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Division of

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# CANCER BIOLOGY AND DIAGNOSIS

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Volume II Extramural Research Program  
October 1, 1983-September 30, 1984

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DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

ANNUAL REPORT

October 1, 1983 through September 30, 1984

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DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

NATIONAL CANCER INSTITUTE

EXTRAMURAL RESEARCH PROGRAM

October 1, 1983 through September 30, 1984

INTRODUCTION:

The Extramural Research Program of the Division of Cancer Biology and Diagnosis covers three specific areas: Tumor Biology, Immunology, and Cancer Diagnosis. These programs primarily utilize investigator initiated grants; however, a small number of contracts which provide important biological resources to the scientific community are also supported.

The total budget of the DCBD Extramural Research Program in fiscal year 1984 was \$142,489,000; with \$71,400,000 in the Tumor Biology Program, \$59,594,000 in the Immunology Program, and \$11,495,000 in the Cancer Diagnosis Program.



## TUMOR BIOLOGY PROGRAM

### Description and Introduction

The Tumor Biology Program supports a broad spectrum of basic biological research to determine what cellular and molecular factors distinguish cancer cells from normal healthy cells and tissues. The supposition is that knowledge of these properties and processes will help us learn how to manipulate or change the biological signals responsible for the aberrant behavior of cancer cells. Ultimately, this should result in more effective methods for the diagnosis, treatment and management of cancer victims.

Within the Tumor Biology Program, there are three major areas of investigation which conveniently correspond to different theories of how to control the development and progression of neoplastic disease. The first is understanding the basic biochemical mechanisms involved in growth control, whether these involve particular external signals that initiate the process of cell division or cellular molecules more directly responsible for the control of DNA replication and metabolism. This kind of information can lead to the development of specific hormonal and drug therapies. The second is studying changes that occur at the molecular level which lead to cancer cell invasion. The invasive behavior of cancer cells is a prerequisite to malignancy, or the ability of tumors to invade surrounding tissues, escape normal host defense mechanisms and become established at multiple secondary metastatic sites of growth. Theoretically, if the invasive properties of malignant tumors can be controlled and these tumors confined to particular sites, metastasis, the major killer in cancer patients, will not occur. Treatment of tumors confined to a single site is usually more successful. The third is to develop detailed biological and biochemical information about the processes which induce cancer cell differentiation. There is good reason to believe that many kinds of cancers will respond to external stimuli and differentiate. If the genetic program of an actively growing cancer could be changed to one of terminal differentiation, then the malignant tumor could be rendered harmless. Although the above emphasis of the Tumor Biology Program in the areas of growth, invasion and differentiation is stated in simple terms, they provide a purposeful way of viewing the role of basic biological research to the ultimate goal of curing cancer.

The kinds of information developed in the Tumor Biology Program provide a foundation for and relate directly or indirectly to nearly every other program area within the National Cancer Institute. The importance of basic tumor biology research to the National Cancer Plan is reflected by the large \$71.4 million commitment of the NCI to this program area in FY 1984 (See Budget Table). Complete listings and summaries of all grants supported by the Tumor Biology Program are included in the attached Appendices.

Scientific progress continues at an extremely rapid pace. The following report selectively discusses areas in which progress has been exceptional and areas where there is considerable promise for future research.

## Oncogenes and Cancer

Research in identifying and characterizing oncogenes has progressed at a rapid pace in the last year, as evidenced by the expanded list of oncogenes that have been discovered and the remarkable findings identifying previously unsuspected biological activities of some oncogene products. This information is summarized in the adjoining table. Many of the original observations suggesting tissue specificity for oncogene expression have not been confirmed, but most of the fundamental criticisms questioning the validity of oncogene assay systems have been allayed. Now it is possible to form hypotheses of how oncogenes are activated to produce transformed cellular growth. An important fact to emphasize, however, is that oncogene research has been entirely focused on genes which contribute to uncontrolled growth phenomenon. No studies in this area have addressed later events in tumor progression which lead to cellular invasion and metastasis.

As noted in last year's report, oncogenes are highly conserved throughout phylogeny. More recently, it has been demonstrated that different oncogenes are expressed in different tissues and at different times during mammalian embryonic development (Muller et al., 1983a; Muller et al., 1983b; Muller et al., 1983c). All of these observations confirm that oncogene products are of critical importance to normal cell functions, especially to normal growth and development. Thus, developing a precise understanding of how oncogenes contribute to uncontrolled growth in cancer is very likely to produce information fundamental to other life processes. It is not too speculative to predict that oncogene research will produce spin-offs in both technology and information which will yield explanations and cures for many other diseases.

To recapitulate, we know that oncogenes are activated in a number of different ways. Over-expression of an oncogene product can overload the checks and balances of a cell and result in uncontrolled growth. Over-expression can be achieved artificially by inserting a transcriptional promoter before an oncogene. These genetically engineered situations have proved that both c-mos and c-ras can transform cells without any structural modification, just increased expression. Viruses which insert into host genomes can provide promoter sequences near c-onc genes and result in increased synthesis of the oncogene products. More efficient transcription also occurs in cancer cells which have undergone chromosome translocations, again involving the abnormal placement of a strong promoter before an oncogene sequence. Over-expression also occurs when a cancer cell somehow achieves tandem amplification of oncogenes on the same chromosome, making multiple copies of the same oncogene available for transcription. Unfortunately none of the mechanisms responsible for the above processes are understood. While over-expression of an oncogene is a common observation, another mechanism also operates -- alteration of the structure of an oncogene protein by point mutation which results in either increased biological activity or a different activity. This observation initially was observed for c-ras in a cell line and could not be confirmed generally in primary human tumors, but recently mammary carcinomas have been induced in rats using the carcinogen N-nitroso-N-methylurea, tumor formation being the result of a single point mutation in the H-ras gene (Sukumar et al., 1983). It has usually been assumed that evolution has produced the most efficient protein products, especially when the amino acid sequences are highly



conserved. Thus, the notion that a somatic point mutation could result in an essential protein with increased activity was not accepted very easily. Coincidentally, an unrelated study which genetically engineered a point mutation in tyrosyl tRNA synthetase resulted in a 100-fold increase in binding activity for the substrate (Wilkinson et al., 1984). Although over-expression of a protein and structural alteration of a protein with increased activity can explain most observations implicating oncogenes in growth transformation, the myc gene does not always conform to any of the above explanations. It has been postulated that small temporal variations in oncogene expression can also result in transformation. The more work which is carried out to understand the tissue-specific expression and increased activity of oncogenes, the more we realize that any of the above possibilities can be observed for any specific oncogene depending upon the specific form of cancer being studied. The general conclusion is that any mechanism which is possible will affect oncogene expression and increase the probability for neoplastic growth.

A major discovery during the last year was the demonstration by two independent laboratories that primary rodent cell cultures are transformed only when transfected by two separate oncogenes. Primary baby rat kidney cells are transformed when co-transfected with the human adenovirus E1A gene and the H-ras gene of the T24 human bladder carcinoma cell line or the polyoma virus middle T gene (Ruley, 1983). While these same genes are individually unable to produce a transformed phenotype in primary cell cultures, which are not far removed from the in vivo situation, they will transform cell lines, cells which have been adapted to a permanent culture environment over many years. Similar results were also observed for primary cultures of rat embryonic fibroblasts (REF) when co-transfected with the human ras gene and the myc gene or the polyoma virus large T gene (Land et al., 1983a). The ras gene and myc gene added alone produced no transformation, but ras did alter REF morphology. Clearly, the ras and myc oncogenes act differently because they are able to achieve phenotypes that neither is able to achieve alone. The above results are important for two reasons. First, new transfection systems have been developed that no longer rely on the mouse NIH/3T3 cell line. Fortunately, despite the weaknesses of the 3T3 assay, all results obtained so far confirm the validity and generality of work performed with the NIH/3T3 system. In fact, the requirement of cotransfection to achieve transformation in primary cultures confirms the original suspicions that 3T3 had already undergone the first permanent change(s) required before transformation could occur. Second, the possibility now exists for following the stepwise process of carcinogenesis/tumorigenesis as cotransfection bioassays are developed and perfected. The implications of oncogene cooperation to the theory of carcinogenesis will be addressed later in relation to other scientific findings.

A second major finding has been the establishment of a direct relationship between growth factors and oncogenes. For many years researchers have been studying numerous protein factors which combine with plasma membrane receptors and activate the cell growth cycle. Therefore, much is known about the different kinds of growth factors, their interactions with receptors and the internal biochemical processes initiated as a result of the receptor-growth factor interaction (see the section of this report entitled Growth Factors). A major leap forward was the discovery that platelet-derived growth factor (PDGF) has amino acid sequence homology with the c-sis oncogene product and that human PDGF and

the c-sis gene product are both structurally similar and immunologically cross-reactive (Weiss, 1983; Doolittle et al., 1983; Waterfield et al., 1983; Robbins et al., 1983). Furthermore, an equally exciting discovery was that the erb-B oncogene product had considerable sequence homology with the human epidermal growth factor (EGF) receptor (Downward et al., 1984). The oncogene protein is a truncated version of the normal receptor, lacking most of the external EGF-binding domain but retaining the transmembrane domain and the domain involved in stimulating the initial internal biochemical processes of cell proliferation. It can be postulated that both the sis oncogene and the erb-B oncogene produce uncontrollable growth by related processes. In the first situation, the cancer cell overproduces a growth factor (PDGF) which continually turns on cell growth by sustained interaction with its own receptor. In the second example, the absence of an EGF binding domain might remove the requirement for EGF-receptor binding and generate a continuous growth signal equivalent to sustained EGF stimulation. The role of growth factors in the cancer process has become established.

The interesting results obtained directly by transforming primary cell cultures with two oncogenes and by establishing a relationship between oncogene products and growth factor activity have been further substantiated by a number of other observations. As suspected, when primary fibroblasts which normally die after twenty doublings in culture are immortalized by carcinogen treatment, the immortalized but not the primary cells can be transformed with onc genes (Newbold and Overell, 1983). A carcinogen must have been responsible for the first genetic change(s) in the carcinogenic process while the transfected oncogene fulfills the last requirement for transformation. A functional relationship between oncogenes is implied by results with carcinogen-induced revertants of NIH/3T3 which are resistant to transformation by viruses carrying the ras, fes or src oncogenes but can be retransformed by viruses carrying the sis, mos or fms oncogenes (Noda et al., 1983). This suggests that the activities of the first three oncogenes converge on a common target which is bypassed by the last three. When a series of human tumors are examined for increased expression of oncogene transcripts, several c-*onc* genes always are transcriptionally active at the same time (Slamon et al., 1984). Although these results could be explained by the presence of heterogeneous populations of tumor cells which express each oncogene separately, they could also support multiple oncogene expression as a prerequisite of uncontrolled growth. An even more interesting correlation is the high frequency of expression of c-*fos* and c-*myc* along with either H-*c-ras* or K-*c-ras*. In every case of multiple onc gene expression, one onc gene codes for a nuclear protein and the other for a plasma membrane protein. As researchers begin to look at different kinds of cancers in greater depth, they are finding elevated levels of several oncogene products coexisting. For example, both ras and myc genes are activated in the HL-60 human promyelocytic leukemia cell line (Murray et al., 1983). Furthermore, mitogens which are now known as oncogene products, such as PDGF, will specifically induce lymphocytes to express c-*myc* in a cell cycle dependent manner, as will other mitogens such as lipopolysaccharide or Concanavalin A (Kelly et al., 1983). As we have noted before in previous reports, many oncogenes exhibit a rare tyrosine-specific kinase activity (See Table). Interestingly, the erb-B oncogene shares DNA sequence homology with genes that code for proteins with known tyrosine kinase activities (Privalsky et al., 1984). All of these observations together provide the first rational opportunity to speculate about the general mechanisms responsible for growth transformation.

