority of the CTL clones recognize determinants influenced by the interaction of the two external domains. In one instance, CTL can recognize a hybrid D^d/L^d molecule (T9-10-3). However, the CTL employ a restricted T cell receptor (TcR) V_β chain family. Other altered MHC class I genes have also been examined including L cells which have been transfected with truncated L^d and D^d genes and express only the $\alpha 3$/TM portion of the molecule. The finding demonstrated that CTL can be generated against truncated MHC gene products. Finally, the role of the MHC α_3 domain has been examined. In some instances, CTL clones which recognize native K^b-transfected L cells do not recognize MHC hybrid molecules using a human MHC α_3 domain. In addition, a single point mutation at amino acid 227 in the H-2D^d molecule leads to the total loss of CTL recognition of that MHC antigen. Thus, the specificity of CTL although predominently determined by the $\alpha_1 + \alpha_2$ domains is critically influenced by α_3.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05118-04 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Regulation of Immune Response to Tumor Cells and Alloantigen

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

PI:	C. C. Ting	Senior Investigator	IB, NCI
Others:	M. E. Hargrove	Microbiologist	IB, NCI
	J. Bluestone	Senior Investigator	IB, NCI
	J. Wunderlich	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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 standerd unreduced type. Do not exceed the space provided.)*

1. Expression and Function of Asialo GM1 (AsGM1) in Alloreactive Cytotoxic T Cells (CTL) and in Lymphokine-Activated Killer (LAK) Cells. AsGM1 was expressed differently in CTL and LAK cells. AsGM1 was expressed on the LAK precursors but its expression disappeared when LAK precursors were fully differentiated into effectors. The reverse was true for CTL, AsGM1 was not expressed on CTL precursors but was readily demonstrable in 3-day-cultured CTL. Among eleven CTL clones tested, AsGM1 was found to be expressed on a majority (7 out of 8) of $L3T4^-$ CTL clones. The cytotoxicity mediated by $AsGM1^+$ cloned CTL was blocked by αAsGM1 or AsGM1 alone, indicating that AsGM1 is involved in the CTL- target interaction to mediate lytic reaction.

2. Tumor Immunology. Activated killer (AK) cells were generated in spleen cell culture derived from tumor bearing hosts (TS). In many aspects, these AK cells resembled LAK cells. The major difference was that the LAK precursors from normal hosts were $AsGM1^+$ cells and the LAK precursors from TS were $AsGM1^-$, suggesting that the latter was in an "activated" state. These findings indicate that in the tumor bearing hosts, tumor cells trigger the activation of LAK precursors, but the same tumor cells may be immunosuppressive which prevents the full differentiation of LAK precursors into LAK effectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05119-04 I

PERIOD COVERED: October 1, 1986 to September 30, 1987

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 Associate	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 20892

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.)*

We demonstrated previously that the recognition repertoires of $L3T4^+$ T helper cells and $Lyt2^+$ T helper cells were very distinct. In contrast to $L3T4^+$ T helper cells, $Lyt2^+$ T helper cells responded only to class I alloantigens. This year, the recognition repertoires of $Lyt2^+$, Th and CTL were investigated.

Both CTL and Th activities of $Lyt2^+$ T cells from B6 ($H\text{-}2^b$) mice against a series of K^b mutant determinants were compared. Although all K^{bm} determinants examined stimulated B6 $Lyt2^+$ CTL precursors, the various K^{bm} determinants differed dramatically in their ability to stimulate B6 $Lyt2^+$ T cells to function as IL-2 secreting helper cells. Particularly, K^{bm6} determinants only stimulated B6 $Lyt2^+$ T cells to become cytolytic but failed to stimulate them to secrete IL-2. These results demonstrated that the recognition requirements for stimulating primary $Lyt2^+$ T cells to secrete IL-2 and to function as Th cells are distinguishable from those for stimulating primary $Lyt2^+$ T cells to become cytolytic and to function as CTL effector cells.

Finally, the role of the thymus in inducing the self-tolerance of $Lyt2^+$ IL-2 secreting Th cells and IL-2 dependent CTL effector cells was assessed by using allogeneic thymus engrafted B10 athymic nude mice. It was demonstrated that those functionally distinct $Lyt2^+$ Th and CTL differed in their tolerization to thymic class I MHC determinants, namely $Lyt2^+$ Th but not CTL are tolerant to class I MHC antigens expressed in the thymus.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05120-04 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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
Others:	S. Irving	Guest Researcher	IB, NCI

COOPERATING UNITS *(if any)*
Ulrich Siebenlist, Laboratory of Immune Regulation, NIAID, NIH

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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.)*

The goal of this project is to broaden our understanding of the physiology and regulation of early events in T cell activation. Lymphocyte metabolism and effector function expression are regulated by antigen/mitogen and lymphokine binding to cell surface receptors. We are investigating consequences of mitogen 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 encode functions that are fundamentally important for the initiation of proliferation and effector function expression in these cells. Known induced early genes include oncogenes (c-myc and c-fos), lymphokines (IL-2, γ-IFN, GM-CSF), and lymphokine receptors (IL-2 receptor), all of which are thought to have significant effects on T cell proliferation and effector function. We have constructed a subtracted cDNA library enriched for genes that are transcriptionally induced within four hours after stimulating peripheral blood T cells with PHA and PMA. After amplification, 20,000 phage were screened with a subtracted cDNA probe enriched for mitogen-induced mRNA's, and 528 positively hybridizing clones were isolated. Cross-hybridization studies show that greater than forty unique genes have been isolated. Although known induced genes (c-myc and IL-2 receptor) are included within these clones as expected, the large majority of clones represent novel, as yet undescribed genes. We have begun characterizing inducible gene sequences with regard to 1) structure, i.e. sequencing analyses, 2) expression pattern analyses that allow broad categorization of potential function, and 3) expression pattern analyses that define distinct patterns of gene regulation. Initial results indicate that a variety of classes of genes have been isolated including T cell-specific, lymphocyte-specific, and proliferation-specific genes. In addition, distinct regulatory networks acting upon these induced genes have been defined by differential kinetics of expression and discriminating effects of the drug cyclosporin A and the HTLV-I encoded trans-activating factor (TATI) upon transcription patterns.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05122-03 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

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INSTITUTE AND LOCATION
NIC, NIH, Bethesda, Maryland 20892

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.)*

This project continues our studies of the cellular interactions involved in skin allograft rejection. The focus of our studies is the ability of phenotypically distinct, class I specific Th cells to participate in the rejection of class I disparate skin grafts. We have previously established the importance of $Lyt2^+$ Th in the rejection of class I disparate grafts. Further, we have shown that $L3T4^+$ class I specific Th fail to participate in the rejection of these grafts because of the failure of a single epidermal cell population to express the determinants necessary for their activation. We are currently exploring the conditions under which these class I specific $L3T4^+$ Th can be activated in vivo. We have taken two approaches: the first involves attempts to increase the expression of the determinants known to trigger this cell on epidermal cells, and second, to trigger these cells by a route other than skin grafting. A second phase of the project explored the abilities of phenotypically distinct Th populaitons to interact with separate effector cell populations in rejecting skin grafts. This was assessed by measuring whether these Th populations could collaborate with effector cells of a different specificity. We have previously demonstrated the ability of $L3T4^+$ Th specific for class II determinants to function in this way. However, $Lyt2^+$ class I specific helpers fail to do so, suggesting that they may induce graft rejection via a "dual function" mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05124-03 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Function of a Porcine Class I MHC Gene in Transgenic Mice

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

PI:	D. Singer	Senior Investigator	IB, NCI
Others:	J. Bluestone	Laboratory Leader	IB, NCI
	R. Hodes	Chief, Immunotherapy Section	IB, NCI
	W. Frels	Agricultural Research Service	USDA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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.)*

A porcine class I major histocompatibility complex (SLA) gene has been introduced into the genome of a C57BL/10 mouse. This transgenic mouse expressed SLA antigen on its cell surfaces and transmitted the gene to off-spring, in which the gene is also expressed. Skin grafts of such transgenic mice were rejected by normal C57BL/10 mice, suggesting that the foreign SLA antigen expressed in the transgenic mice is recognized as a functional transplantation antigen. The cellular basis for this recognition is under investigation.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09200-02 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Production of Mab Specific for B Lymphocyte Receptors for Lymphokines

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

PI:	G. Laszlo	Visiting Fellow	IB, NCI
Other:	H. B. Dickler	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	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.)*

The goal of this project is to identify and characterize B lymphocyte receptors for lymphokines via the production of monoclonal antibodies. A secondary goal is development of monoclonal antibodies which distinguish functionally distinct subpopulations of B lymphocytes. Monoclonal antibody F1-10 detects a determinant preferentially expressed on activated B lymphocytes. Expression peaks 60 hours after activation using LPS. The F1-10 determinant is also expressed on splenic B lymphocytes and a subpopulation of bone marrow cells but at much lower levels and is absent from thymocytes, splenic T lymphocytes, and activated T lymphocytes. Mapping studies using BXD recombinant inbred mice indicate a gene controlling expression of the F1-10 determinant is located on chromosome 17 between hbap-4 and acry-1. These results together with a unique strain distribution pattern suggest that F1-10 is detecting a previously undescribed determinant selectively expressed on activated B lymphocytes which may be a receptor for lymphokines. A new hybridoma screening method has been developed using particle concentration fluorescence which is capable of detecting antibodies binding to molecules which are expressed at very low levels (500-1000 molecules per cell).