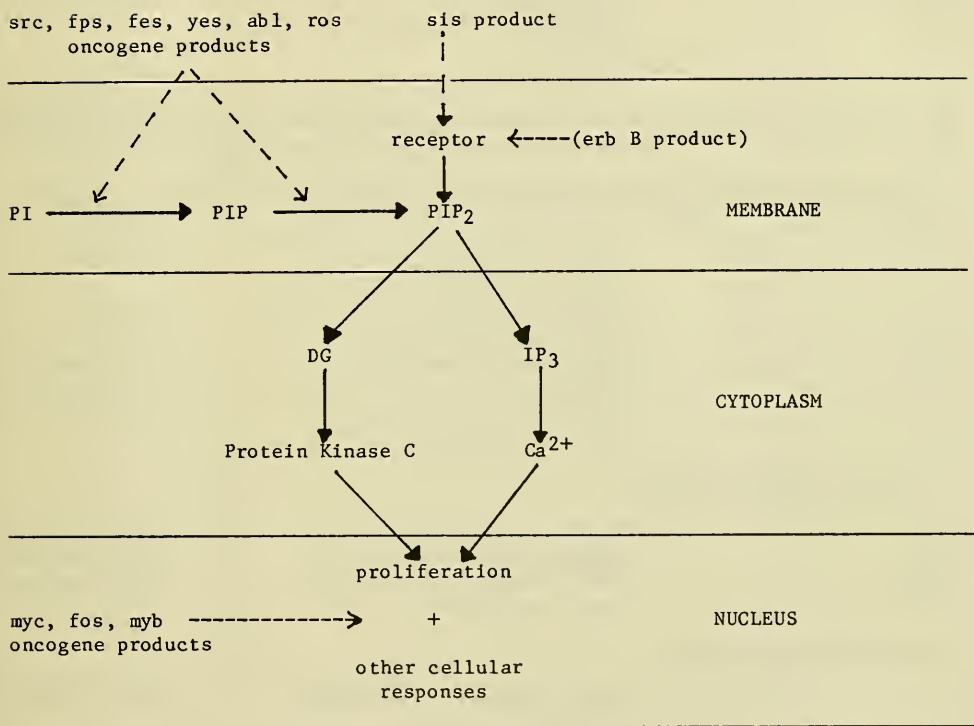


The scientific literature now abounds with speculation and summarizations of how the cooperation between two or more oncogenes is related to mechanisms of action of growth factors (Land et al., 1983b; Marx, 1983a; Marx, 1983b; Marx, 1984a.; Heldin and Westermarck, 1984). Normal diploid cells in culture are under the control of exogeneous growth factors and will cease to divide when these factors are removed. Transformed cells, however, lack certain exogenous growth requirements, supporting the notion that the autonomous growth of cancer cells may be due to the "constitutive expression" of any of the controlling elements along the normal mitogenic pathway. Thus, although it is not a novel idea that transformed cells synthesize, release and respond to their own growth factor, the findings that c-sis codes for PDGF and that c-erb-B codes for an altered EGF receptor protein strongly support this idea. Also, the fact that many oncogenes (e.g. c-myc, c-fos, Large T, ElA) code for nuclear proteins strongly supports the idea that these proteins represent final steps in the cell growth cycle. Plasma membrane proteins coded for by oncogenes such as c-ras, c-src, c-abl and c-erb-B represent initial steps in whatever the complex processes are that signal the nucleus to initiate DNA synthesis. However, although there are many different growth factors and activities associated with oncogene products of the plasma membrane, they all have been associated with the concurrent expression of the c-myc product, which is a nuclear protein. Thus, it is very reasonable to assume that the post receptor pathways of several different mitogens converge at the regulation of expression of genes like c-myc. Changes in proteins of the plasma membrane and the nucleus appear to be requirements of neoplastic transformation. The area which remains the most uncertain is the nature and number of steps occurring as secondary signals between the plasma membrane and nucleus. The tyrosine-specific kinase activities of many oncogenes located both in the plasma membrane and cytoplasm suggest that the idea of a tyrosine kinase cascade should not be dismissed. However, it is possible that scientists have been looking in the wrong place for second messengers transmitting the growth response to the nucleus of the cell. A new body of evidence strongly suggests that a group of minor membrane phospholipids, polyphosphoinositides, play a central role in signal transmission (Marx, 1984b; Michell, 1984). This theory is extremely attractive because it links basic information obtained for many years associating increases in the intracellular concentration of  $Ca^{2+}$  ions to cell growth and activation of protein kinase C. Protein kinase C has a crucial role in signal transduction for a variety of biologically active substances which activate various cellular functions and proliferation (Nishizuka, 1984). The recent finding that the src oncogene phosphorylates phosphatidylinositol, increasing the formation of polyphosphoinositide (Sugimoto et al., 1984), provides a new explanation for the role of oncogene kinases in signal transmission. Thus, a new model representing the relationship of oncogenes to deregulation of cell growth can be summarized as in the adjoining diagram. Oncogene products having kinase activity would serve to control the supply of  $PIP_2$  whereas other oncogenes, such as ras, might be involved in the breakdown of  $PIP_2$ .

The field of oncogene research is so active that there are sure to be additional key developments in the next year. It is likely that other oncogene products will be identified as growth factors or growth factor receptors. EGF and insulin are being studied for this kind of correlation. Other laboratories will be rushing to confirm the possible relationship of their oncogenes to the inositol lipid model which has been discussed above. Understanding the activity

of oncogene products which bind to DNA will be very difficult but critical to our understanding of the regulation of cell proliferation. It would be exciting if the the myc protein product was a protein that bound specifically to the Z-DNA enhancer control sequences (See Section on Z-DNA).

MODEL FOR ONCOGENE ACTIVATION  
OF CELL MITOSIS



PI = Phosphatidylinositol  
 PIP = Phosphatidylinositol phosphate  
 PIP<sub>2</sub> = Phosphatidylinositol diphosphate  
 IP<sub>3</sub> = inositol triphosphate  
 DG = diglycerol

## Families of onc Genes

<u>Nomenclature*</u>	<u>Original Identification</u>	<u>Cellular Location</u>
<u>I<sub>A</sub> Tyrosine kinase activity</u>		
src	Rous sarcoma virus (chicken)	plasma membrane
fps, fes	Fujinami sarcoma virus (chicken, cat)	cytoplasm
yes	Yamaguchi (Y73) sarcoma virus (chicken)	?
ros	UR2 sarcoma virus (chicken)	cytoplasmic membrane
abl	Abelson murine leukemia virus	plasma membrane
fgr	Gardner-Rashed feline sarcoma virus	?
<u>I<sub>B</sub> Nucleotide sequence homology to tyrosine kinase genes - activity unknown</u>		
mos	Moloney sarcoma virus (mouse)	cytoplasm
rel	Reticuloendotheliosis virus strain T (turkey)	?
fms	McDonough feline sarcoma virus	cytoplasm
raf	murine transforming virus	?
erb-B	Avian erythroblastosis virus (chicken)	plasma membrane, (EGF receptor)
mht	Avian carcinoma virus (MH2)	?
mil	Avian carcinoma virus (MH2)	?
<u>II GTP/GDP binding activity</u>		
H-ras	Harvey sarcoma virus (rat)	plasma membrane
K-ras	Kirsten sarcoma virus (rat)	plasma membrane
has/bas	Balb/c sarcoma virus (mouse)	plasma membrane
N-ras <sup>+</sup>	human neuroblastoma	?
<u>III DNA binding activity</u>		
myc	myelocytomatosis virus MC29 (chicken)	nuclear matrix
N-myc <sup>+</sup>	human neuroblastoma	?
myb	avian myeloblastosis virus (chicken)	nuclear matrix
E1A	Adenovirus (DNA virus) (human)	nucleus
Large T	Polyoma virus (DNA virus) (mouse)	nucleus
fos	FBJ osteo sarcoma virus (mouse)	nucleus
<u>IV Growth factor activity</u>		
Blym <sup>+</sup>	human bursal lymphoma	transferrin homology
sis	Simian sarcoma virus (woolly monkey)	PDGF
erb-B	See I <sub>B</sub>	EGF receptor

<u>Nomenclature*</u>	<u>Original Identification</u>	<u>Cellular Location</u>
<u>Other - no known activity</u>		
mam <sup>+</sup>	mammary carcinoma (man, mice)	?
neu <sup>+</sup>	neuroblastoma, glioma (rat)	?
ski	Avian SK V770 virus	?
int-1	mouse mammary tumor virus	?
erb-A	avian erythroblastosis virus (chicken)	cytoplasm
Middle T	polyoma virus (mouse)	plasma membrane

\* All of these acronyms are expressed as v-onc or c-onc depending upon whether a viral or cellular transforming genes is being studied.

+ Identified by transfection - no v-onc counterpart yet identified.



## Growth Factors

It has long been recognized that a characteristic of malignancies is that they are less responsive, both in vivo and in vitro to normal growth regulation than non-neoplastic tissues. Cells that typically require insulin or platelet-derived growth factor (PDGF) or epidermal growth factor (EGF) or multiplication stimulating activity or somatomedins for proliferation lose these factor requirements when they are transformed by viruses or chemicals. Similarly, a number of cell lines derived from tumors are able to continue to proliferate in the absence of added growth factors. It was proposed (Le Larco and Todaro, 1978) that these malignant cells become self-sufficient by producing their own growth factors. This hypothesis has found strong support in the last year with the astounding conclusion cited above under Oncogenes, that the amino acid sequence of the *sis* simian sarcoma virus transforming gene product called p28<sup>sis</sup> closely corresponds to that of human PDGF (Doolittle et al., 1983, Waterfield et al., 1983 and Robbins et al., 1983). Further studies showed that the two proteins share antigenic determinants and structural conformation and exert identical biological functions (Owen et al., 1984) that can only be explained by an ancestral relationship between the two. Swiss mouse 3T3 cells (Deuel et al., 1983) and normal rat kidney cells, transformed by simian sarcoma virus, but not control cells, produce a growth factor that is identical to PDGF. C-*sis* transcripts and PDGF-like proteins have now been demonstrated in human osteosarcoma, glioblastoma and fibrosarcoma cells.

Considerable is also now known about the cell biology of PDGF (Stiles, 1983). It is a basic protein synthesized in blood platelets and composed of at least two closely related peptides, PDGF I and II. PDGF-specific receptors are found on a wide variety of connective tissue cells. The receptor has been partially characterized, has a molecular weight in the range of 185,000 (Heldin et al., 1983) and appears to be a tyrosine-specific kinase that may undergo autophosphorylation. Most connective tissue cells in culture contain an abundance of receptors, up to 350,000 per cell. However, the number of available receptors for PDGF on sensitive human or mouse cells decreases by 50 - 100% when the cells are transformed virally or chemically. This reflects partly the production of new proteins that compete with PDGF for the receptors and partly an actual reduction in receptor number (Bowen-Pope et al., 1984).

An unusual feature of PDGF as a mitogen is that cells require only brief exposure (about 30 minutes) to the factor to become "competent" to induce the subsequent cell proliferation 10 - 12 hours later (Singh et al., 1983); however, the response requires other plasma components or growth factors in addition. A small number (perhaps five) of "early genes" are apparently induced by PDGF during this brief exposure (Cochran et al., 1983). Another protein called pI, which is associated with the nucleus is also rapidly induced by PDGF and has been proposed as the major signal for the initiation of growth in this cell system (Olashaw and Pledger, 1983).

Epidermal growth factor (EGF) has been the model for all growth factor research in the last twenty years. Originally identified as the causative agent in eyelid opening in newborns, it is identical with the hormone called urogastrone. It is a single-chain polypeptide of molecular weight 6000, containing 53 amino acids with three disulfide bonds, synthesized as a large protein precursor.

EGF stimulates active cell proliferation (Carpenter and Cohen, 1979) in epidermis and some epithelial tissues and a variety of cell types in vitro. The entire metabolic pathway of EGF binding to receptors, the internalization and degradation of the complex has been described in detail (Carpenter, 1983; Matrisian et al., 1984; Planck et al., 1984). Initiation of DNA synthesis in EGF-sensitive cells requires at least eight hours of continuous exposure to the factor. Utilizing a human epidermoid carcinoma cell line, A-431, that is very rich in EGF receptors, it was demonstrated that purified EGF receptor protein contains a tyrosine-specific protein kinase activity that autophosphorylates the receptor protein itself. The molecular weight of the receptor is 170,000 (Soderquist and Carpenter, 1983; Schlessinger et al., 1983).

EGF came into special prominence in the past six months when it was reported that an oncogene of a tumor virus of birds (erythroblastosis virus) called erb-B is almost certainly derived from the gene for the cell surface receptor for EGF (Newmark, 1984). The key to this development was the securing of sufficient receptor protein to do amino acid sequencing. The receptor appears to be oriented in the cell with 650 amino acids in the N-terminal, jutting outside the plasma membrane where EGF binds, and 550 amino acids at the carboxy-terminal end inside the membrane. The kinase and the tyrosine that is the phosphorylation site both lie within the cell. The erb-B protein apparently lacks a long stretch of the N-terminal end of the receptor, including the binding domain; thus the oncogene protein cannot bind EGF (Downward, et al., 1984).

EGF is a member of a family of growth factors that can stimulate confluent quiescent cells in monolayer culture to synthesize DNA in the presence of serum protein. The wealth of new information about this factor has become available in the past year in part because, as noted above, the cellular receptor for EGF has identity with the product of the erb-B oncogene. In addition EGF has many similarities with a new growth factor family that is becoming increasingly prominent in cancer biology research, the Tumor Growth Factors (TGF). The TGFs are operationally defined as the factors that render normal "indicator" cells reversibly transformed as evidenced by their new morphology in monolayer cultures or by the acquisition of anchorage-independent colony growth in semi-solid medium. The "indicator" cells are usually normal rat kidney cells or mouse AKR-2B cells.

The first TGF ever described was a sarcoma growth factor (SGF) (DeLarco and Todaro, 1978) derived from conditioned medium of Moloney sarcoma virus-transformed 3T3 cells. The potent biological activity of SGF has now been shown to be the result of two different subtypes of TGFs in the medium (Anzano et al., 1983). Type I, also called alpha or eTGF (for EGF-like) competes for the EGF receptor, stimulates phosphorylation by the endogenous protein kinase, has weak transforming activity and has considerable homology with mouse EGF. Type II also called beta or dTGF (for EGF-dependent) does not bind the EGF receptor but in the presence of EGF or alpha-TGF is a potent stimulus for anchorage independent growth. It was recently shown that beta-TGF causes a rapid increase in membrane receptors for EGF (Assoian et al., 1984) which may explain the synergism seen between the two TGFs. Both alpha and beta-TGFs have been simultaneously partially purified from serum-free medium conditioned by a human melanoma cell line, Hs0294 (Richmond, 1983; Richmond, 1984).

Although beta-TGF can be identified from both normal and transformed cells, alpha-TGF comes only from transformed cells, including virally transformed mouse 3T3 cells, retrovirus-transformed rat fibroblasts and human melanoma cells. One form of alpha-TGF has been highly purified from serum-free medium conditioned by human melanoma cells (Marquardt and Todaro. 1982) and another larger form, from Snyder-Theilen feline sarcoma virus-transformed rat embryo cells (Massague, 1983). The latter has been completely sequenced (Marquardt et al., 1984) and shown to have a molecular weight of 5,600 and to contain fifty amino acids. Most recently the alpha-TGF has been completely synthesized by chemical means and the product shown to have equivalent biological activity to the natural alpha-TGF (Tam et al., 1984).

TGFs can also be demonstrated in extracts of human adenocarcinomas, fibrosarcomas, leiomyosarcomas, Hodgkin's lymphoma, fibroadenomas, leiomyoma and non-neoplastic kidney and lung, by the transformation assay (Nickell et al., 1983). These have been subclassified for their selective activity on mouse or rat cells as TGF $\alpha$  or TGF $\beta$ , respectively. TGF $\beta$ 's are either smaller than 6000 molecular weight (TGF $\beta$ s) or larger than 12,000 (TGF $\beta$ 1), and apparently of the alpha type. It is possible that an interaction of two or more of these various factors play a role in neoplastic development or in maintenance of the transformed phenotype. The finding that some TGFs are expressed in normal tissue suggests that neoplasia involves enhanced expression of otherwise normal gene products.