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09201-02 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	H. B. Dickler	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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.)*

The goal of this project is to characterize interactions between B lymphocyte subpopulations which regulate responses of these cells. Previous work from this laboratory showed that a B lymphocyte hybridoma (2.4G2) produced a low molecular weight substance(s) which triggered B lymphocytes to both proliferate and secrete antibody. This result suggested that certain B lymphocytes might regulate the response of other B cells. Our current studies have shown that large "activated" B lymphocytes obtained directly from mice or B lymphoblasts induced in vitro with $F(ab')_2$ anti-mu significantly augment the responses of small "resting" B lymphocytes to $F(ab')_2$ and lymphokines. Proliferation was augmented 2-4 fold while antibody production was augmented 4-5 fold. This effect was specific for "activated" B lymphocytes in that other cell types did not have this effect. Kinetic experiments revealed that the augmenting signal was effective after stimulation via antigen receptors but prior to the effects of lymphokines. The augmenting effect does not appear to be genetically restricted. Investigation of the nature of the signal revealed that neither supernatants nor plasma membranes from activated B cells alone augmented responses but both together did. These studies suggest that interactions between B lymphocytes are important in regulating humoral immune responses.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09202-02 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charactars or less. Titla must fit on ona lina between tha borders.)*

Characterization of T Cell Receptor Genes in Alloreactive Clones

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

PI:	Hana Golding	Fogarty Visiting Scientist	IB, NCI
Others:	Dinah Singer	Senior Investigator	IB, NCI
	William Biddison	Senior Investigator	NI, N

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.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.)*

A panel of alloreactive T cell clones derived from a single donor has been established and characterized for its fine specificity patterns. It has thus been possible to assign each of the clones to one of four fine specificity groups. In order to attempt to correlate fine specificity with receptor gene utilization, the TcR alpha and beta variable gene segments used by these clones are being analyzed. An alpha variable gene derived from a member of the most common group is being isolated from a restricted cDNA library. Its representation in the other members of the panel will be assessed.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09203-02 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation and Characterization of a Novel H-2 Class I Gene

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

PI:	D. Singer	Senior Investigator	IB, NCI
Others:	S. Rudikoff	Senior Investigator	LG, NCI
	J. Hare	Summer Guest Worker	IB, NCI
	H. Golding	Visiting Associate	IB, NCI

COOPERATING UNITS *(if any)*

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Immunology Branch

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	

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.)*

A new sub-family of H-2 class I genes has been identified in the genome of the C57BL/10 mouse. The family consists of at least two genes which have not been previously identified. One of these, Mbl, has now been extensively characterized. Using a series of recombinant strains of mice, and taking advantage of restriction enzyme polymorphisms, it has been possible to map the Mbl gene to the right of the Qa locus. DNA sequence analysis of Mbl demonstrates that the exon organization of this gene resembles that of other class I genes. Furthermore, it is capable of encoding a transmembrane protein with a structure similar to other class I molecules. However, the level of DNA sequence homology of Mbl to other H-2 genes is no greater than to either human, pig, or rabbit. Furthermore, the over-all organization of Mbl is more similar to man and pig than to mouse. Whereas all previously reported H-2 class I genes have third introns of 1.2-2 kb, Mbl has an intron of 600 bp, similar to those of human and porcine class I genes. Taken together, these data suggest that Mbl may represent a direct descendant of a primordial class I gene, which antedates speciation. In support of this conclusion is the observation that a variety of wild mouse, representing millions of years of evolutionary divergence, contain Mbl in their genomes. Although other members of the Mbl family appear to be expressed, no direct evidence for Mbl expression has been obtained, despite the fact that it is a structurally functional gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09204-02 I

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Function of Accessory Molecules in T Cell Interactions

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

PI:	S. McCarthy	Senior Staff Fellow	IB, NCI
Other:	E. Kaldjian	Howard Hughes Fellow	IB, NCI
	A. Singer	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Immunology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	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.)*

Precursor and effector CTL specific for determinants present on allogeneic MHC class I molecules have previously been shown to bear the Lyt2 cell surface phenotypic marker. Lyt2 is believed to be involved in the function of these cells because antibodies directed against Lyt2 block the cytolytic activity of most in vitro-generated MHC class I allospecific mature CTL. Based on those studies, Lyt2 has been proposed to be either an accessory molecule contributing to antigen specificity, or an "off" signal in mature. CTL. Our studies have focused on the role of Lyt2 during the initial activation of class I-specific CTL in vitro. CTL induced in vitro in the presence of anti-Lyt2 monoclonal antibody are resistant to subsequent blocking of lytic effector function by anti-Ly2 antibody, in sharp contrast to CTL generated in conventional (antibody-free) cultures. We have used limiting dilution assays to demonstrate that anti-Lyt2 monoclonal antibody actively induces the generation of Lyt2-resistant CTL effector cells, and does not merely permit the selective outgrowth of a minority population of Lyt2-resistant precursor CTL. Furthermore, in contrast to conventional class I-specific effector cells, CTL activated in the presence of anti-Lyt2 antibody undergo down-modulation of their cell surface Lyt2, and exhibit an Lyt2-"dull" phenotype. We are currently investigating the functional relationship between antibody-induced down-modulation of Lyt2 and Lyt2-resistant CTL activity. Since CTL with these unusual characteristics are generated only in the presence of anti-Lyt2 antibody, Lyt2 engagement by antibody during activation may initiate an alternative differentiation program in at least some precursor CTL. Thus, Lyt2 may function to generate an "on" signal during CTL activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09205-02 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptor Mediated T 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:	K. S. Hathcock	Chemist	IB, NCI
	D. M. Segal	Senior Investigator	IB, NCI
	M. Taplits	Medical Staff Fellow	IB, NCI
	E. Anglade	Howard Hughes Fellow	IB, NCI
	P. Henkart	Senior Investigator	IB, NCI
	K. Kelly	Senior Investigator	IB, NCI
	J. Bluestone	Senior Investigator	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION
Immunotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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 mechanism of T cell activation has been studied employing both cloned T cell populations and naive heterogeneous T cell populations. Comparison has been made of T cell stimulation by antibodies directed to allotypic determinants on the T cell receptor, T cell activation by specific antigen, and T cell activation by non-receptor-mediated signaling. It has been demonstrated that both cloned and naive T cells can be triggered by the monoclonal antibody F23.1, directed toward determinants on the T cell receptor. Naive $Lyt2^+$ T cells were activated to proliferate in the presence of soluble F23.1, IL-2, and accessory cells. Under the same conditions, $L3T4^+$ naive T cells were unresponsive. These findings thus demonstrated a difference in the activation requirements of T cell subpopulations triggered through T cell receptor determinants. Specific "targeting" of the T cell receptor to accessory cell structures by heteroaggregates of F23.1 coupled to monoclonal anti-Ia antibody were, in contrast, capable of activating both $Lyt2^+$ and $L3T4^+$ T cell subpopulations. The nature of activation signals provided under these diverse conditions is currently under study. In particular, the function of endogenous lymphokines is being analyzed. Parameters of T cell response including specific gene activation and exocytosis of cytoplasmic granules in response to diverse stimuli have been evaluated.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09206-01 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vivo Treatment With Monoclonal Anti T Cell Receptor Antibodies

PRINCIPAL INVESTIGATOR *(List other profassional personnel below the Principal Investigator.) (Neme, title, laboretory, and institute affiliation)*

PI:	J. A. Bluestone	Senior Investigator	IB, NCI
Others:	R. Hirsch	Medical Staff Fellow	IB, NCI
	D. H. Sachs	Chief, Transplantation Biology Section	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION
Transplantation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

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.)*

Monoclonal antibodies against T cell surface markers have been used experimentally in vivo in an attempt to delete T cells and allow for subsequent tolerence to foreign transplantation antigens. One such monoclonal antibody OKT3, directed against the CD3 delta chain of human T cell receptor complex has been shown to have profound immunosuppressive effects on transplantation rejection responses in vivo. We have developed a hamster monoclonal (145-2C11) directed against the CD3 epsilon chain of the murine T cell receptor complex. Like its anti human CD3 counterpart, it can significantly effect transplantation responses in vivo. Animals treated with small quantities of the anti-T3 antibody exhibit prolonged skin graft rejection by as much as three weeks. In addition, cells removed from anti-T3 treated mice are unresponsive to a variety of alloantigens including both class I and class II. Future studies will be designed to examine the role of in vivo treatment with anti T3 on bone marrow engraftment and abrogation of GVH and HVG responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09207-01 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
T Cell Immune Deficiency in Mice and Humans With Autoimmune Disease

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

PI:	G. M. Shearer	Senior Investigator	IB, NCI
Other:	C. S. Via	Medical Staff Fellow	IB, NCI
	G. C. Tsokas	Senior Investigator	ARB, NIADDKD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.5	0.2

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 spece provided.)*

Spleen cells from Mrl-lpr mice gradually develop a loss of $L3T4^+$ T helper cell function that is age-dependent which is concomitant with the appearance of anti-DNA antibodies and of suppressor cells that selectively suppress only $L3T4^+$ T helper cell function. This observation contrasts with Mrl +/+ mice that exhibit none of the above during the same time period. Similar results were observed in the $(NZBxNZW)F_1$ autoimmune strain.