#### Tumor Cell Enzymes Involved in Metastasis

Among the most relevant biological questions posed today by cancer research is why and how do tumor cells depart from their primary tumor and establish new metastatic tumors at distant locations? Although only an estimated 0.001% of detached cells are successful in this process, they are the tumor cells that are the principle obstacle to cancer cure. Answers to the question are not yet in hand but some interesting new information about one process involved in metastasis, the role of degradative enzymes, has been reported in the past year. These enzymes are involved in the early malignant cell escape into the circulatory system and the later escape out of it, into a new tissue site. The invasive cell actually cuts through the extracellular matrix by forcing a hole in the vascular endothelium and boring on through the basement membrane by digesting it away (White, 1983). Enzymes may also be involved in the protective aggregation of platelets around circulating tumor cells (Honn et al., 1983), and localized enzymatic activity induced by angiogenic agents may be the key to neovascularization in developing tumors (Kalebic et al., 1983 and Gross et al., 1983). Proteases and other degradative enzymes are normal cellular components sequestered from the contents of the cell in lysosomes. The release of such enzymes outside the cell or to the cell surface is usually carefully regulated. Tumor cells produce directly and indirectly the specific enzymes they need to destroy the extracellular matrix and in the proper sequence. The four major groups of connective tissue proteins are:

- 1) glycoproteins, including fibronectin, laminin, chondronectin, etc.
- 2) proteoglycans such as the glycosaminoglycans chondroitin, keratan and heparan sulfate and heparin, and the core proteins



- 3) collagen, interstitial types I, II and III and basement membrane types IV and V.
- 4) elastin, an amorphous highly insoluble protein resistant to proteolysis  
(Jones and De Clerck, 1982)

There is however, no typical connective tissue because the quantitative and qualitative distributions of these tissue proteins vary with the tissue type. This thus serves to demonstrate how complex a process it is for a tumor cell to penetrate tissue barriers. Fibronectin and laminin are susceptible to a variety of serine proteases including plasmin and elastase and the cysteine proteases, the cathepsins. Degradation of the proteoglycans requires both proteases and enzymes like hyaluronidase. Collagen is rather protease-resistant, however specific metalloproteases (collagenases) exist for each collagen type. Hydrolyzed collagen fibrils are susceptible to a number of proteases. Elastin is also proteolysis resistant except to specific elastases.

The serine protease called plasminogen activator (P.A.) may be the enzyme most closely associated with metastasis. It is involved in early events in the enzyme cascade as it converts serum plasminogen to the important protease, plasmin. No clear correlation has been observed, however, between P.A. levels and the metastatic ability of a cell (Nicolson, 1982). Another difficulty not yet resolved is whether the tumor cell itself or participating normal host tissue actually produces the enzyme. Recently it was demonstrated that normal human diploid fibroblasts in culture can be induced to produce P.A. by a diffusible factor from malignant mouse cells (Davies et al., 1983). This polypeptide inducer is now being characterized. This suggests an interesting new mechanism for local proteolysis in which tumor cells elicit the help of adjacent normal cells to elaborate necessary enzymes. In contrast, the inhibition of P.A. activity in one cell by another has also been recently demonstrated (Liu, et al., 1984). Two cell lines of a rat neuroblastoma, isolated from the same tumor, are different from each other in that "D" cells are tumorigenic and have high levels of P.A., "AC" cells are stem cells without P.A. activity. When D and AC cells are co-cultivated the enzyme activity is nearly extinguished. This inhibition is not mediated by a soluble factor but requires cell-cell contact. It is not surprising that an enzyme of such biological importance as P.A. is subject to regulation by a variety of effectors. Glucocorticoids have been implicated in the regulation of plasminogen activator in rat hepatoma cells in culture first as inducer of a specific cell inhibitor of P.A. and second in a paradox, that the steroids enhance stimulation of P.A. by cyclic nucleotides (Gelehrter et al., 1983). In MCF-7 cells both natural and synthetic estrogens induce P.A. activity in parallel with cell growth. Antiestrogens suppress both P.A. activity and growth (Katzenellenbogen et al., 1984).

An important direct test of the involvement of P.A. in tumor dissemination has been made recently using antibodies against P.A.. Human carcinoma cells inoculated onto the chorioallantoic membrane of a developing chick egg typically invade the embryo and develop as pulmonary metastases. The antibodies administered to the embryo either delayed or prevented the establishment of metastatic foci (Ossowski and Reich, 1983).

One model system in popular use for studying metastatic properties is the B16 mouse melanoma. Among a number of B16 sublines compared, the ability to enzymatically degrade sulfated glycosaminoglycans (heparan sulfate) present in extracellular matrix correlated well with the ability to form metastatic colonies in the lung. This suggests the presence of a specific endoglycosidase that helps degrade the walls of pulmonary blood vessels (Nakajima et al., 1983). Comparison of cathepsin B activity between solid subcutaneous tumors of B16 F1 (with low metastatic potential) and B16F10 (highly metastatic) (Sloane et al., 1982) also showed good correlation with metastatic potential. In this case the successful metastatic cell, endowed with cathepsin B may be better able to survive the perils of the circulatory system. This enzyme acting directly or indirectly on platelets causes their aggregation and may thus shield the tumor cells and facilitate their arrest in a new location.

Separation of rabbit VX-2 carcinoma into the two cell types, E cells (epithelial-like) and F-cells (fibroblast-like) has provided some new information about collagenase production by tumor cells (Dabbous et al., 1983a) in serum-free media. The F-cells have a normal karyotype and thus probably represent a cell population contributed by the normal host tissue. The E-cells represent the true tumor tissue. Both cell types produce both active and latent collagenase when co-cultured, but the F-cells lose this function when in pure cultures after several passages whereas pure E-cells keep expressing collagenase activity. The authors concluded that the tumor cells (E-cells) stimulate collagenase production and release from neighboring fibroblastic stromal cells through a factor released into the medium. This factor may also be present in the serum of rabbits carrying VX-2 carcinoma tumors (Dabbous et al., 1983b).

### Angiogenesis

Another supportive role for degradative enzymes in insuring the maximum survival of malignant tumor cells is in the process of angiogenesis. The basal lamina of certain capillaries must be destroyed in order that new blood vessel sprouts can be formed and the endothelial cells must then penetrate surrounding tissue to extend the capillary network. Bovine capillary endothelial cells in culture produce increased amounts of both plasminogen activator and latent collagenase when exposed to any of several angiogenic factors (Gross et al., 1983). In a comparable system utilizing fetal bovine endothelial cells, both type IV and V collagenase were induced by an angiogenic factor which also promoted cell migration toward it. Type IV and V collagens are components of basement membrane and metalloproteinases capable of degrading them have not previously been identified in endothelial cells.

Since the generation of a new vascular system is critical for the development of a tumor, any agent that interferes with angiogenesis has potential therapeutic use. The recent demonstration (Folkman et al., 1983) that the administration of heparin and cortisone in test animals prevented metastasis and even caused regression of large tumor masses has been greeted with great interest. The antitumor effect of heparin-cortisone is specific in that growth of new microvessels is prevented. The problem of heparin's anticoagulant activity when administered subcutaneously has been overcome since heparin administered orally is degraded

into highly active fragments synergistic with cortisone in preventing angiogenesis. Although not effective against all types of experimental tumors, this drug combination is being pursued as a possible new class of pharmaceutical agents.

### Differentiation of Hematopoietic Tumor Cells

The process of cell differentiation is another area of research emphasis in the Tumor Biology Program. From studies of cell biology it is known that when a cell becomes committed to a program of maturation it loses the ability to proliferate. Agents which could force continually proliferating neoplastic cells into a pathway toward differentiation might be exploited for chemotherapy. Perhaps the best tumor model system for studying the details of differentiation is the hematopoietic system which provides, between bone marrow and blood, representative cells of nearly every stage in the lineage of hematopoiesis. Recent progress has focused on purification of cells characteristic of these various stages of maturation and attempting to purify their regulatory factors. Unlike the recent successes in sequencing growth factors for epidermal and mesenchymal cells whereby a new understanding of their cell biology was gained, the growth factors for human hematopoietic cells (except erythropoietin) are not highly purified. The problem of identifying the maturation stage of hematopoietic cells is also very difficult. Primitive stem cells do not actively proliferate in vitro, do not have specific antigenic determinants and probably lack receptors for any common biological modifiers because once receptors for a modifier develop, the stem cell becomes a "committed" progenitor cell willing to begin expressing specific characteristics. Stem cells apparently shift from multipotential (able to produce progenitor cells of different hemopoietic lineages) to bipotential to monopotential during commitment, depending on the available regulatory molecules. Immature cells at any point in these early stages may be the target of a transforming agent and that clone will subsequently expand into some variety of clinical leukemia.

The mouse models, both normal and leukemic, currently in use have contributed the most information to this research area. Four different molecular regulators, of proliferation and maturation have been described and purified to homogeneity: granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage-CSF (M-CSF), granulocyte-CSF (G-CSF) and multipotential-CSF (Multi-CSF) (Metcalf, 1983; Johnson and Nicola, 1984). The kind of effect each factor has can be implied from its name. These factors are critical for proliferation of progenitor cells, however, G-CSF may be most important because of its potent differentiating activity, forcing cells to enter either the granulocytic or macrophage pathway. Its activity on granulocyte precursors is so rapid the possibility has been raised that G-CSF may modify one of the newly synthesized daughter chromatids in the dividing precursor cell during S phase, causing immediate commitment and a granddaughter cell with modified characteristics.

There are three leukemia cell lines of human origin that have been most intensively studied over the past five years. They are each homogeneous cell populations *in vitro*, but when transplanted into immunodeficient mice they form myelosarcomas. The first described was the K-562 line (Lozzio and Lozzio, 1975), established from a patient with chronic myeloid leukemia; however, the sublines



contain erythroleukemic cells that synthesize red blood cell markers. Under different culture conditions, this is a pluripotent line and progenitors of either the granulocytic, monocytic, erythrocytic, megakaryocytic or lymphocytic lines can be induced (Lozzio et al., 1983).

The next line isolated was the HL-60, from the blood of a woman with acute promyelocytic leukemia (Gallagher et al., 1979). These cells are more mature than most of the other cell models and represent the later stages of granulocyte maturation. They are easily triggered by various agents (DMSO, chemotherapeutic drugs, retinoic acid) to become granulocytes; however, tumor promoting phorbol diesters induce HL-60 to differentiate to macrophage-like cells. The mechanism of these inductions is not at all clear. It was recently noted that HL-60 cells have amplified myc genes as do a number of actively proliferating tumor cells.

The most recently described myeloid line, KG-1 (Koeffler and Golde, 1978), was derived from the bone marrow of a man with erythroleukemia. The cell line is composed of myeloblasts that are quite resistant to any of the common inducers, however phorbol diesters promote the development of macrophage-like characteristics. In general, murine colony stimulating factors are inactive on any of these human leukemic cell lines. The KG-1 line secretes a CSF-like growth factor which seems to stimulate its own self-renewal however this factor has not yet been purified (Koeffler, 1983).

Although it may be over optimistic to believe that a leukemic cell with its abnormal chromosomes, deletions and translocations could really undergo normal poiesis, it is reasonable that some cells with more normal karyotypes might revert to diploidy. In the least, the self-renewal program of the most premature leukemia cells may be turned off and the origin of the growing population of tumor cells thus eliminated.

### Introduction of Foreign Genes Into Mice

The use of genetically engineered mice to study gene expression and regulation has a number of advantages over the use of tissue culture cell lines. The major advantage is that the biological system is a whole animal and not subject to the criticism that in vitro observations have no relevance to in vivo physiology. Other advantages are also important to note: (1) a gene of known origin and sequence becomes inserted into the germ line and thus is present in the same chromosome location of every cell in the animal's body; (2) the gene can be retrieved whenever necessary; and (3) sequential changes in gene DNA structure and expression can be studied in different tissues during the development and aging of the animal. The technological advances in the last few years in handling fertilized eggs and achieving development in foster mothers combined with state-of-the-art molecular genetic technology are offering a whole new approach to studying gene regulation.

One of the biggest obstacles to studying genetically engineered mice is that of controlling which tissues will express the gene activity. There have been two major reports recently which study this problem. A group of scientists in England have microinjected a cloned rabbit globin gene into mouse eggs and studied its chromosome location, inheritance and tissue expression (Lacy et al., 1983). In five transgenic mouse lines investigated, the foreign globin gene integrated at

one or two different loci, was amplified up to as many as forty copies in a tandem array but failed to express itself in erythroid cells. There was an inappropriate low level expression in skeletal muscle of one line and testes in another line. Significantly, however, the integrated genes were stably inherited as a single Mendelian marker. Another study has achieved greater success by obtaining expression of a microinjected immunoglobulin gene in what appears to be a tissue-specific fashion (Brinster et al., 1983). The microinjected immunoglobulin gene was expressed in the appropriate target tissue, spleen, but not in the liver. The injected gene was expressed equivalently in every line of transgenic mice investigated; thus, the chromosome location and copy number appear to have little bearing on expression. It was concluded that the signals for tissue-specific expression already were contained within the cloned DNA which was introduced into the original mouse eggs.

The above studies have stimulated a great deal of speculation about what controls the success or failure of tissue-specific expression (Hogan, 1983; Marx, 1983c). Once again the well-studied immunoglobulin genes become a good model system for investigating biological phenomenon of general importance. The possible role of tissue-specific transcription in cell differentiation and malignant transformation is addressed in experiments which identify short DNA sequences required for high level expression of the heavy chain immunoglobulin gene after introduction into mouse myeloma cells by DNA transfection (Gillies et al., 1983). The immunoglobulin gene with its enhancer sequence was expressed in myeloma cells, the tissue of origin, but not in fibroblasts which were derived from a different tissue. For the first time enhancer elements have been identified in normal cellular genes of eukaryotes. It turns out that regulatory enhancer segments in the DNA increase the rate of transcription for many eukaryotic promoters (Boss, 1983). Previously, enhancer elements had been identified only in viruses and, although all viral enhancer sequences contain a similar core element, the rest varies considerably and appears to control the species- and tissue-specificity of the virus. An interesting feature of enhancers is that, within reason, their distance upstream or downstream from the promoter does not alter activity. Thus, they can be located before or after exons or within introns. There is also the possibility that more than one enhancer element can contribute in some additive fashion to promoter activity.

Understanding how to select for the right combination of enhancers, promoters and flanking sequences may insure tissue-specific expression of a gene wherever it happens to integrate. Enhancers which are not regulated by chromosome position may explain why myc oncogenes in some human tumors even when oriented in the opposite transcriptional direction relative to immunoglobulin genes are still activated. The ability to create transgenic mice in which oncogenic DNA sequences are expressed in a tissue-specific fashion will provide excellent experimental model systems for studying the development of different kinds of malignancies.