Human patients with SLE exhibit a similar selective loss of CD4 T helper cell function.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09208-01 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Human T Cell Responses by Adherent 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

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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.)*

Depletion of human peripheral blood leukocytes (PBL) of Leu M3$^+$ cells by adherence to plastic and sephadex G10 results in the abrogation of proliferative (^{3}H) and cytotoxic T lymphocyte (CTL) responses to influenza A virus (FLU), but in an elevation of ^{3}H and CTL responses to HLA alloantigens (ALLO). This loss of the FLU T cell response was attributed to removal of antigen-presenting cells (APC), whereas the elevation of the ALLO response was shown to be due to a suppressor cell (or a suppressor inducer cell) that is contained in the Leu M3$^+$ adherent cell population. Suppressor activity was inactivated by culturing either unfractionated PBL or adherent cells with viruses, including influenza A, measles and mumps viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09209-01 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Homologous Peptides from HIV gp41 and HLA Class II Bind CD4 on Human T Cells

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

PI:	H. Golding	Visiting Associate	IB, NCI
Others:	D. Singer	Senior Investigator	IB, NCI
	F.A. Robey	Senior Investigator	NIDR
	B. Golding	Senior Investigator	DBP, FDA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

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.)*

The CD4 molecule has been identified as the receptor for HIV envelope protein. Recently, the natural ligand for CD4 on antigen presenting cells has been tentatively localized to the N-terminal domain of the beta chain of MHC Class II. It ws postulated that the MHC Class II and HIV bind the non-polymorphic CD4 via similar conserved regions. A hydrophilic septamer was identified displaying a high degree of homology between gp41 of HIV and the beta-1 domain of HLA-DR and -DQ. Both the HIV and MHC Class II derived sepatmers were synthesized. Incubation of these peptides, but not control peptides, with CD4 positive cells at 37°C for 45 min resulted in reduced binding of anti-CD4 antibodies (OKT4, OKT4a and Leu3) to the cells. This peptide mediated reduction of binding to CD4 could be blocked in the presence of chloroquine. The binding of antibodies directed against other surface antigens, were unaffected by pre-incubation with the peptides. The temperature requirement and the sensitivity to chloroquine suggest that the peptides induced partial modulation of the CD4 molecules via receptor mediated endocytosis. In addition, flow cytometry showed that biotinylated chicken albumin conjugates of the peptides can bind directly to CD4 bearing CEM cells, but not to a CD4 negative CEM mutant or to B cell lines. This binding could be partially inhibited in the presence of mouse monoclonal anti-CD4 antibodies. Therefore, these findings suggest that the homologous regions of HIV and MHC Class II, which we have identified, may be the sites involved in binding of AIDS virus and MHC Class II antigens to CD4 on human T cells. Rabbit antiserum and murine mAb specific for the HIV derived peptide were found to recognize intact inactivated virions and also stain native human class II molecules on B cell lines and on murine L cell transfected with DR, DQ and DP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09210-01 I

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Induction of Class I MHC Gene Expression by Ethanol

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

PI:	D. Singer	Senior Investigator	IB, NCI
Others:	M. Kolber	Medical Staff Fellow	IB, NCI
	L. Parent	Guest Researcher	IB, NCI
	R. Wall	Guest Researcher	G.W.U. Hospital

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

Ethanol enhances expression of cell surface class I MHC antigens in a variety of cell lines. In an embryonic cell line, this increase is up to 10-fold. Ethanol treatment of L cells also induced increased cell surface expression of MHC antigens, with a concomitant increase in steady-state levels of RNA. This effect is promoter dependent and restricted, since not all gene products are elevated. The effective ethanol concentration (1%) is physiologically attainable. In a study of chronic alcoholics, it was found that the levels of Class I MHC antigens on their PBL was significantly higher than in normal controls.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 09211-01 I

PERIOD COVERED
October 1, 1986 to December 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Type Specific Regulation of the T Cell Receptor β Chain

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

PI:	K. Kelly	Lab Leader	IB, NCI
Others:	M. Kearns	Guest Researcher	IB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Immunology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

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.)*

The genetic regulatory mechanisms that govern tissue specific expression of the T cell receptor β chain have been investigated utilizing an in vitro model of cell type specificity. A transient expression system has been used to assay the transcription of a genomic TCF β chain gene (including 5 kb 5' of a rearranged $V_{\beta 1}$-J-C clone) in T cells, fibroblasts, and a variety of hematopoietic tumor cells. DNA sequencing of the $V_{\beta 1}$ leader and an additional 400 bp 5', in conjunction with S1 nuclease protection assays, has identified the start site of transcription. Also, a putative regulatory hexamer, CTTTCT, that is conserved in several human and murine V_{β} genes has been identified approximately 250 bp 5' to the mRNA cap site. Transfection efficiencies were normalized by determining the mRNA levels of a truncated histone gene contained within the plasmid vector as a tissue nonspecific control. Unlike immunoglobulin genes, expression of a rearranged $C_{\beta 1}$ gene was found in nonlymphoid cells. However, it was observed that T cells show a minimum of a 3 fold preferential expression of the β chain as compared to fibroblasts and monocytic cells. Furthermore, a 1.5 kb deletion in the 5' region of the $J_{\beta 1}$-$C_{\beta 1}$ intron does not preclude β chain expression in T cells or fibroblasts. Tissue specific regulation of T cell receptor β chain expression does not appear to require gene sequences within this region of the intron, but may be influenced by a conserved region of the gene 5' to the promotor.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03200-18 LCBGY

PERIOD COVERED

October 1, 1986 through September 30, 1987

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)*

PI:	L. W. Law	Chief, Lab. of Cell Biology	LCB, NCI
OTHER:	E. Appella	Medical Officer (Res.)	LCB, NCI
	V. J. Hearing, Jr.	Research Biologist	LCB, NCI
	E. A. Robinson	Chemist	LCB, NCI
	S. J. Ullrich	Sr. Staff Fellow	LCB, NCI
	W. D. Vieira	Microbiologist	LCB, NCI

COOPERATING UNITS *(if any)*

Memorial Sloan-Kettering Cancer Center, New York, NY
Pittsburgh Cancer Institute, Pittsburgh, PA
Frederick Cancer Research Facility, Frederick, MD

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

Class I (restricted) and Class II (crossreacting) tumor antigens of the trans-plantation rejection type (TATA), and of tumor antigens (TA) assayed by in vivo and in vitro techniques and of the immune responses they evoke have received major emphasis. 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. 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.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 03229-18 LCBGY

PERIOD COVERED

October 1, 1986 through September 30, 1987

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)*

PI:	E. Appella	Medical Officer (Res.)	LCB, NCI
OTHER:	M. J. Darsley	Visiting Fellow	LCB, NCI
	S. K. Moore	Sr. Staff Fellow	LCB, NCI
	E. A. Robinson	Chemist	LCB, NCI
	S. J. Ullrich	Sr. Staff Fellow	LCB, NCI
	K. Ozato	Res. Microbiologist	LDMI, NICHD
	E. D. Korn	Chief, Lab. Cell Biol.	LCB, NHLBI

COOPERATING UNITS *(if any)*

Weizmann Institute of Sci., Rehovot, Israel
ENEA-Euratom Immunogenetics, Lab. of Pathology, C.R.E., Casaccia, Rome, Italy

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Chemistry

INSTITUTE AND LOCATION

NCI, NIH, BETHESDA, MD 20892

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.)*

The major area of our research involves the molecular structure of histocompatibility antigens, T cell suppressor receptors and tumor antigens using a combination of protein and DNA sequencing in conjunction with peptide and nucleotide synthesis. Site directed mutagenesis and recombinant DNA constructs were used to elucidate the contribution of individual amino acids as well as of the individual 1/ 2 domains of class I antigens toward conformational serological epitopes and CTL reactivity.

The complete cDNA sequence of the and chains of the T cell receptor of a T suppressor clone was determined and was found to use the same pool of gene segments used in T-helper and cytotoxic cells. These clones will be used to investigate the role of these genes in conferring suppressor activity.

Residues 105-120 of hen egg lysozyme were found to correspond to the immunodominant epitope recognized by I-E^d restricted T cell hybridomas. By using various synthetic peptides, individual amino acids involved in antigen/T-cell receptor and antigen/I-E^d molecule interactions were determined.

A tumor-specific tranplantation antigen was identified as a heat shock protein consisting of two isoforms; both isoforms were found to be members of multigene families dispersed on several chromosomes. Complete nucleotide sequence of one isoform and partial nucleotide sequence of the second isoform were determined and genomic clones for both isoforms have been isolated. The regulation of the synthesis of three isoforms were examined in normal and transformed cells at the transcriptional and translational level. Further analysis should permit identification of the molecular changes in these antigens which elicit cell mediated immunity.

DNA sequences predicted to have aberrant helices with hairpin stems and loops have been crystallized and preliminary X-ray structures have been solved.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09100-4 LCBGY

PERIOD COVERED

October 1, 1986 through September 30, 1987

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)*

PI:	Vincent J. Hearing, Jr.	Research Biologist	LCB, NCI
Other:	Lloyd W. Law	Chief, Lab. of Cell Biology	LCB, NCI
	Ettore Appella	Medical Officer (Res.)	LCB, NCI
	Mercedes Jimenez-Atienzar	Visiting Associate	LCB, NCI
	Koichiro Kameyama	Visiting Fellow	LCB, NCI

COOPERATING UNITS *(if any)*

Columbia University, New York, NY
Georgetown University Medical Center, Washington, D.C.
Pittsburgh Cancer Institute, Pittsburgh, PA

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Chemistry

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

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

This project is aimed at characterizing: (1) the host immune response to malignant melanoma, and the role it plays in the progression of tumor growth and metastatic spread; (2) tumor specific proteins produced by melanoma cells in vivo and in vitro, to determine the mechanism of their formation, to examine the impact of their expression on tumor growth, and to study the feasibility of utilizing their specificity for the immunoassay and immunotherapy of melanoma; (3) the role of cell surface proteases in the cascade of events leading to metastatic spread of tumors; (4) the control mechanisms involved in the regulation of pigment production in normal and in transformed melanocytes. The 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 tumor rejection (TSTA); these antigens have a specificity restricted to melanoma cells. One murine melanoma however (S91) has a unique TSTA, and studies are underway to characterize its antigen(s). We have shown that immunized mice produce high titers of melanoma-specific cytotoxic antibodies, and that this B cell response may account for the observed tumor rejection, since we have been unable to demonstrate any T cell response, thus far. We have found that surface urokinase activity significantly affects the metastatic potential of melanoma cells, suggesting that this protease plays an important role in the metastatic sequence. We have produced and utilized monoclonal antibodies specific for the melanocyte specific enzyme, tyrosinase, to examine cellular control mechanisms functional in the response of melanocytes to varying environmental stimuli which affect pigmentation, such as melanocyte stimulating hormone.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08525-11 LIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Zbar	Chief, Cellular Immunity Section	LIB NCI
	T. Borsos	Chief, Laboratory of Immunobiology	LIB NCI
OTHER:	G. Glenn	Medical Staff Fellow	LIB NCI
	T. Yano	Visiting Fellow	LIB NCI

COOPERATING UNITS *(if any)*

	B. Szende	Semmelweis Medical University, Budapest, Hungary

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Cellular Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, NIH, Frederick, Maryland 21701

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.)*

The primary objective of this project was to evaluate the therapeutic efficacy of various biologic response modifiers in animals with primary cancer as a guide for treatment of human cancer. This project has been terminated.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08528-11 LIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

COOPERATING UNITS *(if any)*

J. Talmadge	Program Resources Inc., NCI-FCRF
B. McEwen	Upjohn Pharmaceutical, Kalamazoo, Michigan

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Cellular Immunity Section

INSTITUTE AND LOCATION

NCI-FCRF, NIH, Frederick, Maryland 21701

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

B

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

The goals of this project are to analyze the genetic mechanisms by which immunogenic tumors escape host immune responses and the genetic basis of transplantation antigens of chemically-induced tumors.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08552-21 LIB

PERIOD COVERED

October 1, 1986 to September 3, 1987

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	Office of the Chief	LIB NCI
OTHER:	A. Circolo	Visiting Associate	LIB NCI
	M. Kirschfink	Visiting Fellow	LIB NCI
	S. Hosoi	Visiting Fellow	LIB NCI

COOPERATING UNITS *(if any)*

Department of Biochemistry, University of Lausanne

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, FCRF, Frederick, MD 21701

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.)*

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.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08577-02 LIB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Restriction fragment length polymorphisms in normal and neoplastic human tissues

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:	H. Brauch	Visiting Fellow	LIB NCI
	T. Yano	Visiting Fellow	LIB NCI
	M. Lerman	Expert	LIB NCI
	G. Glenn	Medical Staff Fellow	LIB NCI
	M. Linehan	Surgery Branch	DCT NCI

COOPERATING UNITS *(if any)*

John Minna	Navy Medical Oncology Unit, NCI
Bernard J. Poiesz	SUNY Health Sciences Center, Syracuse
George D. Sorenson	Dartmouth Medical School
Peter Schwartz	Yale University School of Medicine

LAB/BRANCH: Laboratory of Immunobiology

SECTION: Cellular Immunity Section

INSTITUTE AND LOCATION: NCI-FCRF, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	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 primary objective of this project is to compare restriction fragment length polymorphisms (RFLPs) in DNA extracted from normal and neoplastic human tissues. The initial study will focus on renal cell carcinoma and small cell lung cancer. We will look for evidence of deletion of specific chromosomal loci (change in RFLP from heterozygosity to homozygosity).

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08575-15 LIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Inflammation

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

PI:	E. Leonard	Chief, Immunopathology Section	LIB NCI
OTHER:	Antal Rot	Visiting Fellow	LIB NCI
	Teizo Yoshimura	Guest Researcher	LIB NCI

COOPERATING UNITS *(if any)*

L. Henderson — Litton Bionetics Basic Research Program, FCRF

LAB/BRANCH

Laboratory of Immunobiology

SECTION

Immunopathology Section

INSTITUTE AND LOCATION

NCI, NIH, FCRF, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.3	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.)*

Investigations in the Immunopathology Section are on chemotactic and other immune effector responses of leukocytes. The emphasis is on chemotaxis, a mechanism by which cells are attracted to inflammatory sites, delayed hypersensitivity reactions and growing tumors. The project includes chemistry and biology of bacterial derived chemotactic factors, characterization of a serum protein that modulates macrophage motility, and definition of functional subpopulations of blood monocytes.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05190-07 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Monoclonal Antibodies Define Carcinoma Associated and Differentiation Antigens

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

Jeffrey Schlom	Chief	LTIB, DCBD, NCI
Patricia Horan Hand	Chemist	LTIB, DCBD, NCI
Jean Simpson	Medical Staff Fellow	LTIB, DCBD, NCI
Ricardo Parker	BTP	LTIB, DCBD, NCI
Shashi Shrivastav	Visiting Fellow	LTIB, DCBD, NCI
Masahide Kuroki	Visiting Associate	LTIB, DCBD, NCI
Alfredo Molinolo	Visiting Fellow	LTIB, DCBD, NCI
Kai Chang	Visiting Fellow	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

P. Noguchi, Bureau of Biologics, FDA
W. Johnston, Dept. of Pathology, Duke Univ.

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.3	4.3	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.)*

These studies involve the generation and utilization of monoclonal antibodies (MAbs) to identify and characterize human carcinoma associated antigens and differentiation antigens of mammary and colonic epithelium. These MAbs are being used to better understand the cell biology and pathogenesis of human carcinomas, and to provide reagents for use in several aspects of the management of human carcinomas. These include: detection of occult tumor cells; further defining the degree of differentiation of "normal", dysplastic, and carcinoma cell populations; serum antigens assays; and radiolocalization of primary and metastatic carcinoma lesions in situ (and potentially therapy) using radiolabeled monoclonal immunoglobulins and fragments. These studies are divided into four areas of investigation: (I) The generation and characterization of an MAb that defines a novel tumor associated antigen (TAG-72); (II) The development and characterization of MAbs to a repertoire of epitopes on carcinoembryonic antigen (CEA) which are differentially expressed among carcinoma cell populations; (III) The generation and characterization of MAbs to proteins associated with metastatic cell populations, and (IV) The definition and characterization of breast and colon differentiation antigens.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09009-06 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Augmentation of Tumor Antigen Expression

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

John W. Greiner	Cancer Expert	LTIB, DCBD, NCI
Fiorella Guadagni	Visiting Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

D. S. Pestka, Institute of Molecular Biology, Hoffman La Roche, Nutley, NJ;
Dr. P. Fisher, Columbia University, New York, NY;
Dr. P. Noguchi, Bureau of Biologics, FDA

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	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 spece provided.)*

Antigen heterogeneity within human tumor cell populations can be attributed somewhat to the intrinsic ability of the cells to modulate the level of expression of certain tumor antigens by: 1) cell cycle kinetics 2) clonal variability and 3) cell-to-cell communication in three-dimensional organoids. Studies were carried out to establish that exogenously administered biological response modifiers, such as the recombinant human interferons, can overide the intrinsic modulation of these antigens resulting in an increase in the percent of the cell population expressing the monoclonal-antibody (MAb) defined tumor antigen as well as increasing the amount of antigen expressed per cell. Utilizing eight different human breast tumor cell lines, it was shown that both recombinant human leukocyte (alpha) and gamma (type I and II) interferons were effective in augmenting several MAb-defined cell surface tumor antigens. In addition, alpha interferon was shown to increase the expression of TAG-72 on human breast tumor cells isolated from a patient's pleural effusion. Additional studies demonstrated that the in vivo administration of recombinant leukocyte (clone A) interferon was effective in increasing the amount of tumor antigen expressed by a human colon xenograft in situ and also augmented the localization of a radiolabeled MAb to the tumor site. Thus, these studies may lead to the new strategies designed to use recombinant interferon as an adjunct for MAb-binding to human carcinoma cell population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05233-06 LTIB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Purification of and Radioimmunoassays for Human Carcinoma Associated Antigens

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

David Colcher	Supv. Microbiologist	LTIB, DCBD, NCI
Donald Sheer	BTP Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI
Patrizia Ferrone	Visiting Fellow	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	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.)*

A competitive radioimmunoassay (RIA) for a tumor associated antigen (termed TAG-72) identified by monoclonal antibody B72.3 has been established. The distribution of TAG-72 in human tissues has been shown to be highly specific for carcinomas with no significant reactivity to normal tissues. The RIA was used to examine sera from patients with colorectal carcinomas, other malignancies and normal sera. A mean of 2.2 units/ml of TAG-72 was found in the normal sera. When a cut-off level of 3 standard deviations above the mean level of TAG-72 found in normals is used, no patient with inflammatory disease or other benign colon diseases exhibited abnormal levels of TAG-72. Thirty-five percent of sera from advanced colon cancer patients and patients with other carcinomas were positive for TAG-72. A second RIA using a solid-phase matrix to establish a multi-determinant assay using radiolabeled B72.3 was also established. This assay detected significant ($\geq$IOU/ml) in approximately 60% of patients with colorectal carcinomas. Comparison of the TAG-72 levels in sera with antigens recognized by the monoclonal antibodies currently used to screen sera of carcinoma patients clearly demonstrated that TAG-72 is different from the other antigens, and that TAG-72 can be found in some sera where no antigen is detected by the commercially available MAb RIAs.