### Z-DNA - Regulation of Gene Expression

What appeared to be an odd discovery of no particular biological significance five years ago may be critical to understanding how the structure of DNA is related to the regulation of gene expression (Kolata, 1983; Widom, 1984). Normally, DNA exists in a right-handed, double-stranded conformation which is called B-DNA.

Dr. Alexander Rich and his colleagues discovered that when the DNA molecule consists of nucleotide sequences of alternating purine and pyrimidine residues, the molecule can assume a new conformation, which they termed Z-DNA (Wang et al., 1979). This unusual conformation of the DNA molecule is also double-stranded but is much more tightly coiled in a left-handed rather than a right-handed orientation, the backbone zigzagging down the molecule. Since the formation of B-DNA requires less energy than Z-DNA, these investigators began to suggest that Z-DNA might be involved in the control of gene expression. Clearly, understanding the mechanism of gene expression is important to cancer as well as many other disease processes.

There are several lines of evidence which intriguingly attribute regulatory significance to the Z-DNA conformation and which relate strongly to other areas of research. In last year's annual report, information was presented which supported methylation of DNA as a potential mechanism for switching genes on and off, 5-methylcytosine being implicated as a target/product of this process. A group of investigators at the National Institutes of Health have observed that when certain cytosine-guanine sequences are methylated in eukaryotic DNA, genes nearby are activated, and when they are not methylated, the genes are inactive. Interestingly, the Z-DNA conformation is stabilized when the 5 position of cytosine-guanine residues is methylated (Fujii et al., 1982). Furthermore, for over fifteen years histones, small proteins of molecular weight 10,000 to 15,000, were believed to be the elements in the nucleus of the cell which controlled gene expression, but now it is believed that larger proteins present in smaller quantities are involved in the regulation of specific gene expression. Significantly, proteins of molecular weight 70,000 to 150,000 can be isolated which bind selectively to Z-DNA and not to B-DNA (Nordheim et al., 1982). These could be the regulatory proteins that scientists have sought for so many years; they bind to the Z-DNA and hold it in the Z-conformation.

To further investigate the importance of the Z-conformation of DNA, investigators have employed new technology and a well-defined viral model system. Monoclonal antibodies were developed which reacted specifically with Z-DNA and a method was developed to cross-link these antibodies to the DNA binding sites (Moller et al., 1982). With this technology available it was quickly demonstrated that fluorescently labeled antibodies bind to transcriptionally active genes in the macronucleus of protozoa (Lipps et al., 1983) and that polytene chromosomes of *Drosophila* contain regions of Z-DNA (Pardue et al., 1983). With the knowledge that Z-DNA was present in cellular DNA, investigations turned to the SV40 virus, a monkey tumor virus, all of whose genes and control regions are completely characterized. After exposure to the most favorable conditions for Z-DNA formation, the Z form was observed only in the control regions of the viral DNA and specifically in a segment of the DNA known to contain the transcriptional enhancer sequence (Nordheim and Rich, 1983). A survey of other known viral genomes also demonstrated that alternating stretches of purines and pyrimidines, the Z-DNA forming regions, were in the regulatory regions of the viruses. If the alternating stretches of SV40 were modified by mutation to reduce the probability of Z-DNA formation, the virus grew very slowly. Thus, Z-DNA is generally found in normal eukaryotic DNA and specifically in enhancer regions that serve as entrance sites for RNA polymerases which are critical for regulating gene transcription.



The discovery of Z-DNA and its prevalence in different kinds of biological systems opens up completely new approaches for studying gene regulation. It is now possible to study how RNA polymerases attach to the DNA molecule before transcription and to investigate the nature and mechanism of action of proteins potentially critical in the control of gene expression. This rapid transition from fundamental studies of DNA structure to studies of gene expression would not have been possible without the development of new biotechnologies and past investment of resources into structural and functional studies of tumor viruses.

The possible involvement of Z-DNA segments in transcriptional enhancement may have some relationship to oncogene activation. Anything that modifies the Z-DNA potential, whether by mutation or translocation, could enhance or inhibit the effectiveness of a particular regulatory sequence. Oncogenes express transforming potential when their normal functions are no longer regulated either quantitatively, qualitatively or temporally.

### Summary

The discussions in this report probably raise as many questions as they answer. Even the explanations that seem simple are not simple at all. For example, it is apparent that simple autocrine production of a growth factor cannot render a cell "transformed"; in fact, maintaining a constant high concentration of standard growth factors in the medium usually makes normal cells in culture insensitive to these regulatory molecules. The tumor cell has no comprehension of this desensitization process. These are finely tuned systems where a single amino acid change in protein structure can make a large difference in biological activity; it is not surprising that very sophisticated techniques have been required to begin unraveling the complex stepwise process of tumorigenesis.

We can now be confident that cancer biology is using state-of-the art technology to understand the most sophisticated biological processes. In the past, tumor cell systems were often deemed too complex, too unpredictable, too heterogeneous for really rigorous research. Now they may be the real key even to answering fundamental questions about normal cell functions. Oncogene probes have become the reagent of choice for investigations in all types of experimental systems. The scientists supported by this Program have the technological tools available to explore their most creative ideas; thus, we predict a continued high rate of progress.

FISCAL YEAR 1984 EST.  
TUMOR BIOLOGY PROGRAM  
SUMMARY BY SUB CATEGORY (DOLLARS IN THOUSANDS)

	NON-COMPETING		COMPETING		TOTAL	
	No.	Amount	No.	Amount	No.	Amount
Cell Surface	81	\$9,002	26	\$3,403	107	\$12,405
Enzymes	23	2,763	11	1,511	34	4,274
Peptide Hormones	17	1,908	4	496	21	2,404
Steroids	18	2,167	5	678	23	2,845
Membrane Organelles	7	1,290	4	616	11	1,906
Ribosomes & Polyribosomes	4	721	0	0	4	721
r-RNA	11	1,491	2	437	13	1,928
r-RNA	4	453	2	113	6	566
t-RNA	12	1,659	2	263	14	1,922
Growth Factors*	22	2,586	16	2,373	38	4,959
Nucleus	16	1,766	4	463	20	2,229
Contractile Elements	9	917	4	563	13	1,480
Development & Differentiation*	58	7,353	16	2,052	74	9,405
Cell Growth, Cell Division*	21	2,286	13	1,710	34	3,996
Clonogenic Cell Genetics	15	1,981	0	0	15	1,981
Inheritance of Neoplasms	3	389	0	0	3	389
Plasmids, Viruses	4	301	0	0	4	301
In Vivo & In Vitro Tumor Lines	4	524	6	525	10	1,049
Difficult to Classify	3	600	1	190	4	790
Oncogenes**	0	0	14	1,833	14	1,833
<b>SUB TOTAL</b>	<b>332</b>	<b>40,157</b>	<b>130</b>	<b>17,226</b>	<b>462</b>	<b>57,383</b>
Program Projects	13	8,807	5	5,127	18	13,934
Conferences	0	0	10	83	10	83
<b>SUB TOTAL</b>	<b>13</b>	<b>8,807</b>	<b>15</b>	<b>5,210</b>	<b>28</b>	<b>14,017</b>
<b>TOTAL</b>	<b>345</b>	<b>48,964</b>	<b>145</b>	<b>22,436</b>	<b>490</b>	<b>71,400</b>

These areas include considerable oncogene research.  
This area includes those grants which are primarily focused on oncogene research.



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Knowles Wistar Institute of Anatomy and Biology

R01 CA18801 Glycolipid Metabolism and Tumorigenesis  
Morre Purdue University, West Lafayette

R01 CA19130 The Surface Membranes of Normal and Cancer Cells  
Warren Wistar Institute of Anatomy and Biology

R01 CA19144 Membrane Changes Caused by Tumor Virus Transformation  
Buck Wistar Institute of Anatomy and Biology

R01 CA20026 Glycolipids of Normal and Transformed Cells  
Hakomori Fred Hutchinson Cancer Research Center

R01 CA20421 Mutants Altered in Glycosylation of Soluble & Membrane Proteins  
Krag Johns Hopkins University

R01 CA20424 Murine Ascites Tumor Cell Glycoproteins  
Goldstein University of Michigan at Ann Arbor

R01 CA20668 Immunomicrospheres for Cell Membrane Research  
Rembaum California Institute of Technology

R01 CA21246 The Roles of Laminin and Entactin in Cell Adhesion  
Chung University of Pittsburgh

R01 CA21359 Cell Interactions During Malignant Transformation  
Bertram Roswell Park Memorial Institute

R01 CA21404 Molecular Effectors of Enzymatic DNA Synthesis  
Modak Sloan-Kettering Institute for Cancer Research

R01 CA21463 Steroid Correction of the Cell Matrix in Neoplasia  
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R01 CA21722 Membrane Pathology in Carcinogenesis  
Scott Mayo Foundation

R01 CA21923 Oligosaccharide Structure and Function in Recognition  
Baenziger Washington University

R01 CA22451 Contact Behavior of Developing and Transformed Cells  
Trinkaus Yale University

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R01	CA36481	Cathepsin B-Like Cysteine Proteinases and Tumor Invasion Sloane Wayne State University
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Ramachandran University of California, San Francisco

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           Sorenson                   Dartmouth College
- R01 CA36399      Lineages in Mammary Cell Transformation  
           McGrath                   Michigan Cancer Foundation
- R01 CA36526      Expression of Human Chorionic Gonadotropin Genes  
           Kourides                   Sloan-Kettering Institute for Cancer Research
- R01 CA36718      Mechanism of Ectopic Hormone Synthesis by Tumor Cells  
           Cox                        University of Nebraska Medical Center
- R01 CA37370      Ectopic Hormone Synthesis in Pheochromocytoma Cells  
           Goodman                   New England Medical Center Hospital

STEROIDS

- R01 CA02758      Steroid Metabolism in Tumors and Normal Tissues  
           Kandutsch                  Jackson Laboratory
- R01 CA08315      Steroid Induced Changes in Cultured Malignant Cells  
           Melnykovych               University of Kansas, College of Health Sci. and Hosp.
- R01 CA13410      Mechanism of Hormone Action on Target Cells in Culture  
           Sonnenschein              Tufts University
- R01 CA15135      Histones in Cell Differentiation and Carcinogenesis  
           Zweidler                   Fox Chase Cancer Center
- R01 CA15776      Prostatic Differentiation and Sex Hormone Metabolism  
           Leav                       Tufts University
- R01 CA16091      Biochemical Control in Adrenocortical Carcinoma Cells  
           Sharma                    University of Tenn. Center for Health Sciences
- R01 CA17323      Glucocorticoid-Resistant Leukemic Lymphocytes  
           Munck                     Dartmouth College
- R01 CA19907      Physiology of Pituitary Cell Glucocorticoid Binding  
           Harrison                   Vanderbilt University
- R01 CA24347      Hormonal Control of Proliferation of Malignant Thymocytes  
           Thompson                  University of South Carolina at Columbia
- R01 CA25365      Hormonal Regulation of Cultured Endometrial Cells  
           Gerschenson               University of Colorado Health Sciences Center
- R01 CA26617      Estrogen Mediated Pituitary Tumor Cell Growth  
           Sirkasku                   University of Texas Health Sciences Center, Houston



- R01 CA27702 Sex Hormones, Cancer and the Immune System  
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- R01 CA29497 An Adrenal Tumor: Cytochrome P-450 and Steroidogenesis  
Hall Worcester Foundation for Experimental Biology
- R01 CA29808 Molecular Mechanism of Desensitization  
Iyengar Baylor College of Medicine
- R01 CA30253 A Study of Tropic Hormone Action in Carcinoma Cells  
Mason University of Texas Health Science Center, Dallas
- R01 CA31046 Mechanism of Estrogen Action on Melanocyte Function  
Beattie University of Illinois at Chicago
- R01 CA32178 Hormonal Control of Fibrinolysis in Gynecologic Tissue  
Sherman Sloan Kettering Institute for Cancer Research
- R01 CA32226 Steroid Resistance in Human Leukemic Cells  
Harmon U.S. Uniformed Services Univ. of Hlth. Sci.
- R01 CA32767 Steroid Receptors in Human Gynecologic Carcinoma Cells  
Crickard State University of New York at Buffalo
- R01 CA34860 Corticosteroids: Cytokinetic and Biochemical Studies  
Braunschweiger AMC Cancer Research Center and Hospital
- R01 CA36146 Studies of Regulation in E. Coli Extended to Lymphomas  
Bourgeois-Cohn Salk Institute for Biological Studies
- R01 CA36370 Tumor-Associated DNA Movement  
Selsing Brandeis University
- R23 CA38327 Tumor-Associated Renal Phosphate Wasting  
Adams University of Connecticut Health Center

#### MEMBRANEORGANELLLES

- R01 CA06576 Biochemical Cytology of Normal and Malignant Tissues  
Novikoff Yeshiva University
- R01 CA08964 Energy Metabolism in Normal and Tumor Cells  
Racker Cornell University, Ithaca
- R01 CA10951 Control of Enzymatic Phosphate Transfer in Mitochondria  
Pedersen Johns Hopkins University
- R01 CA12858 Lysosome Biogenesis: Normal and Tumor Cells  
Stahl Washington University
- R01 CA25360 Respiration-Coupled Transport Processes in Tumor Cells  
Lehninger Johns Hopkins University



- R01 CA25633 A New Assay for Transformed Cells  
Mangel University of Illinois, Urbana-Champaign
- R01 CA27809 Pathways of Energy Metabolism in Malignancy In Vitro  
Sauer Mary Imogene Bassett Hospital
- R01 CA28677 Transport in Cholesterol-Rich Tumor Mitochondria  
Coleman New York University
- R01 CA31265 Cation Translocation in Normal and Tumor Cells  
O'Neal University of Oklahoma, Norman
- R01 CA32742 Glucose Catabolism in Neoplastic Tissues  
Pedersen Johns Hopkins University
- R01 CA32946 Transport-Regulated Calcium Metabolism in Tumor Cells  
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#### RIBOSOMES AND POLYRIBOSOMES

- R01 CA04186 Molecular Structure of Nucleic Acids and Proteins  
Rich Massachusetts Institute of Technology
- R01 CA08416 Form and Function of Nuclear and Cytoskeletons  
Penman Massachusetts Institute of Technology
- R01 CA16608 Translation Control in Reticulocytes and Leukemic Cells  
Hardesty University of Texas, Austin
- R01 CA21663 Intermediary Metabolism in Animals and in Man  
Henshaw University of Rochester

#### M-RNA

- R01 CA12550 RNA Synthesis and Transport in Mammalian Cells  
Martin University of Chicago
- R01 CA16006 RNA and Growth Control in Animal Cells  
Darnell Rockefeller University
- R01 CA20124 Messenger RNA of Normal and Malignant Human Cells  
Saunders University of Texas System Cancer Center
- R01 CA22302 Analysis of Gene Regulation by Nuclear Transplantation  
Lucas State University of New York, Stony Brook
- R01 CA23226 Gene Expression in Regenerating and Neoplastic Livers  
Fausto Brown University

- R01 CA24273 Expression of Globin Genes--Erythroleukemia Cells  
Rovera Wistar Institute of Anatomy and Biology
- R01 CA25078 Poly(A) Polymerase and mRNA Processing  
Jacob Pennsylvania State University Hershey Medical Center
- R01 CA26790 Phenotypic Variation and Neoplastic Progression  
Peterson Children's Hospital Medical Center, Northern California
- R01 CA27607 Coordinated Gene Expression in Mammalian Cells  
Lee University of Southern California
- R01 CA31810 Control of mRNA Processing in Normal and Transformed Cells  
Rottman Case Western Reserve University
- R01 CA31894 Control of RNA Synthesis by Carcinogens and Hormones  
Jacob Pennsylvania State University Hershey Medical Center
- R01 CA33643 Mechanisms of Regulation of Cell Proliferation  
Getz Mayo Foundation
- R01 CA33953 mRNA Turnover in Differentiating Leukemia Cells  
Volloch Boston Biomedical Research Institute
- R01 CA39066 Embryonal Carcinoma Growth and Differentiation  
Linney Duke University

T-RNA

- R01 CA13591 Chemical Studies on Tumor Nucleic Acids  
Randerath Baylor College of Medicine
- R01 CA20683 Control Mechanisms in Human Tumor Cells--Small RNAs  
Eliceiri St. Louis University
- R01 CA20919 tRNA Q Base Metabolism in Normal and Tumor Cells  
Katze University of Tennessee Center for Health Sciences
- R01 CA28395 tRNA Methylation in Normal and Neoplastic Rat Tissues  
Leboy University of Pennsylvania
- R23 CA37836 Tumor Specific Differences in tRNA Methyltransferases  
Reinhart Philadelphia College of Pharmacy-Science
- R23 CA38015 Base-Pairing of Cytoplasmic LMW RNAs in Regulation  
Maxwell North Carolina State University, Raleigh

## DNA

- R01 CA14835 DNA Polymerases in Normal and Neoplastic Human Cells  
Korn Stanford University
- R01 CA15044 Pathogenetic Determinants of Human CNS Tumors  
Manuelidis Yale University
- R01 CA15187 DNA Synthesis: Regulation in Normal and Cancer Cells  
Baril Worcester Foundation for Experimental Biology
- R01 CA16790 DNA Transcription Control in Normal and Cancer Cells  
Maio Yeshiva University
- R01 CA17287 Mechanism and Role of Gene Amplification in Mammals  
Hogness Stanford University
- R01 CA23365 DNA Polymerases in Normal and Cancer Cells  
Chang U.S. Uniformed Services Univ. of Hlth. Sci.
- R01 CA24158 Rates of DNA Synthesis in Normal and Transformed Cells  
Collins Virginia Commonwealth University
- R01 CA26391 Molecular Pathology of Leukemia and Lymphoma  
Coleman University of Kentucky
- R01 CA34462 Role of Double Minutes and HSR Markers in Tumor Cells  
George University of Pennsylvania
- R01 CA34784 The Molecular Genetics of DHFR Gene Expression  
Melera Sloan-Kettering Institute for Cancer Research
- R01 CA34793 Amplified Genes in Differentiation-Resistant HL-060 Cells  
Gallagher University of Maryland at Baltimore
- R23 CA34898 Cytoskeletal and Membrane Associations of B Cell Ig  
Rosenspire Sloan-Kettering Institute for Cancer Research
- R23 CA35703 Cytoplasmic Control of Normal and Neoplastic Cell Growth  
Gutowski University of Connecticut Health Center
- R23 CA36735 Deoxyribonucleoside Triphosphate Metabolism  
Albert University of Chicago

## GROWTH FACTORS

- R01 CA11176 Factors Required for Mammalian Cell Division  
Holley Salk Institute for Biological Studies
- R01 CA15744 Spontaneous Transformation and Progression in Cell Lines  
Rubin University of California, Berkeley

R01 CA16816 Mechanism of Chemical Carcinogenesis In Vitro  
Moses Mayo Foundation

R01 CA17620 Growth Control in Normal and Neoplastic Cells  
Smith University of Nebraska, Lincoln

R01 CA21566 Anti-Tumor Invasion Factors Derived From Cartilage  
Kuettner Rush University

R01 CA22410 Ceruloplasmin and Copper Metabolism in Cancer  
Linder California State University, Fullerton

R01 CA23043 Peptide Transforming Factors from Transformed Cells  
Ozanne University of Texas Health Science Center, Dallas

R01 CA24071 Studies of the Receptor for Epidermal Growth Factor  
Carpenter Vanderbilt University

R01 CA27217 Growth Factors and Receptors in Chemical Transformation  
Moses Mayo Foundation

R01 CA27466 Endothelial Colony-Stimulating Activity  
Quesenberry University of Virginia, Charlottesville

R01 CA28110 Nerve Growth Factor Function--Secretion by Cancer Cells  
Young University of Florida

R01 CA29101 Characterization of a Liver Specific Growth Promotor  
La Brecque University of Iowa

R01 CA30101 Structure and Function of Platelet-Derived Growth Factor  
Antoniades Center for Blood Research

R01 CA31279 Epidermal Growth Factor: Interactions with Cell Receptor  
Haigler University of California; Irvine

R01 CA31615 Growth Factors in Normal and Neoplastic Hematopoiesis  
Adamson University of Washington

R01 CA31796 Effect of Glia Maturation Factors on Tumors  
Lim University of Iowa

R01 CA33209 Leech Antimetastatic Factors: Isolation and Action  
Gasic Pennsylvania Hospital

R01 CA34162 Growth Factors and Cellular Transformation  
Scher Children's Hospital of Philadelphia

R01 CA34470 The Role of Epibolin and Serum in Cancer Cell Spreading  
Stenn Yale University

R01 CA34472 Ca-Growth Control in Neoplastic and Nonneoplastic Cells  
Tucker Johns Hopkins University

R01 CA34568 Control of Tumor-Induced Vascularization  
Fenselau Miami Heart Institute

R23 CA34590 Biochemistry of a Melanoma Growth Stimulation  
Richmond Emory University

R01 CA34610 Intracellular Targets of Transforming Growth Factors  
Massague University of Massachusetts Medical School

R01 CA34809 Tumor Promotion and Epidermal Growth Factor Receptors  
King University of Illinois at Chicago

R01 CA35373 Hepatopoietins, Liver Regeneration and Carcinogenesis  
Michalopoulos Duke University

R01 CA36306 Monokines Which Regulate the Production of CSA  
Bagby Oregon Health Sciences University

R01 CA36544 Synthetic Transforming Growth Factors  
Tam Rockefeller University

R01 CA36595 Human Tumor Culture Lines in Defined Media  
Sato W. Alton Jones Cell Science Center

R01 CA36740 Regulation of Myelopoiesis by Acidic Isoferritins  
Broxmeyer Indiana University-Purdue University at Indianapolis

R23 CA36908 Growth Factors and Human Bladder Cancer  
Messing University of Wisconsin, Madison

R01 CA37392 Cartilage and Chondrosarcoma-Derived Growth Factors  
Klagsbrun Children's Hospital (Boston)

R01 CA37393 Growth and Migration of Capillary Endothelial Cells  
Zetter Children's Hospital Medical Center

R01 CA37395 Angiogenesis: A Control Point in Animal and Human Tumors  
Folkman Children's Hospital (Boston)

R01 CA37877 Tyrosine Phosphorylation and the Control of Cell Growth  
Frackelton Massachusetts General Hospital

R01 CA39053 Growth Regulation of Polyamine Synthesis  
Morris University of Washington

R01 CA39099 Cytoplasmic Factors in Cellular Growth  
Bucher Boston University

R01 CA39181 Cellular Actions of Transforming Growth Factors  
Magun Oregon Health Sciences University

R01 CA39193 The Hormonal Regulation of Normal Cell Growth  
Rossow Institute for Medical Research, San Jose



NUCLEUS

- R01 CA12226 Metabolism of NC-Methylarginines and Neoplasia  
Paik Temple University
- R01 CA12877 Function of H1 Histone Phosphorylation  
Langan University of Colorado Health Sciences Center
- R01 CA13195 Histone ADP-Ribosylation and HeLa Cell Replication  
Smulson Georgetown University
- R01 CA16346 Molecular Control of Chromatin Transcription  
Axel Columbia University
- R01 CA16910 Chromosome Aberrations in Myeloproliferative Diseases  
Rowley University of Chicago
- R01 CA17782 Tumor-Enriched Nonhistone Chromatin Proteins  
Reeck Kansas State University
- R01 CA18455 Isolated Chromosomes in Genetics and Cancer Research  
Wray Baylor College of Medicine
- R01 CA21927 Chromatin Structure of Normal and Malignant T Cells  
Maizel University of Texas System Cancer Center
- R01 CA24546 Relation of Histones to DNA in Normal and Cancer Cells  
Kornberg Stanford University
- R01 CA25055 Cytogenetics of Clonal Neoplasias  
Hecht Southwest Biomedical Research Institute
- R01 CA28679 Chromosomal Organization of Dihydrofolate Reductase Gene  
Biedler Sloan-Kettering Institute for Cancer Research
- R01 CA29476 Clonal Karyotypic Evolution in Human Solid Tumors  
Trent University of Arizona
- R01 CA31024 Fine Structural Chromosomal Defects in Acute Leukemia  
Yunis University of Minnesota of Minneapolis-St. Paul
- R01 CA33011 Chromatin Proteins of Embryonal Carcinoma Cells  
Oshima La Jolla Cancer Research Foundation
- R01 CA33314 Fine Chromosomal Defects in Non-Hodgkin's Lymphoma  
Yunis University of Minnesota of Minneapolis-St. Paul
- R01 CA34003 Nuclear cAMP Binding Proteins in Morris Hepatomas  
Rosenberg Albany Medical College
- R01 CA34775 Mapping Chromosomes and Genes in Relation to Leukemia  
Chaganti Sloan Kettering Institute for Cancer Research

- R01 CA34783 Monoclonal Antibodies to Mitotic Cells  
Rao University of Texas System Cancer Center
- R23 CA34831 Gene Mapping of Chromosome 3 and Small Cell Carcinoma  
Miller University of Colorado Health Sciences Center
- R01 CA35829 Histone in Virally-Infected and Transformed Cells  
Jackson Medical College of Wisconsin
- R01 CA37193 Human Leukemia/Lymphoma Specific Changes in Chromatin  
Mears Columbia University

#### CONTRACTILE ELEMENTS

- R01 CA05493 Leukopoietic Mechanisms  
De Bruyn University of Chicago
- R01 CA15544 Effect of Microtubular Proteins on Cell Surfaces  
Berlin University of Connecticut Health Center
- R01 CA23022 Studies of Mitosis in Normal and Neoplastic Cells  
Brinkley Baylor College of Medicine
- R01 CA29405 Studies on Prostacyclin and Tumor Metastasis  
Honn Wayne State University
- R23 CA31460 Contractile Protein Function in Normal and Transformed Cells  
Kiehart Johns Hopkins University
- R01 CA31760 Intermediate Filaments in Normal and Transformed Cells  
Goldman Northwestern University
- R01 CA33265 Tropomyosin Subunits: Normal and Transformed Cells  
Warren University of Miami
- R01 CA34282 Biochemical Mechanisms of Cellular Invasion  
Rifkin New York University
- R01 CA34709 Lymphoma Metastasis/Role of Endothelial Cell Recognition  
Butcher Stanford University
- R01 CA34763 Mutant B-Actin Gene Structure and Function in Neoplasia  
Leavitt Linus Pauling Institute of Science and Medicine
- R01 CA35738 Tropomyosins in Normal and Transformed Cells  
Matsumura Cold Spring Harbor Laboratory
- R23 CA35954 Cytochalasin/Probes of Cytoskeletal Function  
Krafft Syracuse University at Syracuse
- R01 CA36498 Cytoskeleton-Associated Proteins of Lung Carcinomas  
Bernal Dana-Farber Cancer Institute

DEVELOPMENT AND DIFFERENTIATION

- R01 CA02662      Investigations on Teratocarcinogenesis  
Stevens                                  Jackson Laboratory
- R01 CA10095      Gene Action and Cellular Differentiation in Culture  
Silagi                                      Cornell University Medical Center
- R01 CA13047      Control Mechanisms of Differentiation and Malignancy  
Friend                                      Mount Sinai School of Medicine
- R01 CA13533      Ectopic Placental Proteins in Cancer  
Sussman                                    Stanford University
- R01 CA14054      Malignant Behavior and Cellular Antigen Expression  
Klein                                        Caroline Institute
- R01 CA14319      Glycoconjugates and Nervous System Cell Differentiation  
Schengrund                                Pennsylvania State University Hershey Medical Center
- R01 CA15222      Hepatoma AFP: Model of Glycosylation in Malignancy  
Smith                                        University of Vermont and State Agriculture College
- R01 CA15619      Normal and Malignant Hematopoietic Cell Replication  
Cline                                        University of California, Los Angeles
- R01 CA16368      Control of Differentiation of Erythroleukemic Cells  
Skoultchi                                    Yeshiva University
- R01 CA16720      Gene Regulation and Interaction--Normal and Malignant Cells  
Klinger                                      Yeshiva University
- R01 CA17389      C-Cell Hyperplasia and Medullary Thyroid Carcinoma  
Wolfe                                        Tufts University
- R01 CA17575      Erythroid Differentiation in Friend Leukemia Cells  
Housman                                    Massachusetts Institute of Technology
- R01 CA18375      Hemopoietic Stem Cells and Induced Differentiation  
Goldwasser                                University of Chicago
- R01 CA19492      Terminal Transferase in Mammalian Hemopoietic Tissue  
Coleman                                    University of Kentucky
- R01 CA21967      Normal/Neoplastic Phosphatases: Comparative Structures  
Fishman                                    La Jolla Cancer Research Foundation
- R01 CA22294      Quantitative Studies on Granulocyte Differentiation  
Kinkade                                      Emory University
- R01 CA22556      Differentiation of Granulocytes and Macrophages  
Metcalf                                      Walter and Eliza Hall Institute of Medical Research