TAG-72 has been purified from extracts of a human colon carcinoma xenograft in athymic mice using molecular sieving and antibody affinity chromatography. It has an apparent molecular weight of $\geq 10^6$ daltons, and has many properties similar to mucins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09008-06 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Localization of Human Tumors in Athymic Mice with Labeled Monoclonal Antibodies

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

David Colcher	Supv. Microbiologist	LTIB, DCBD, NCI
Mario Roselli	Visiting Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI
Patrizia Ferrone	Visiting Fellow	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

O. Gansow, Radiation Oncology, DCT, NCI;
A. Keenan and S. Larson, Department of Nuclear Medicine, CC, NIH;
J. Carrasquillo, Department of Nuclear Medicine, CC, NIH

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Laboratory of Tumor Immunology and Biology

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Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

A

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

Monoclonal antibody B72.3 binds to human breast and colon tumor associated antigens. IgG was purified and radiolabeled with I-125, and I-131 and In-111 without loss of immunoreactivity. The radiolabeled antibody was injected into athymic mice bearing antigen positive human colon tumors or an antigen-negative melanoma as a negative control. With iodinated B72.3 IgG activity in the tumor rose for the first 2 days and remained constant over the 19 day period of study. Tumor-to-normal tissue ratios rose over this period of time with ratios of approximately 18:1 for liver, spleen and kidney at 7 days. At 19 days approximately 40% of the radiolabeled B72.3 IgG was found in the tumor. Various chelates (MA, CA, SCN-Bz-EDTA and SCN-Bz-DTPA) were attached to B72.3 IgG and radiolabeled with In-111. Comparative biodistribution and imaging studies were performed using all four chelates showed that the tumor uptake of radio-label expressed as a percentage of the injected dose per gram was very similar when three of the chelates were ligated to the B72.3 IgG (30% ID/g). The uptake by normal organs, especially the liver, was greater when MA-DTPA, CA-DTPA, and SCN-Bz-EDTA chelate-B72.3 IgG was used in comparison to that found with the B72.3-SCN-Bz-DTPA. Tumor to liver ratios rose over time with the In-111-B72.3-SCN-Bz-DTPA complex reaching approximately 5:1 by 72h. The tumor to liver ratios for the other MAb-chelate complexes, on the other hand, ranged from only 1.3:1 to 2.5:1. Tumors could easily be identified in scintigraphic images with all the chelate-antibody complexes. However, a progressive accumulation of activity in the abdominal organs, predominantly in the liver, was seen with the MA-DTPA, CA-DTPA and SCN-Bz-EDTA chelate-antibody complexes. This uptake was very prominent with these chelate-MAb complexes but was virtually absent in the mice injected with B72.3-SCN-Bz-DTPA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09018-03 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Clinical Trials with Radiolabeled Antibodies

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

Jeffrey Schlom	Chief	LTIB, DCBD, NCI
David Colcher	Supv. Microbiologist	LTIB, DCBD, NCI
Jean Simpson	Medical Staff Fellow	LTIB, DCBD, NCI
Mario Roselli	Visiting Fellow	LTIB, DCBD, NCI
Alfredo Molinolo	Visiting Fellow	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

S. Larson, Chief, Nuclear Medicine, NIH
W. Sindelar, Chief, Colorectal Surgery, Surgery Branch, NCI
G. Bryant, Laboratory of Pathology, NCI

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

A

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

Accurate detection and anatomic localization of both primary and metastatic lesions remains one of the major problems in the management of most human carcinomas. We have recently initiated clinical trials at the NIH Clinical Center to detect and localize colorectal carcinoma lesions using radiolabeled monoclonal antibody (MAb) B72.3. Parameters that are being systematically investigated concerning both the efficiency of MAb localization and the efficiency of gamma scanning of carcinoma lesions include: (a) effect of MAb dose and specific activity of radionuclide coupled MAb; (b) comparison of the use of intact IgG, $F(ab')_2$, and Fab'; (c) choice of radionuclide; (d) route of inoculation; (e) size, location, and other inherent properties of the tumor mass such as antigen content; (f) the presence of circulating antigen; (g) the presence and/or absence of human anti-murine Ig antibodies; (h) metabolism of MAb and fragments; (i) combinations of MAbs. It is hoped that these studies will also aid in establishing a rational basis for the subsequent therapeutic use of a particular MAb, either coupled to toxins, via effector cell-mediated or complement-mediated mechanisms, or using MAbs radiolabeled with one of a variety of isotopes. This latter goal can be accomplished by direct analyses of biopsy material (both tumor and normal tissues) from patients receiving radiolabeled MAb to define the "radiolocalization index" or potential "therapeutic index" (i.e., the ratio of the amount of MAb bound [via cpm] per gram of tumor tissue to that bound per gram of normal tissues).

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09012-04 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Monoclonal Antibodies to Detect Occult Carcinoma Cells

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

Jean Simpson	Medical Staff Fellow	LTIB, DCBD, NCI
Noriaki Ohuchi	Visiting Fellow	LTIB, DCBD, NCI
Alfredo Molinolo	Visiting Fellow	LTIB, DCBD, NCI
Kai Chang	Visiting Fellow	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Drs. W. Johnston and C. Szpak, Department of Pathology, Duke Univ., Durham, NC;
Dr. F. Gorstein, Department of Pathology, Vanderbilt Univ., Nashville, TN

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

A

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

Monoclonal antibodies (Mabs) have been utilized with immunohistochemical methods for the (a) detection of occult carcinoma in surgical and cytology preparations, (b) phenotyping of malignant cell populations, (c) differentiation of histologic tumor types, and (d) identification of various cellular products. Mabs with selective reactivity against tumor-associated antigens have specifically been adapted for use with cytologic preparations including cytospins, membranes, and fine needle aspiration biopsies (FNAB) for the detection and differentiation of carcinomas from benign cell types and other cancerous lesions.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09021-01 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Cloning of Tumor Associated Antigens

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

Judy Kantor	Expert	LTIB, DCBD, NCI
Rosette Tran	Visiting Fellow	LTIB, DCBD, NCI
Jian Xiang	Visiting Fellow	LTIB, DCBD, NCI
Shannon Dixon	Microbiologist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The use of monoclonal antibody technology has allowed the identification and partial characterization of human carcinoma-associated antigens as well as differentiation antigens of mammary and colon epithelium. MAbs provide powerful tools for use in the diagnosis and management of human carcinomas. They have been used for screening and diagnostic purposes for the detection of tumor associated antigens in blood serum assays, and for the radio-localization of primary and metastatic carcinoma lesions in-situ. Using radiolabeled monoclonal immunoglobulins and fragments, a powerful therapy regime is being developed.

The overall goal of this project is to molecularly clone and identify the genes that encode the tumor associated antigens TAG-72 and CEA. We have recently initiated studies for the cloning of TAG-72. These studies include: 1) the construction of cDNA libraries from LS174 mRNA in several prokaryotic and eukaryotic expression vectors. 2) the cotransfection of LS174 tumor DNA with plasmids carrying drug selectable markers into mammalian cells. 3) the construction of oligomeric probes to the peptide sequence of TAG-72 for use as hybridization probes.

Additional studies have recently been initiated to study the regulation of CEA expression in colon and breast tumor cell lines after interferon induction. Several DNA probes have been developed to use in Northern blot analysis of poly A selected mRNA from these cell lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09017-03 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Oncogene Expression in Human Carcinomas

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

Patricia Horan Hand	Chemist	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI
Noriaki Ohuchi	Visiting Fellow	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 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.3	2.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Several distinct and high-conserved genes comprise the ras gene family of rodents and humans, i.e., rodent Harvey and Kirsten, and human Harvey, Kirsten and neuroblastoma. Transformation, either by a point-mutation resulting in a change in one amino acid of the 21 kDa ras gene product (p21), or by increased expression of ras p21, has been demonstrated to be mediated by members of this gene family. We have reported the development of direct binding liquid competition radioimmunoassays for the detection and quantitation of the ras oncogene and proto-oncogene products. Using these radioimmunoassays and ras p21 purified from Escherichia coli containing the full-length T24 mutant human Harvey ras gene protein product as a standard, we have defined the actual amount of ras p21 per µg of total cellular protein, or per cell, in various ras transformed and "normal" mammalian cell lines. Absolute levels of Ha-ras p21 have also been determined in human breast and colon carcinomas, benign lesions, and/or their respective normal tissues using the radioimmunoassays. Enhanced Ha-ras expression was documented in 66% of breast and 100% of colon carcinomas as compared with their normal counterparts, with levels in breast carcinomas ranging from 18.4 to 51.7 pg ras p21 per ug protein. Some dysplastic lesions of the breast and colon also contained elevated Ha-ras p21. Relative levels of Ha-ras p21 expression, detected by competition RIA, correlated with percent Ha-ras p21 positive cells as determined by immunohistochemical assays. Using liquid competition RIA and immunohistochemical assays, it has been shown that levels of ras p21 expression did not always correlate between primary and metastatic colon lesions of the same patient. The use of the quantitative RIA and semiquantitative immunohistochemical assays, in concert with cDNA probes for identification of specific ras pointmutated oncogenes or protooncogenes, may now provide the means for definitive quantitative analyses of ras p21 in human carcinomas and benign lesions.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08226-11 LTIB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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 & Tumorgenesis

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

B.K. Vonderhaar	Research Chemist	LTIB, DCBD, NCI
C. Dati	Visiting Fellow	LTIB, DCBD, NCI
E. Ginsburg	Biologist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
~~Dr. Kathleen Antol, Clarke College, Dubuque, IA~~
Dr. Sandra Haslam, Michigan State University, East Lansing, MI
Dr. Randy Whitcomb, Developmental Endocrinology, NICHD
Dr. Susan Bates, Pediatrics Branch, NCI

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Experimental Oncology Section

INSTITUTE AND LOCATION
NCI, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.35	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

X 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 and in development growth and maintenance of mammary tumors. Studies include: 1) examination of the role of epidermal growth factor and mammary gland-derived growth factors in lobuloalveolar development of the mouse mammary gland, 2) 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, 3) examine the hormonal conditions in vitro which induce production of autocrine growth factors by normal mammary tissue and breast cancer cell lines, 4) examine the effect of sialadenectomy on mammary tumor incidence and growth in mice as well as production of pre-neoplastic hyperplastic alveolar modules.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08274-06 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

B.K. Vonderhaar	Research Chemist	LTIB, DCBD, NCI
Suzanne Ziska	Staff Fellow	LTIB, DCBD, NCI
Ratna Biswas	Visiting Fellow	LTIB, DCBD, NCI
Claudio Dati	Visiting Fellow	LTIB, DCBD, NCI
Erika Ginsburg	Biologist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Dr. Rhoda Maneckjee, Medical Oncology Branch, NCI, Bethesda, MD
Dr. Anthony Capuco, USDA, Beltsville, MD

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Experimental Oncology Section

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.9	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.)*

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 subunits of the receptor, 3) characterization of the nature of the interaction of Tamoxifen with membrane bound receptors related to the lactogen receptor, and 4) define the relationship of monoclonal antibody B6.2 to human lactogenic hormone receptors.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09022-01 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Cytoskeletal Proteins in Oncogenic Transformation and Human Neoplasia

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

Herbert L. Cooper	Chief, Cell & Molec. Phys. Section	LTIB, DCBD, NCI
Basudev Bhattacharya	Visiting Fellow	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 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	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.)*

Studies have continued on the role of tropomyosin (TM) suppression in neoplastic transformation. The mechanism of suppression of TM synthesis by retroviral oncogene expression is being explored. We have obtained evidence that TM suppression in fibroblasts transformed by retroviral oncogenes is due to the action of alpha-transforming growth factor produced as a consequence of oncogene expression. In mouse mammary epithelial cells constitutively expressing activated c-Ha-ras, Tm synthesis was not suppressed, but accumulation of newly synthesized TM was suppressed, apparently due to accumulation of actin and TM in abnormal ratios in the cytoskeleton. TM expression was also studied in a panel of established human breast cancer lines. In all but one case abnormalities in tropomyosin expression were observed in the tumor cell lines, suggesting that derangement of TM expression may be a frequent event in human neoplasia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09006-05 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Studies on the Nature and Function of the Phosphoprotein, Prosolin.

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

Herbert L. Cooper	Chief, Cell and Molec. Phys. Sect.	LTIB, DCBD, NCI
Richard Braverman	Chemist	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 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.3	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.)*

Studies have continued on the unique phosphoprotein, pp17, which undergoes rapid phosphorylation in HL60 promyelocytic leukemia cells in response to treatment with phorbol ester (TPA). We have identified the non-phosphorylated form of this protein as a major cytosolic protein of Mr 18.4K, pI 5.9, and have named it 'prosolin'. Within 15 min of treatment of HL60 cells with TPA nearly 50% of pre-existing prosolin is phosphorylated, making this event one of the earliest and most significant biochemical changes resulting from TPA treatment in HL-60 cells. Studies with peripheral blood lymphocytes and malignant lymphoid cell lines suggest that synthesis of prosolin is associated with rapid growth of hematopoetic cells, while phosphorylation of prosolin may be associated with rapid cell responses associated with reduction of DNA synthesis and expression of genes involved in differentiated cell function. Amino acid sequencing and cDNA cloning of prosolin are in progress.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04848-16 LTIB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
RNA Tumor Viruses: Replication, Transformation, and Inhibition in Cell Cultures

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

R. Bassin	Chief, Biochem. of Oncogenes Sect.	LTIB, DCBD, NCI
K. Yanagihara	Visiting Fellow	LTIB, DCBD, NCI
G. Tortora (half time)	Visiting Fellow	LTIB, DCBD, NCI
H. Cooper	Chief, Cell. and Mol. Phys. Sect.	LTIB, DCBD, NCI
D. Salomon	Research Biologist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*
Dr. C. Lechene, Harvard University, Boston, Mass.
Dr. S. Egan, University of Manitoba, Winnipeg, Canada
Dr. L. Benade, American Type Culture Collection, Rockville, MD.

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Biochemistry of Oncogenes Secion

INSTITUTE AND LOCATION
NCI, Bethesda, Maryland 20892

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.)*

We are continuing to investigate the properties of cells resistant to transformation by specific retroviral oncogenes. New methods for the selection and study of resistant cells have been developed. This includes the use of new mutagens and new selective procedures. Following re-infection of the original resistant cell lines with Ha-MuSV, dot blot analysis shows that resistant cells do not block v-ras mRNA synthesis but must be altered at some point closer to the transformation event. New methods for developing resistant cells have been worked out as have new selective agents. We are currently trying to isolate more resistant cells using these methods.

We are studying a new murine virus that induces solid tumors in mice. We have worked with this isolate in cell culture where it transforms cells and appears to be defective. Initial dot blot analysis indicates that this isolate is related to v-Ha-ras.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 09003-05 LTIB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Alpha Transforming Growth Factors in Rodent and Human Mammary Carcinomas

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

David Salomon	Supv. Res. Biologist	LTIB, DCBD, NCI
Fortunato Ciardiello	Visiting Fellow	LTIB, DCBD, NCI
William Kidwell	Chief, Cell Cycle Reg. Sec.	LTIB, DCBD, NCI
Robert Bassin	Chief, Biochemistry Oncogenes Sec.	LTIB, DCBD, NCI
Robert Callahan	Chief, Oncogenetics Sec.	LTIB, DCBD, NCI
Herbert Cooper	Chief, Cellular and Molecular Physiology Sec.	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)* Dr. Marc Lippman, Medicine Branch, NCI
Dr. James Tam, Dept. of Biochemistry, Rockefeller Univ., New York, NY
Dr. Ryk Derynck, Dept. of Molecular Biology, Genentech, Inc., San Francisco, CA
Dr. Mary Lou McGeady, Otsuka Pharmaceutical Co., Rockville, MD

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

SECTION
Biochemistry of Oncogenes Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

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.)*

Experiments are being conducted to determine the distribution and role of alpha transforming growth factors (αTGFs) in normal and malignant rodent and human mammary epithelial cells. Primary dimethylbenz(α)anthracene (DMBA) and nitrosomethylurea (NMU) rat mammary adenocarcinomas possess three to seven-fold more biologically active and immunoreactive αTGFs than transplantable DMBA-I and NMU-II carcinomas. Moreover, a specific 5.0 kb mRNA species could be detected in the primary DMBA and NMU tumors following hybridization of poly A(+) RNA to a human and mouse αTGF cDNA probes but not in the DMBA-I or NMU-II tumors. Ovariectomy produced a two to three-fold decrease in the level of αTGFs in the primary DMBA tumors which was preceeded by a loss in the expression of αTGF mRNA. Mouse mammary epithelial which have been transformed with a point-mutated c-Ha-*ras* proto-oncogene, NMuMG/*ras*^{H} cells, become resistant to the growth promoting effects of EGF because these cells have an increased capacity to synthesize αTGF mRNA. Human breast cancer cell lines are also producing αTGF and possess αTGF mRNA. In MCF-7 cells, the level of production of αTGF and TGFα mRNA expression can be enhanced by estrogens and blocked by anti-estrogens. In addition treatment of MCF-7 cells with a polyclonal anti-human TGFα antibody or with a monoclonal antibody against the human EGF receptor will inhibit the growth of these cells *in vitro*. Elevated levels of immunoreactive αTGF and αTGF mRNA can be detected in approximately 65% of primary human breast carcinomas. There is a strong positive correlation between the presence of αTGF mRNA and the presence of functional estrogen receptors in a subset of these tumors. These results suggest that TGFα may function as an autocrine growth factor for a subset of rodent and human mammary tumor cells which are estrogen responsive and that the activation or overexpression of a *ras* proto-oncogene may also control the level of TGFα production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05148-08 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mammary Tumorigenesis in Inbred and Feral Mice

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

Robert Callahan	Chief, Oncogenetics Section	LTIB, DCBD, NCI
Gilbert Smith	Microbiologist	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Dr. Christine Kozak, LVD, NIAID, NIH
Dr. Michael Potter, LG, DCBD, NCI, NIH
Dr. John Silver, LVD, NIAID, NIH

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NCI, NIH, Bethesda, Maryland 20892

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

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.)*

We have identified a new common integration locus (designated int-3) for the mouse mammary tumor virus (MMTV) in MMTV (CZECHII) induced mammary tumors. Our current data show that 8 out of 44 CZECHII tumor DNAs contain a viral insertion at this site. MMTV integration at int-3 has been found to induce a 6kb species of RNA in addition to the previously described 2.4kb RNA species. Both RNAs correspond to DNA sequences adjacent to the int-3 locus. In 10 out of the 44 CZechII tumor DNAs, the int-1 locus was found to be occupied by an MMTV genome. One of these tumor DNAs also had an insertion of the int-2 locus. Analysis of the int loci in tumor DNAs from CZECHII mice infected with another strain of MMTV as well as other strains of MMTV infected mice shows that the frequency of int activation is a function of the virus strain as well as the genetic background of he host. In other work we have found that MMTV infected CZECHII mice develop mammary gland hyperplastic aveolar nodules (HAN). Currently hyperplastic outgrowth (HOG) lines are being developed from MMTV infected CZECHII.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 04829-13 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

The Identification and Characterization of Human Genes Associated with 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
Iqbal Ali	Visiting Associate	LTIB, DCBD, NCI
Renato Mariani-Costantini	Visiting Associate	LTIB, DCBD, NCI
Georgio Merlo	Visiting Fellow	LTIB, DCBD, NCI
Danielle Liscia	Visiting Associate	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Dr. Carlo Croce, Wistar Institute, Philadelphia, PA
Dr. Rosette Lidereau, Rene Huguenin Centre, St. Cloud, France

LAB/BRANCH

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Oncogenetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	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 continued our efforts to identify and characterize frequent genetic changes associated with primary human breast tumor DNAs. In 20% of the tumor DNAs (n=56), heterozygosity of multiple loci on chromosome 11p was lost. The somatic loss of these sequences has a significant correlation with histopathological grade III tumor ($P<.006$), estrogen and progesterone receptor negative tumors ($P<.02$ and $P<.002$, respectively) and patients which develop distalmetastasis ($P<.05$).