R01 CA23097 Embryo-Derived Teratocarcinoma  
Damjanov Hahnemann University

R01 CA24241 Differentiation in a Malignant Neural Tumor  
Pantazis University of Iowa

R01 CA24479 Polyamine Metabolism and Neuroblastoma Differentiation  
Chen Rutgers, The State University, New Brunswick

R01 CA24488 The Controlled Initiation of Neoplasms in Drosophila  
Hanratty University of California, Irvine

R01 CA25098 Alpha-Fetoprotein Regulation in Fetal and Cancer Liver  
Chiu University of Vermont and State Agriculture College

R01 CA25512 Modulators of Granulopoiesis from Human Cell Lines  
Brennan University of Rochester

R01 CA25966 X-Chromosome Activity in Teratocarcinoma Stem Cells  
Martin University of California, San Francisco

R01 CA25972 Self-Renewal in Normal/Leukemic Hemopoietic Stem Cells  
Metcalf Walter and Eliza Hall Institute of Medical Research

R01 CA26038 Differentiation and Proliferation of Myeloid Cells  
Koeffler University of California, Los Angeles

R01 CA26656 Cell Culture Analysis of Human Epithelial Neoplasia  
Rheinwald Dana-Farber Cancer Institute

R01 CA28050 Regulation of Alpha-Fetoprotein Gene Expression  
Tilghman Institute for Cancer Research

R01 CA28287 Driving Forces for Nutrient Transport in Tumor Cells  
Smith University of Texas Health Science Center, San Antonio

R01 CA28427 EGF and Its Receptors in Embryonic Differentiation  
Adamson La Jolla Cancer Research Foundation

R01 CA28656 Differentiation of Capillary Endothelial Cells  
Auerbach University of Wisconsin, Madison

R01 CA29894 Human Teratocarcinoma-Derived Cell Lines  
Andrews Wistar Institute of Anatomy and Biology

R01 CA29895 Antiproliferative Effects of Interferons  
Baglioni State University of New York at Albany

R01 CA30393 Endocrine Regulation of Melanoma Cell Differentiation  
Fuller Texas Tech University

R01 CA31042 Developmental Regulation of B Globin Gene Expression  
Lo University of Pennsylvania



R01 CA31271 Differentiation and Stroma-Induction in Neural Tumors  
Rubinstein University of Virginia, Charlottesville

P01 CA31768 Leukemia Cell Systems: Induction of Differentiation  
Rifkind Sloan Kettering Institute for Cancer Research

R01 CA31937 Control of Melanoma Cell Differentiation: Genetic Study  
Graf Cornell University Medical Center

R01 CA31945 K-562: A Human Pluripotent Leukemia Stem Cell Line  
Lozzio University of Tennessee, Knoxville

R01 CA32152 Growth and Differentiation of Human Melanocytes  
Eisinger Sloan Kettering Institute for Cancer Research

R01 CA32186 REC-DNA Analysis of Human Hematopoietic Differentiation  
Salser University of California, Los Angeles

R23 CA32260 Differentiation and Malignancy in Neural Cell Culture  
Krystosek University of Colorado Health Sciences Center

R23 CA32733 Modulation of Normal and Abnormal Human Myelopoiesis  
Abrahm University of Pennsylvania

R01 CA33000 Glycoproteins in Differentiation and Oncogenesis  
Fukuda La Jolla Cancer Research Foundation

R01 CA33021 Isolation of Tumor Genes from Human Lung Carcinomas  
Perucho State University of New York, Stony Brook

R01 CA33065 Immunobiology of UVL-Induced Tumors  
Daynes University of Utah

R01 CA33579 Growth and Differentiated Function of Keratinocytes  
Green Harvard University

R01 CA33664 The In Vitro and In Vivo Regulation of Hemopoiesis  
Cronkite Associated University-Brookhaven National Laboratory

R01 CA33800 Pathobiology of Chemically Induced Teratocarcinoma  
Speers University of Colorado Health Sciences Center

R01 CA33895 Glycoproteins in Normal and Leukemic Cell Differentiation  
Fukuda La Jolla Cancer Research Foundation

R01 CA33946 Teratocarcinoma Cytoskeletal Proteins  
Oshima La Jolla Cancer Research Foundation

R01 CA34181 RNA's of Lymphoma and T Cell Differentiation Antigens  
Rothenberg California Institute of Technology

R23 CA34186 Human Immature Megalaryocytes and Hematologic Neoplasms  
Long University of Michigan at Ann Arbor



R01 CA34230 Onco-Developmental Gene Control: Alpha-Fetoprotein  
Sell University of Texas Health Science Center, Houston

R01 CA34759 Molecular Basis of Oncogenesis and Differentiation  
Tereba St. Jude Children's Research Hospital

R01 CA34826 Oncogenes and Growth Factors in Pre-B Cells  
Ozanne University of Texas Health Science Center, Dallas

R01 CA34891 Molecular Basis of Differentiation and Neoplasia  
Roeder Rockefeller University

R01 CA35150 Chromosome Translocations and Ig Genes in Human Leukemia  
Nowell University of Pennsylvania

R01 CA35326 Expression of Exogenous Genes in Teratocarcinoma  
Gautsch Scripps Clinic and Research Foundation

R01 CA35367 Embryonic Control of Neuroblastoma and Melanoma  
Pierce University of Colorado

R01 CA35517 The Role of cAMP in Leukemic Cell Differentiation  
Fontana West Virginia University

R01 CA35533 Epigenetic Regulation of the Chondrosarcoma  
Miller University of Colorado Health Sciences Center

R01 CA35823 Human Lymphocytic Leukemia Oncogenes/Gene Products  
Kennett University of Pennsylvania

R01 CA36122 Neuroblastoma Transfection and Transformation  
Gilbert Mount Sinai School of Medicine

R01 CA37675 Teratocarcinoma Stem Cell Adhesion  
Gabel Wesleyan University

R23 CA37727 Induction of Leukemic Cell Maturation  
Tsiftoglou Beth Israel Hospital

R01 CA37874 Effect of Proteases in Erythroid Cell Differentiation  
Scher Cuny-Mount Sinai School of Medicine

R23 CA37887 Malignant Potential of the Components of Wilm's Tumors  
Garvin Medical University of South Carolina

R01 CA37918 Hemin Transport into Differentiating Leukemic Cells  
Glass Beth Israel Hospital (Boston)

R01 CA38189 Bone-Bone Marrow Interaction  
Lee University of Washington

R01 CA38405 Activation of Primordial Germ Cells to Form Teratomas  
Damjanov Hahnemann University



R01 CA32172 Regulation and Inhibition of Polyamine Metabolism  
Hoffman University of Louisville

R01 CA32952 Phosphotyrosine and the Control of Cell Growth  
Ross Wistar Institute of Anatomy and Biology

R01 CA33505 Cell Cycle Specific Control of Cellular Differentiation  
Yen University of Iowa

R01 CA33764 Analysis of Melanoma Growth and Regression  
Amoss Texas Agri. and Mech. University College Station

R01 CA34460 Cell Cycle Control--The Role of Monovalent Cation Fluxes  
Adelberg Yale University

R01 CA34512 Regulation of 2-5A-Dependent RNase Levels By Interferon  
Silverman U.S. Uniformed Services Univ. of Hlth. Sci.

R01 CA35469 Collagenase Function and Activity in Malignant Tumors  
Takahashi Yeshiva University

R01 CA35789 Regulation of Gene Expression by Interferons  
Sen Sloan Kettering Institute for Cancer Research

R01 CA36063 Malignant Tumor Metastasis: Role of Vitamin K Metabolism  
Fasco New York State Department of Health

R01 CA36464 Myelopoietic Regulation By Lactoferrin and Transferrin  
Broxmeyer Indiana University-Purdue University at Indianapolis

R01 CA36487 The Role of Calcium in Cell Growth Regulation  
Dedman University of Texas Health Science Center, Houston

R01 CA36535 Regulation of Cellular Growth in Multicellular Spheroids  
Freyer University of California

R01 CA36784 Calcium and Cell Cycle Control in Human Fibroblasts  
Tupper Syracuse University at Syracuse

R01 CA36913 Biology and Therapy of Poorly Nourished Tumor Cells  
Tannock Ontario Cancer Treatment and Research Foundation

R01 CA37391 Anticoagulants, Vitamin K, and Tumor Cell Growth  
Hauschka Children's Hospital (Boston)

R01 CA37673 Regulation of Expression of the Thymidine Kinase Gene  
Deininger Louisiana State University Medical Center, New Orleans

R01 CA37789 Polyamine Metabolism and Colon Cancer  
Luk Johns Hopkins University

R01 CA38016 Altered Nutritional Requirements for Growth  
McClure W. Alton Jones Cell Science Center

SOMATIC CELL GENETICS

- R01 CA12130      Cytoplasmic Inheritance in Normal and Tumor Cells  
           Harris                                      University of California, Berkeley
- R01 CA16631      Epithelial Cell Growth and Function: A Genetic Study  
           Meiss                                        New York University
- R01 CA16754      Hybridization, DNA Function, Mutation in Cell Culture  
           Littlefield                                Johns Hopkins University
- R01 CA19401      Genetic Analysis of Human Malignancy  
           Stanbridge                                University of California, Irvine
- R01 CA20741      Biology of Human Fibrosarcoma  
           Croce                                        Wistar Institute of Anatomy and Biology
- R01 CA24828      Genetic Analysis of Tumorigenesis  
           Sager                                        Dana-Farber Cancer Institute
- R01 CA28559      Study of Malignant Transformation: A Genetic Analysis  
           Athwal                                      University of Medicine & Dentistry of New Jersey
- R01 CA30643      Genetic Bases for the Transformed Phenotype  
           Ozer                                         Hunter College
- R01 CA30938      Structural and Functional Analysis of Cloned MHC Gene  
           Weissman                                    Yale University
- R01 CA31553      Cytogenetics and Molecular Biology of Human Neuroblastoma  
           Biedler                                    Sloan Kettering Institute for Cancer Research
- R01 CA31649      Transformation Proteins of Non-Virally Induced Tumors  
           Weinberg                                   Massachusetts Institute of Technology
- R01 CA31777      BUdR Dependence, Malignancy, and Differentiation  
           Davidson                                    University of Illinois at Chicago
- R01 CA31995      Retroviral Oncogenes: Analysis of Cellular Homologues  
           Sheiness                                    Louisiana State Univ. Med. Ctr., New Orleans
- R01 CA32580      Biochemical Genetics of Mammalian Nucleoside Transport  
           Ullman                                      University of Kentucky
- R01 CA33108      Cell Transforming Genes of T- and B-Lymphocyte Neoplasms  
           Lane                                        Dana-Farber Cancer Institute
- R01 CA36521      Genetics of Non-Burkitt B Cell Lymphomas  
           Croce                                        Wistar Institute of Anatomy and Biology

## INHERITANCE OF NEOPLASMS

- R01 CA32832     Cytogenetics of Familial Medullary Thyroid Carcinoma  
           Wurster-Hill                     Dartmouth College
- R01 CA33093     Recombinant Inbred Mouse Strains and Cancer  
           Taylor                             Jackson Laboratory
- R01 CA37381     Transforming Genes of Benign and Malignant Colon Tumors  
           Augenlicht                     Montefiore Hospital and Medical Center

## PLASMIDS, VIRUSES

- R01 CA11526     Tumor-Inducing Substance of Agrobacterium Tumefaciens  
           Kado                               University of California, Davis
- R01 CA18604     The Mechanism of Tumorigenesis by A. Tumefaciens  
           Matthysse                     University of North Carolina, Chapel Hill
- R01 CA19402     Molecular Genetics of Agrobacterium Plasmids  
           Farrand                         Loyola University Medical Center
- R01 CA29474     Cytology, Biochemistry of Viral-Specific Proteins  
           Buchanan                     Massachusetts Institute of Technology

## IN VIVO AND IN VITRO TUMOR LINES

- R01 CA11683     Coenzymes and Nucleic Acids Metabolism  
           Kaplan                         University of California, San Diego
- R01 CA17229     Keloids: An In Vitro Model of Tumor Growth Regulation  
           Russell                         Meharry Medical College
- R01 CA29078     Cellular Origins of Hepatic Preneoplasias  
           Iannaccone                     Northwestern University
- R01 CA32134     B16 Melanoma Metastasis Model System  
           Stackpole                     New York Medical College
- R01 CA32318     Antigenic Analysis of Hematopoiesis  
           Civin                            Johns Hopkins University
- R01 CA33027     Differentiation and Chemotherapy in Human Gliomas  
           Yung                             University of Texas System Cancer Center
- R01 CA33305     Genetic Basis for Spontaneous Cancer and Aging  
           Rodriguez                     University of Texas System Cancer Center



- R23 CA37238      New Investigator Research Award  
Lloyd                      University of Michigan at Ann Arbor
- R01 CA37778      SL12 T-Lymphoma: A New Model for Gene Control in Tumors  
Macleod                      University of California, San Diego
- R01 CA38110      Pathobiology of Metastasis in a New Melanoma Model  
Berkelhammer                      AMC Cancer Research Center and Hospital