Our data suggests that the most frequently deleted region lies between the β Globin and PTH loci. In situ RNA:RNA hybridization on frozen sections of primary breast and colon carcinomas was used to examine cmyc and cHras-1 expression. In the breast carcinomas high levels of cmyc RNA expression; with few exceptions, correlates with amplification of the gene. The expression of cmyc and cHras-1 in colon carcinomas is aberrantly regulated in several cases. Their expression is not a function of the proliferative capacity of the tumor. In addition no genetic alterations of these genes could be detected which explain the patterns of expression observed.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 09023-01 LTIB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

Syed Kashmiri	Expert	LTIB, DCBD, NCI
Robert Callahan	Chief, Oncogenetics Section	LTIB, DCBD, NCI
Jeffrey Schlom	Chief	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Tumor Immunology and Biology

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Oncogenetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	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 recently initiated a program to develop chimeric antibodies of defined specificity. Such immunoglobin molecules which could be ideal for radiotherapy and radioimaging will include chimeric antibodies with murine variable region and human constant region. Additionally, we plan to generate isotype switch variant of specific monoclonal antibody genes. We will also attempt to manipulate antibody genes that may lead to a change in Fc fragment of the antibody molecule resulting in a desirable change in the biological effector function. Our plans also include generation of pared down antibody molecules (Fab'_2, Fab and FV). Such molecules can be used for antigen clearance without complement activation. Among other desirable variants of antibody molecules that we will attempt to generate by gene manipulation will include, a) antibodies with improved affinity for the target, b) molecules with altered carbohydrate content, and c) antibody molecules that can specifically bind to certain enzymes or toxic substances.

To attain our objectives we will initially attempt to clone the cDNA copies of messages for light and heavy chain genes of immunoglobulin synthisized by hybridomas against tumor associated antigens. We will characterize these clones and will attempt expression of the cDNA constructs by inserting them in appropriate vectors and transfecting the construct into myeloma cells. Subsequently, we will attempt to clone the rearranged genomic DNA segments encoding heavy and light chain genes of hybridomas.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 05216-16 LTIB

PERIOD COVERED

October 1, 1985 to September 30, 1987

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

cAMP Receptor Protein in Cancer Growth Control

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

Y.S. Cho-Chung	Chief, Cellular Biochemistry Section	LTIB, DCBD, NCI
T. Clair	Chemist	LTIB, DCBD, NCI
P. Tagliaferri	Visiting Fellow	LTIB, DCBD, NCI
D. Katsaros	Visiting Fellow	LTIB, DCBD, NCI
G. Tortora	Visiting Fellow	LTIB, DCBD, NCI
S. Ally	Guest Worker	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Dr. W.R. Miller, U. of Edinburgh
Dr. L. Neckers, LP, DCBD, NCI
Dr. S.O. Doskeland, U. of Bergen, Norway
Dr. R.K. Robins, Nuclei Acid Research Institute, Costa Mesa, CA

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

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Cellular Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

Cyclic AMP (cAMP) in mammalian cells functions by binding to cAMP receptor protein, the regulatory subunit of cAMP-dependent protein kinase. The cAMP receptor protein has two different cAMP binding sites, and cAMP analogs that specifically bind to either one of the two binding sites are known as Site 1-selective (C-2 and C-8 analogs) and Site 2-selective (C-6 analogs), respectively. Further the Site 1- and Site 2-selective analogs in combination produce synergistic enhancement of the binding to cAMP receptor protein and protein kinase activation in vitro.

Application of these in vitro findings to demonstrate cAMP analog-mediated response in vivo, in intact cells or tissues has been scarce. Moreover, virtually all past studies of cAMP-regulation of cell growth employed a few, early known, cAMP analogs which are weakly active for protein kinase and effective only at unphysiological high mM concentrations. The site-selective cAMP analogs which are many-fold more active for protein kinase have never been tested for their growth regulatory effect.

We, therefore, investigated the growth regulatory effect of site-selective cAMP analogs on a spectrum of human cancer cell lines nd the in vivo growth of rodent mammary tumors. The analog effect on the cell growth will be correlated with the response of cAMP receptor protein present in the cancer cells. The goal of this study is to elucidate the growth regulatory mechanism of cAMP analogs which can be extrapolated to the treatment of human cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08281-05 LTIB

PERIOD COVERED

October 1, 1985 to September 30, 1987

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

The Regulatory Mechanism of Oncogene Expression

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

Y.S. Cho-Chung	Chief, Cellular Biochemistry Section	LTIB, DCBD, NCI
T. Clair	Chemist	LTIB, DCBD, NCI
P. Tagliaferri	Visiting Fellow	LTIB, DCBD, NCI
D. Katsaros	Visiting Fellow	LTIB, DCBD, NCI
G. Tortora	Visiting Fellow	LTIB, DCBD, NCI
R. Bassin	Chief, Biochemistry Oncogenes Section	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Dr. J.D. Corbin, Howard Hughes Medical Inst. Lab., Vanderbilt U., Nashville, TN
Dr. R.K. Robins, Nucleic Acid Research Inst., Costa Mesa, CA
Dr. H. Fan, University of California, Irvine, CA

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

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Cellular Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In spite of the relatively large body of information concerning the molecular structure of retrovirus oncogenes and their specific protein products, little is known about the mechanisms by which they transform cells. Moreover, protooncogenes, the cellular counterpart of retroviral oncogenes, have been found in normal cells but their expression is low and the mechanism for this low expression is not known. It is conceivable that substances that increase tumor development by apparently increasing cellular proliferation do so by altering the quantitative or temporal expression of cellular oncogenes. Understanding the cellular mechanisms governing the expression of both viral and cellular oncogenes would therefore provide an insight into the mechanism of neoplastic cell growth and tumor development. 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.

To investigate factors that affect phenotypic reversion of transformed cells, we have chosen a cell line transfected with transforming ras gene of Ha-MuSV, clone 13-3B-4 of NIH 3T3 cells. The effect of cAMP on the transcriptional activities of the wild type and deleted M-MuLV LTRs was also studied. We also used human cancer cell lines. The goal of this study is to investigate the effect of intracellular regulatory factors, such as cyclic nucleotides, hormones, and growth factors on the expression of cellular oncogenes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08280-04 LTIB

PERIOD COVERED

October 1, 1985 to September, 30, 1987

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

Enhancement of Oncogene Expression and Mammary Cancer

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

Y.S. Cho-Chung	Chief, Cellular Biochemistry Section	LTIB, DCBD, NCI
T. Clair	Chemist	LTIB, DCBD, NCI
S. Ally	Guest Researcher	LTIB, DCBD, NCI
D. Katsaros	Visiting Fellow	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Dr. W.R. Miller, U. of Edinburgh, Scotland
Dr. B.E. Haley, U. of Wyoming, Dept. of Biochemistry, Laramie, Wyoming
Dr. H. Abou-Issa, Ohio State U., Dept. of Surgery, Columbus, Ohio

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Cellular Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.0	0.7

CHECK APPROPRIATE BOX(ES) B

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

☐ (a1) Minors

☐ (a2) Interviews

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

Over twenty distinct transforming genes have been identified in the genomes of oncogenic retroviruses. Each of these oncogenes has a homologue in the chrosomal DNA of a 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 hypothesis is that deregulation of oncogene expression may be a possible general mechanism for the induction of neoplasia in humans. Our efforts have been concentrated on the cellular homologue of the ras gene, the oncogene carried by Harvey and Kirsten Sarcoma viruses. In this study we are investigating the role of ras gene expression in the induction of rat and human mammary carcinomas. In a study of more than 200 human breast carcinomas, we have observed elevated expression of c-rasH in 70% of estrogen and progesterone receptor positive tumors and 40% of estrogen and progesterone receptor negative tumors. Whereas, an amplified or rearranged c rasH gene has not been detected in human mammary carcinomas. Thus, the mechanism by which c-rasH gene expression is deregulated in these tumors remain to be determined. To study the mechanism of the enhanced c-rasH gene expression, we will determine the c-rasH expression in growing and growth-arrested human breast cancer cells (MCF-7), growing vs regressing rat mammary tumors, hormone-dependent vs hormone-independent tumors, and the mammary gland of rodents during normal development and chemical or viral carcinogenesis. The goal of this proposal is to provide us a fundamental basis for better understanding the mechanisms by which oncogenes involved in the neoplastic development and growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CB 08249-07 LTIB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