#### CONFERENCES

- R13 CA02809      Cold Spring Harbor Symposia on Quantitative Biology  
Watson                      Cold Spring Harbor Laboratory
- R13 GM33534      Introduction of Macromolecules into Eukaryotic Cells  
Ruddle                      Gordon Research Conferences
- R13 GM33655      Summer Conference on Calcium and Cell Function  
Vanaman                      FASEB
- R13 AM33976      International Stem Cell Symposium  
Palek                      St. Elizabeth's Hospital of Boston
- R13 CA35980      Symposium on the Cell Surface in Development and Cancer  
Steinberg                      American Society of Zoologists
- R13 CA36756      Intermediate Filaments: Structure and Search for Functions  
Wang                      New York Academy of Sciences
- R13 CA38111      Gordon Research Conference on Cancer, 1984  
Nathan                      Gordon Research Conferences
- R13 CA38546      1984 Gordon Research Conference on Nucleic Acids  
Tjian                      Gordon Research Conferences
- R13 GM39218      Summer Conference: Somatic Cell Genetics  
Chasin                      FASEB

#### PROGRAM PROJECTS

- P01 CA10893      Cancer Research Center  
Busch                      Baylor College of Medicine
- P01 CA15823      Program in Developmental Biology of Cancer  
Pierce                      University of Colorado Health Sciences Center
- P01 CA19265      Chromosome Metabolism in Cancer Biology  
Ultmann                      University of Chicago

P01 CA21901 Studies of Normal and Malignant Cell Membranes  
Roseman Johns Hopkins University

P01 CA22376 Control of Gene Expression: Normal and Neoplastic  
Feigelson Columbia University

P01 CA22427 Molecular Analysis of Malignant Transformation  
Pardee Dana-Farber Cancer Institute

P01 CA23052 Program Project on Athymic Mice and Human Tumors  
Kaplan University of California, San Diego

P01 CA23076 Regulatory Mechanisms in Tumor Biology  
Mueller University of Wisconsin, Madison

P01 CA25875 Cell Differentiation and Cancer  
Croce Wistar Institute of Anatomy and Biology

P01 CA26712 Molecular Analyses of Cellular Proteins and Their Genes  
Hynes Massachusetts Institute of Technology

P01 CA28853 Pathophysiology of Metastasis  
Holyoke Roswell Park Memorial Institute

P01 CA28896 Cell-Matrix Interactions in Neoplasia and Development  
Ruoslahti La Jolla Cancer Research Foundation

P01 CA29545 Interferon, Differentiation and Oncogenesis  
Carter Hahnemann Medical College & Hospital of Philadelphia

P01 CA29569 Gene Organization and Expression in Eukaryotes  
Sambrook Cold Spring Harbor Laboratory

P01 CA32737 A Program in Medical Oncology  
Golde University of California, Los Angeles

P01 CA34936 A Mutational Model for Childhood Cancer  
Strong University of Texas System Cancer Center

P01 CA37589 Cell Culture Factors and Their Relation to Cancer Biology  
Sato W. Alton Jones Cell Science Center

DIFFICULT-TO-CLASSIFY

R01 CA09247 Partial Subsidy for the Journal of Cancer Research  
Handschumacher American Association for Cancer Research

R01 CA22062 A Bone Resorptive Protein from Cancer Ascites Fluid  
Nimberg Boston University

R01 CA25298 Biology of Human Cutaneous Malignant Melanoma  
Clark University of Pennsylvania

R01 CA27120 Interferon System: Action, Induction and Regulation  
Ts'O Johns Hopkins University

ONCOGENES/TRANSFECTION

R01 CA26663 Cell Transformation by RSV  
Weintraub Fred Hutchinson Cancer Research Center

R01 CA28946 Transfection by Endogenous Human Transforming Genes  
Cooper Dana-Farber Cancer Institute

R01 CA35911 Expression and Structure of Invertebrate Oncogenes  
Wadsworth Worcester Foundation for Experimental Biology Inc.

R01 CA36246 Chromosome Translocated Oncogenes and Neoplasia  
Marcu State University New York, Stony Brook

R01 CA36327 Isolation of Transforming Genes in Murine Thymomas  
Pellicer New York University

R01 CA36355 Expression of Oncogenes and IgA Genes in Transformed Cells  
Sonenshein Boston University

R01 CA36827 Oncogenes in Physiologic and Pathologic States  
Slamon University of California, Los Angeles

R01 CA36928 Structure-Function Studies of Altered Oncogenic Proteins  
Buchanan Massachusetts Institute of Technology

R23 CA37038 Role of GTP in RAS Oncogene Induced Transformation  
Halliday University of California, Berkeley

R01 CA37165 C-MYC Rearrangements in Human Hematopoietic Neoplasias  
Dalla Favera New York University

R01 CA37222 Properties of Cellular and Viral SRC Genes  
Parker Columbia University

R01 CA37702 The Function of the RAS Oncogene Homolog in Yeast  
Tatchell University of Pennsylvania

R01 CA37866 Analysis of Human Oncogene Polymorphisms  
Krontiris Tufts University

R01 CA38047 DNA Rearrangements in Momulv Induced Thymomas  
Tsichlis Fox Chase Cancer Center

R01 CA39186 Genetics of Hematopoietic Cancers  
Sakaguchi University of Texas Health Science Center, San Antonio

CONTRACT RESEARCH SUMMARY

Title: Morris Hepatoma Resource Program

Principal Investigator: Dr. Lynnard J. Slaughter  
 Performing Organization: Howard University  
 City and State: Washington, D.C.

Contract Number: NOI-CB-14345  
 Starting Date: 6/30/81 Expiration Date: 6/29/84

Goal: To maintain eleven Morris hepatomas representative of the spectrum of rapidly to very slow-growing tumors in stock rats and provide them on request to laboratories for research purposes.

Approach: The hepatomas are propagated by serial transplantation in rats and periodically monitored by enzyme profiles and assay of specific metabolites to assure stability of each line. Requests for any of the hepatomas are filled, depending on availability, by injecting tumor tissue into host rats purchased by the requestor and then shipping them to his/her laboratory by air freight.

Progress: Since the inception of the contract 4734 tumor-rats and 256 controls were supplied to investigators on request. The actual use is broken down as follows:

<u>Fast-growing</u>	<u>Tumor Rats</u>	<u>Slow-Growing</u>	<u>Actual Use</u>
5123tc	233	9618A	317
7777	587	7787	176
3924A	1832	16	127
44	330	20	171
8999	123		

Intermediate-Growing

7800	624		
5123D	214	Controls (Non-tumor-bearing)	256

Tumor-bearing rats have been sent to approximately 76 different investigators, in the U.S., Canada and Europe.

Significance to Cancer Research: Each of these hepatomas has specific characteristics that make it the tumor of choice for certain research projects. A number of NCI grants in the areas of enzymology, intermediary metabolism and molecular biology utilize this liver tumor system.

Project Officer: Judith M. Whalen  
 Program: Tumor Biology Section  
 FY 84 Funds: \$ 0

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## IMMUNOLOGY PROGRAM

The Immunology Program of the National Cancer Institute supports research that contributes to an understanding of the role of the immune system in the development, growth and spread of tumors. The specific areas of investigation supported by the Program include:

- ° The synthesis and structure of myeloma proteins in animals and man.
- ° The synthesis, structure, and function of antibodies capable of reacting with tumor cells, agents which induce tumors, and agents used in the treatment of tumors.
- ° The synthesis, structure, and function of humoral factors other than antibody which participate in, activate and/or regulate the immune response to tumors. This would include complement, interferon, lymphokines, lymphoid cell growth factors, helper factors, suppressor factors, etc., as they are involved in immune responses to tumors.
- ° The immunobiology of lymphocytes which participate in antitumor responses including their development, heterogeneity, interactions, and functions.
- ° The immunobiology of monocytes and macrophages which participate in antitumor responses including their development, heterogeneity, interactions, and functions.
- ° The identification, isolation, and characterization of cell surface determinants of lymphocytes and macrophages which are involved in the responses of these cells to tumors.
- ° The identification, isolation, and characterization of cell surface determinants on tumor cells which serve as target antigens for the immune response.
- ° The immunobiology of malignancies of the immune system (lymphomas and leukemias) including studies of immunologic markers for the classification and characterization of neoplastic cells and their normal counterparts.

- ° Immunobiology of sarcomas, carcinomas, and melanomas including studies of immunologic markers for the classification and characterization of tumor cells and their normal counterparts.
- ° Immune surveillance against the development of tumors of various origins by all immune mechanisms (e.g. T cell immunity, macrophage reactivity, natural killer cell activity).
- ° Immunopathology studies on the host-tumor interaction.
- ° Immune status of tumor-bearing animals and man including studies on immunostimulation, immunosuppression, and the effects of disease course on immune function.
- ° Bone marrow transplantation (BMT) in man and animals as a treatment for cancer when the emphasis is on understanding how BMT affects or is affected by the immune system.
- ° Immunotherapy in animal models including studies on specific and non-specific stimulation of the immune system using natural and synthetic agents when the emphasis is on understanding how the therapy affects or is affected by the immune system.
- ° Immunotherapy including preclinical and clinical protocols where the main emphasis is upon the study of immune parameters, immune mechanisms, and other immunologic concerns rather than upon a therapeutic result. Included are studies on specific and nonspecific stimulation of the immune system using natural and synthetic agents.

The Immunology Program supports a broad spectrum of research in the areas of basic immunology and tumor immunology. The number of grants funded in each category with their costs are identified in Table 1 of this report. Since the Immunology Program funded 444 grants and expended approximately 59 million dollars during FY 1984, this report serves to highlight selected areas of research and should not be considered comprehensive.

The development of hybridoma technology and the availability of well-characterized monoclonal antibodies have made major contributions to immunology research as well as to many other areas of basic research, in addition to their great potential for the diagnosis and therapy of cancer. Monoclonal antibodies have become powerful tools to allow the immunologist to dissect the many inter-related aspects of the immune response and to examine the biochemical nature

and functional reactivity of cell surface determinants on tumor cells and on cells of the immune system. This report will highlight aspects of research on cytotoxic lymphocytes, both cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. In addition, some new developments in research on leukemia-associated antigens will be discussed.

The Immunology Program Annual Report for FY '83 specifically highlighted research on the Acquired Immune Deficiency Syndrome (AIDS). Research in this area will be discussed, but not described in great detail, in this year's Annual Report. The recent discovery of the role of human T cell leukemia virus-III (HTLV-III) as the potential etiologic agent of this disease has had a major impact on the analysis and interpretation of data from many ongoing research programs. There has not yet been enough time to integrate these new findings and to evaluate their impact on ongoing studies.

### CYTOTOXIC T LYMPHOCYTES

The killing of target cells by antigen-specific T lymphocytes is a multi-step process which involves antigen recognition and adhesion of cytotoxic T lymphocytes (CTL) to target cells, delivery of the lethal hit and target cell lysis. Following the sequence of events, the CTL can detach and engage in further killing encounters. The CTL itself, as well as bystander cells, are unharmed in the killing reaction. The molecular basis of CTL-mediated killing has been an area of intense investigation. Research in this field has recently been reviewed by Wyrta Heagy (CA 35986) and colleagues (Martz et al., 1983), and by Timothy Springer (CA 31798) and colleagues (Springer et al., 1983).

The ability to expand and characterize cloned populations of T lymphocytes has been an important technological advance for immunologists and immunogeneticists. While a variety of techniques can potentially enrich desired effector populations, none can approach the homogeneity which is achieved by studying the progeny of a single immunocompetent cell. As a result, T cell clones have been utilized to study a broad range of immunologic and immunogenetic questions. This field has been recently reviewed by Flomenberg and Dupont, 1983 (CA 22507).

### Lymphocyte Function Antigens

In studies of CTL in the mouse system, monoclonal antibodies to the Lyt-2,3 and the lymphocyte function-associated (LFA-1) antigens on CTL, but not monoclonal antibodies to many other types of surface structures, were found to inhibit CTL killing of target cells. The LFA-1 antigen appears to have a general function in mouse T lymphocyte immune interactions, since monoclonal antibodies to this antigen block not only allogeneic and xenogeneic CTL-mediated killing but also T helper cell antigen-specific proliferative responses and T cell-dependent B cell responses. These antibodies block CTL killing by binding to the effector CTL rather than to the target cell and block the formation of CTL-target conjugates (Springer et al., 1982).

In related studies of human CTL, Steven Burakoff (CA 34129) and Timothy Springer (CA 31798, 31799) have prepared monoclonal antibodies to cytotoxic T lymphocytes reactive to the human major histocompatibility complex (MHC) antigen HLA-DR and screened these antibodies for inhibition of CTL-mediated killing. Although it had been generally accepted that cytotoxic T lymphocytes bear the T8 antigen and that helper T lymphocytes bear the T4 antigen, it has been demonstrated that CTL directed against HLA Class II (HLA-DR) antigens express T4 but not the T8 antigen (Meuer et al., 1982). Studies of the binding of these monoclonal antibodies to four types of cell surface molecules -- LFA-1, LFA-2, LFA-3, and HLA-DR -- demonstrated the ability of these monoclonal antibodies to inhibit CTL killing, suggesting that these corresponding cell surface molecules participate in the CTL-target cell interaction. Biochemical characterization of these cell surface molecules indicated that the LFA-1 antigen contains two polypeptide chains of molecular weight 177,000 and 95,000 daltons which are noncovalently associated. LFA-1 is present on both B and T lymphocytes and marks subpopulations that differ in quantitative expression. Human LFA-1 appears to be the homologue of mouse LFA-1. Human LFA-2 is of molecular weight 49,000 daltons with a minor component of molecular weight 36,000 daltons. It is expressed on CTL lines but not on a B cell line, and in peripheral blood is preferentially expressed on T lymphocytes. Human LFA-3 is of molecular weight 60,000 daltons and is expressed on both B and T lymphocytes (Sanchez-Madrid et al., 1982).