William R. Kidwell	Chief, Cell Cycle Regulation Sect.	LTIB, DCBD, NCI
David Salomon	Supv. Res. Biologist	LTIB, DCBD, NCI
Sanjeeva Mohanam	Visiting Fellow	LTIB, DCBD, NCI
Sue Liu	Bio. Lab. Tech	LTIB, DCBD, NCI
Brunella SanFilippo	Visiting Fellow	LTIB, DCBD, NCI

COOPERATING UNITS *(if any)*

Rick Derynck	Genentech
Richard Grosse	Akadamie der Wissenschaffen der DDR

LAB/BRANCH

Laboratory of Tumor Immunology and Biology

SECTION

Cell Cycle Regulation Section

INSTITUTE AND LOCATION

NCI, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	0.5	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Mechanisms for the escape of breast cancer cells from normal growth controls are being evaluated, both in human and rodent tumor model systems. Two growth promoting and two growth inhibiting activities, present in and made by mammary tissues, have been detected. One of these, MDGFI, a human factor, was found to be secreted into the growth medium by primary cultures of normal, benign and malignant human mammary epithelium. On average, the malignant cells made about three times as much MDGFI as did the normal cells. A second growth promoter, transforming growth factor alpha (TGFα) was also made by both normal cells and by carcinoma in situ. In rodent tissues, a change in TGFα production was found as a function of the stage of progression of the tumors. Normal cells produced readily detectable TGFα levels, whereas production by adenocarcinomas was significantly more. In the most advanced tumors, those with high metastatic potential, TGFα production was nearly zero. These results were confirmed both by radioimmunoassay and by Northern blot analysis of poly A+ mRNA. In vivo and in vitro studies demonstrated that estrogens regulated the production of TGFα by rodent mammary adenocarcinomas, a finding consistent with the depletion of TGFα mRNA following ovariectomy. TGFβ one growth inhibitor made by mammary tissues, was found by bioassay, and by Northern blot hybridization to be produced in equivalent amounts by both normal and malignant rodent and human mammary epithelium. A second inhibitor, a 13 Kd acidic protein, was found to be high in normal but low in malignant mammary cells. This factor has been purified about 5000 fold and partial sequence determined. Nanogram amounts inhibit normal and malignant mammary cell growth in vitro and also dramatically lower the production of extracellular matrix proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08212-13 OD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 chererters 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 Investigetor.) (Name, title, laboretory, and institute effiliation)*

PI:	Shelby L. Berger	Research Chemist	OD DCBD NCI
Other:	William H. Eschenfeldt	Senior Staff Fellow	OD DCBD NCI
	Marc S. Krug	Staff Fellow	OD DCBD NCI
	Alvaro Leone	Visiting Fellow	OD DCBD NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
OD, DCBD

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

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.)*

Clones for human prothymosin α have been identified in cDNA libraries from staphylococcal enterotoxin A-stimulated normal lymphocytes and from simian virus 40-transformed human fibroblasts. The 1198-base-pair fibroblast clone has been sequenced. The encoded protein is highly acidic and shares greater than 90% sequence homology with rat prothymosin α. The peptide "hormone" thymosin α_1 appears at positions 2-29 of the prothymosin α amino acid sequence. There is no signal peptide. Prothymosin α is inducible and enjoys broad tissue specificity. In two systems, the mitogen stimulated resting lymphocyte and the serum deprived NIH 3T3 cell upon serum restitution, an increase in the level of prothymosin α mRNA accompanies cell growth. There are at least four genes, three of which have been isolated from cosmid libraries. There is no evidence that prothymosin α serves as a precursor for secreted thymic peptides or that its function specifically involves modulation of the immune system. Rather, prothymosin α appears to play a role in cell proliferation.

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

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 05526-19

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
P53: 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	Supervisory Chemist	OD DCBD NCI
Other:	C. Dale Smith	Visiting Associate	OD DCBD NCI
	M. John Louis	Visiting Fellow	OD DCBD NCI
	V. W. McFarland	Chemist	OD DCBD NCI
	K. Chandrasekaran	Visiting Scientist	OD DCBD NCI

COOPERATING UNITS *(if any)* Pierre May, CNRS, Villejuif, France; Frank Hetrick, University of Maryland; Anton Jetten, National Institute of Environmental Health Sciences; David Winterbourne, St. George's Hospital, University of London

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NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.2	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.)*

A sequence homologous to the mammalian "oncogene", p53 was detected in fish showing great evolutionary conservation. During the embryonal development of the chicken the decrease of this nuclear phosphoprotein was traced to a posttranscriptional step in the mRNA processing, eventually accounting for the decline in the steady state level of the protein. Similar declines in p53 mRNA were found in two different types of induced differentiation in culture of the rabbit tracheal epithelial cells. This together with earlier experiments on retinoic acid induced differentiation of embryonal carcinoma cells, indicates that the decline of p53 mRNA is a common correlate of the cellular differentiation processes. Upon stimulation of adrenergic receptors with isoprotenerol a very rapid and great increase in p53 (and also of c-fos and c-myc) mRNA was observed in rat parotid acinar cells.

Numerous SV40 transformed murine and human cell lines were found in which a stable p53 is not in complex with the T antigen or with any other protein, demonstrating that other, yet unknown mechanisms can result in the stability and thus elevated level of the p53 protein.

In spontaneous transformation of mouse cells the elevated level of p53 and its half life was unrelated to the cellular tumorigenicity as it was the mRNA levels of p53 and also of all the major hitherto recognized proto-oncogenes. However, in the spontaneous transformation a very significant (10/10) correlation was recognized between spontaneous tumorigenecity and a specific change in the heparan sulfate structure. The same heparan sulfate change was recognized before in SV40 transformation of cells. The clonal analysis of the cell systems and the method of selection we present for variant cells for their ability to colonize in the host could be invaluable by allowing a systematic analysis of the natural evolution of tumors.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 00941-31 OD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Other Factors Affecting Marrow Transplantation on Irradiated Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Delta S. Uphoff Research Biologist OD DCBD NCI

COOPERATING UNITS *(if any)*
None

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

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.)*

Successful marrow transplantation requires not only genetically compatible donors but the elimination of normal or malignant hematopoietic cells of the recipient without destruction of other vital tissues. Procedures for irradiating experimental animals were recommended by the International Commission on Radiological Units and Measures. The investigation of physical factors affecting successful marrow transplantion in inbred mice has demonstrated that the ICR recommendations were obsolete and other basic concepts of radiation biology were invalid. Exposure-rate effects and exposure-rate + absorption significantly altered the repair capabilities of normal and malignant hematopoietic cells. Repair was initiated only during exposure and the pattern of repair required only 10 min. to become established. Successful repair of hematopoietic cells interfered with establishment of the marrow graft resulting in partial chimerism or complete reversal to recipient genotype. Significant differences in results occurred with simultaneous 2 direction, dorsal, ventral and reciprocal alternate dorsal + ventral and ventral + dorsal exposures to X-rays. Similar effects are being found using gamma radiation from Ces 137. Cellular repair has usually been investigated using tissue culture system rather than intact animals which gave new incite into this phenomenon. There must be changes in the way physical factors are reported when dealing with biological systems to insure reproducibility of experimental data. In addition the universal practice of converting roentgens as calibrations in air to Grays as absorbed tissue doses should be abandoned in all cases were exact measurements are impractical.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CB 08901-3 OD

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Samuel W. Luborsky Chemist OD DCBD NCI

COOPERATING UNITS *(if any)*

Kenneth M. Yamada, Chief, Membrane Biochemistry Section, LMB, DCBD, NCI

LAB/BRANCH
OD, DCBD

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

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.)*

Cells of most vertebrate species are anchorage dependent and undergo mitosis and proliferate only when firmly attached to a surface. Such cells often secrete their own adhesion proteins (e.g., fibronectin (FN)), which facilitate their adhesion to extracellular substrate surfaces or to other appropriate cells. The mechanism of cell adhesion is little understood. Recently, synthetic peptides derived from the sequence of FN have been used for competitive inhibition of its functions in vitro. We have started to examine this system for the possible effects of homologous and heterologous peptide associations upon peptide function and receptor binding. The degree of peptide aggregation could be measured by sedimentation equilibrium experiments in the analytical ultracentrifuge. We determined that sedimentation equilibrium was achieved in an overnight centrifugation run in phosphate buffered saline, pH 7.2. Such studies were carried out on these peptides and the percent dimer determined for each peptide at a series of peptide concentrations. For such small peptides, the effects of changes in amino acid composition or sequence on peptide properties and percent dimer were significant and easily seen. We hope the results of these studies will help us to better understand the mechanism of cell adhesion.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 08903-1 OD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Structure of Thyroid Hormone Precursors

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

PI:	Sidney Shifrin	Chemist	OD DCBD NCI
	Evelyn F. Grollman		LBM NIADDK
	Sonia Quatih Doi		LBM NIADDK

COOPERATING UNITS *(if any)*
Richard Montali, D.V.M., National Zoological Park, Washington, D. C.

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INSTITUTE AND LOCATION
NIH, NCI, Bethesda, MD 20892

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 spece provided.)*

The thyroid proteins isolated from FRTL cells and from a human goiter are being characterized by chemical methods and by physicochemical methods. The proteins extracted from a Bongo obtained from the National Zoo resemble the human goiter proteins in many of its properties.

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