Functional studies indicate that these monoclonal antibodies block cytotoxicity by both T4<sup>+</sup> and T8<sup>+</sup> CTL, the proliferative responses to phytohemagglutinin (PHA) and the mixed lymphocyte response (MLR). Anti-LFA-1 monoclonal antibody has been found to block NK cell-mediated cytotoxicity. LFA-2 has been found to be a determinant on the sheep red blood receptor and is specific for T cell functions. LFA-3 is a widely distributed antigen present on both hematopoietic and non-hematopoietic tissues, yet appears to be involved only in T cell functions. Monoclonal antibodies to LFA-1 and LFA-2 inhibit function by binding to effector cell (CTL) surface molecules, whereas anti-LFA-3 appears to block by binding to the surface of the target cell (Krensky et al, 1983a). The broad distribution of each of these determinants makes them unlikely to be part of an antigen-specific receptor. The possibility that anti-LFA-3 monoclonal antibody can also block function at the effector level is being studied. Whereas monoclonal antibodies to Class I histocompatibility antigens HLA-A,B block cytotoxicity by T8<sup>+</sup> CTL and anti-HLA-DR monoclonal antibodies block cytotoxicity by T4<sup>+</sup> CTL, anti-LFA-3 monoclonal antibodies block cytotoxicity by both sets of CTL, suggesting that LFA-3 may play a role different from T4 and T8 in this CTL-target interaction. Monoclonal antibodies OKT3, OKT4 and OKT8 (anti-T3, T4 and T8, respectively) have been shown in earlier work to inhibit lysis by human CTL (Meuer et al., 1982). Studies of the anti-LFA antibodies indicate that in addition to the antigen-specific interactions between the T cell antigen receptor and MHC antigens expressed on the target cell (H-2 in mouse, HLA in human), other T cell and target target cell surface molecules appear to mediate accessory interaction. The ability of anti-accessory molecule monoclonal antibodies to inhibit cytotoxicity lends further support to the hypothesis that an "adhesive strengthening" process accompanies antigen-specific recognition (Krensky et al., 1984). From these studies it appears that CTL-target conjugation involves two steps: a) specific immunologic recognition involving an interaction between the CTL receptor and target antigen and b) adhesion strengthening which depends on the interaction of antigen non-specific cell surface structures that form receptor ligand pairs between effectors and targets. It appears that LFA-1, -2 and -3 are involved in this process.



Carl Ware (CA 35638), in collaboration with Steven Burakoff and Timothy Springer, has produced additional monoclonal antibodies reactive with human LFA-1 and has found three unique and three partially overlapping epitopes on this antigen as defined by competitive cross-inhibition binding assays (Ware et al., 1983). In contrast, antibodies against mouse LFA-1 all recognize a common or shared epitope. Anti-LFA-1 monoclonal antibodies reversibly inhibited CTL reaction by slowing the initial rate of cytolysis, suggesting that these antibodies inhibit CTL functions by specific blockade of a functionally relevant molecule; the data suggest that the mechanism of anti-LFA-1 blocking was not through a non-specific "blanketing" effect of antibody on the CTL surface. It is not yet clear whether anti-LFA-1 monoclonal antibodies modulate LFA-1 on the cell surface or inhibit CTL function by direct steric hindrance of a functional site. LFA-1 may function as a general cell/cell interaction molecule contributing to the overall avidity of the T cell antigen receptor, promoting firm contact between the CTL and the target cell membranes. Further studies on the LFA-1 molecule have revealed that the combination of anti-LFA-1 and anti-T8 monoclonal antibodies is synergistic, both in magnitude and dose, in their ability to inhibit CTL killing. Pools of monoclonal antibodies reactive with distinct epitopes on either LFA-1 or T8 antigens did not inhibit either synergistic or additive effects alone, but did when combined. Anti-LFA-1 or anti-T8 showed no ability to modulate either antigen, alone or in combination. Other antibodies to CTL antigens also did not act synergistically, indicating that this interaction was specific. Dr. Ware and colleagues have suggested the possibility that LFA-1 and T8 may functionally and perhaps physically link during the cytolytic process (Ware et al., 1984).

Dr. Springer and colleagues have recently determined some similarities between macrophage and T lymphocyte-mediated immunity. Two human cell surface molecules with surprisingly similar structures, LFA-1 and Mac-1/OKM1, have recently been found to be important in cytolytic T lymphocyte-mediated killing and in complement receptor function respectively. As previously mentioned, monoclonal antibodies to LFA-1 block antigen-specific CTL-mediated killing and T-helper cell responses as well as natural killing. LFA-1 participates in the  $Mg^{++}$ -dependent adhesion step of CTL-mediated killing. Monoclonal antibodies to human Mac-1 block adhesion by myeloid cells mediated by the complement receptor Type 3 (CR3). Mac-1 may thus be identical to the CR3. Mac-1 appears to be identical to OKM1 and Mbl antigens in cell distribution and structural characteristics. These antigens are expressed on monocytes, granulocytes and natural killer cells and, in contrast to LFA-1, are absent from lymphocytes. A structural relationship between molecules implies similarities in the molecular mechanisms underlying their function and Dr. Springer has shown that LFA-1, OKM1/Mbl, and a third novel molecule termed P190,95 each contain non-covalently associated subunits, some of which are identical (Sanchez-Madrid et al., 1983a,b).

#### Lymphocyte Differentiation and Other Antigens

In his studies of the role of HLA Class II Ia-like (DR) surface membrane antigens in T cell-mediated cytotoxicity, Robert Todd (CA 39064) and co-workers have developed and characterized a monoclonal antibody, 9-49, that inhibits Ia-related cellular interactions, including binding of T cells to antigen-pulsed



macrophages, T cell proliferation in response to soluble protein antigens, as well as cellular auto- and alloantigens, generation of cytotoxic activity in response to Class II antigens, and allo/autoreactive CTL activity by T4<sup>+</sup> inducer lymphoblasts. This IgG2a monoclonal antibody binds to an invariant determinant on the Ia(DR) molecule. Proliferation and CTL activity of a T8<sup>+</sup> clone of CTL is unaffected by monoclonal antibody 9-49 (Todd et al., 1984). The recognition of the high degree of polymorphism that exists within the Ia system has come in part from the development of monoclonal antibody reagents specific for portions of single Ia structural epitopes. Many of these antibodies are specific for invariant portions while others identify products of specific allelic forms controlled by a sublocus. The fact that several monoclonal anti-Ia antibodies do not block all Ia-related functions has suggested that only certain Ia epitopes or portions of the Ia molecular structure are functionally active. This new antibody, 9-49, is specific for a non-polymorphic determinant associated with a human Ia(DR) antigen and is particularly interesting because of its broad inhibitory effect on several immune interactions that involve Ia antigens. It is still not clear why some monoclonal anti-Ia reagents block function while others do not, but the availability of this new monoclonal antibody will provide a powerful tool for answering this question.

John Hansen (CA 39548), Walter Newman (CA 36643) and colleagues have developed two monoclonal antibodies which bind to Tp50, a human T lymphocyte surface protein associated with the E-rosette receptor, and have demonstrated that these antibodies bind to Tp50 epitopes in close proximity. In functional studies, both antibodies caused a similar degree of antigenic modulation, inhibited T cell proliferative responses and inhibited cytotoxic T lymphocyte function without affecting cells that mediate antibody-dependent cell-mediated cytotoxicity (ADCC). The antibodies were different, however, in that antibody 9.6 inhibited E-rosette formation and NK cell-mediated lysis, whereas antibody 35.1 did not. These results could not be attributed to differences in antibody class or binding characteristics and additional studies on binding to T cells from non-human primates further supports the conclusion that they bind to distinct epitopes of Tp50 (Martin et al., 1983).

Dr. Hansen and colleagues have reported another new monoclonal antibody, 60.3, which recognizes a cell surface molecular complex, Lp95-150, common to most leukocytes. The binding of this antibody inhibits the lytic function of both alloreactive CTL and NK cells. It inhibits lymphocyte transformation in response to soluble antigens, mitogens and allogeneic cells and it also blocks the migration of neutrophilic polymorphonuclear granulocytes. The antigen immunoprecipitated by this antibody consists of multiple polypeptide chains. Three major chains have been identified (Beatty et al., 1983). The relationship of this antibody to the previously described monoclonal antibodies to lymphocyte function antigens is unclear. The Lp95-150 cell surface molecular complex may be involved in a membrane-associated step common to the activation of cells of diverse lineages. Inhibition occurs at the level of the effector cell and it appears that the effect does not occur at the level of specific recognition because this antibody blocks lysis of both alloreactive CTL and NK cells, which are fundamentally distinct in two major respects: a) CTL recognize specific alloantigens whereas NK cells do not, and b) antigen recognition by CTL is restricted by MHC gene products, whereas antigen recognition by NK

cells is not. Therefore, it is unlikely that the Lp95-150 molecular complex could function as the distinct antigen receptor for both CTL and NK cells. Their results suggest that antibody 60.3 blocks lysis either by inhibiting activation of the lytic process or by interfering with the delivery of lytic hit.

John Hansen, in collaboration with Edward Clark (CA 34199) and others, has reported the results of testing a panel of 77 anti-T cell monoclonal antibodies which were exchanged under the auspices of the First International Workshop on Leukocyte Differentiation Antigens (November 1982). Immune precipitation experiments confirmed that 15 of these antibodies reacted with a 32,000 to 45,000 dalton heteromultimeric cell surface molecular complex, homologous to the murine Lyt-2,3 antigens and designated Tp32. Anti-Tp32 antibodies can interfere with the lytic activities of CTL and it has been proposed that Tp32 molecules function as associative recognition structures in restricting elements that facilitate effector target binding. With cross-blocking experiments it was possible to demonstrate that the panel of antibodies tested recognized at least seven different epitopes of Tp32 molecules (Martin et al., 1984). From flow-microfluorometric analysis of human peripheral blood T cells stained with anti-Tp32 antibodies, it was possible to define two subpopulations of Tp32<sup>+</sup> cells that were quantitatively distinct in the surface density of Tp32 molecules. Bice Perussia (CA 37115), Giorgio Trinchieri (CA 20833) and colleagues have found that Tp32 "bright" cells express T cell antigens recognized by monoclonal antibodies OKT1 and OKT3, whereas the Tp32 "dull" cells do not. The two populations were also functionally distinct in that the dull cells had NK and ADCC activity, whereas the bright cells did not (Perussia et al., 1983a). Many of the antibodies in this panel demonstrated the same pattern of reactivity as Leu-2a/OKT8. The data suggest that antibody-mediated inhibition of lytic function was not caused solely by hindrance of an active site or that the active site encompasses a large portion of the molecule because the antibodies tested bound to at least seven spacially distinct epitopes and all were capable of partial inhibition of target lysis by CTL. It is possible that the functional inhibition is caused by antibody-induced perturbations in the cell surface distribution of Tp32 molecules. The Tp32 molecules of cells from different CTL lines might vary in their susceptibility to cell surface modulation which may account for some of the variation between CTL lines and the degree to which lytic activity can be inhibited by antibody binding. The complexity of Tp32 molecules has been demonstrated in a variety of ways, and much work remains to be done to resolve the role of these and other molecules in CTL function.

As previously discussed, human HLA Class II histocompatibility antigens can also serve as target determinants for CTL generated in vitro in mixed lymphocyte cultures (MLC) and it appears that long-term cultured CTL lines specific for HLA-DR are OKT4<sup>+</sup>. Similar data have been obtained by studying alloactivated T cell clones, and indicate that the T4 antigen is directly involved in recognition of the Class II target by the effector cells, while the T8 antigen relates to the recognition of Class I targets. However, Bo Dupont (CA 22507) and colleagues have developed nine long-term cultured CTL lines with HLA(DR) target specificity, five of which have the Leu-3a/OKT4 phenotype, while four have the Leu-2a/OKT8 phenotype (Flomenberg et al., 1983a). Seven of the nine CTL lines described had activity against K562, an NK cell target, in addition to alloscytotoxicity restricted to DR antigen. Cytotoxic effector cells with

activity against NK targets have been previously documented to be present after mixed lymphocyte culture. Their studies indicated that the majority of CTL lines with Class II target specificity have the Leu-2a<sup>+</sup>3a<sup>+</sup> phenotype. Monoclonal antibody anti-Leu-3a can block DR-specific cytotoxicity by Leu-3a<sup>+</sup> CTL lines, but this blocking can vary from partial to complete inhibition. NK cytotoxicity mediated by Leu-2a<sup>+</sup>3a<sup>+</sup> CTL lines is not blocked by monoclonal antibody anti-Leu-3a. Some Leu-2a<sup>+</sup>3a<sup>-</sup> CTL lines may also recognize Class II antigens. However, these effector cells may be directed against a presently undefined target determinant distinct from HLA-A,B,C (Class I) or DR (Class II). In further studies, Dr. Dupont and colleagues have developed T cell clones which were cytotoxic for human HLA Class I target antigens. Specificity was based on target cell panel studies and inhibition by monoclonal antibodies to Class I determinants. Eight CTL clones were Leu-2<sup>+</sup>3<sup>-</sup> and their cytotoxicity was inhibited by antibody to the Leu-2 antigen. Two clones expressed the Leu-2<sup>+</sup>3<sup>+</sup> phenotype and were not inhibited by anti-Leu-2a or anti-Leu-3a antibodies. These studies indicate that Class I specific cytotoxic T cells are distributed in both T cell subsets, though predominantly in the Leu-2<sup>+</sup>3<sup>-</sup> group. In addition, these studies suggest that the Leu-3 molecule may not function in identical fashion in Leu-3<sup>+</sup> cytotoxic cells which recognize Class I target antigens as in those which recognize Class II targets. Their findings illustrate that the Leu-2 and Leu-3 markers of T cell subsets do not distinguish in absolute fashion between the functions performed by individual T cell clones or between the types of histocompatibility antigens which they recognize (Flomenberg et al., 1983b).

Dr. Dupont's group, in collaboration with Carlo Russo (CA 32635) and Soldano Ferrone (CA 32634), have characterized four HLA Class I-specific helper-independent cytotoxic T lymphocyte clones and have found that human Class I histocompatibility antigens encoded by both HLA-A and B loci may induce alloproliferative responses. Those T cell clones which proliferate in response to Class I antigens may also exhibit cytotoxic responses to cells bearing the same determinant. Blocking studies with monoclonal antibodies toward HLA Class I antigens have demonstrated that these responses are triggered by determinants on the Class I molecule itself and these determinants are spatially close to those which influence serologic allospecificity. The T cells which proliferate in response to Class I antigens, like those which mediate Class I specific cytotoxicity, are distributed into both the Leu-2<sup>+</sup>3<sup>-</sup> and Leu-2<sup>+</sup>3<sup>+</sup> subsets. These findings suggest that the Class I molecules themselves are the weak MLC stimulatory determinants which have previously been mapped to the HLA-A and B regions of the MHC (Flomenberg et al., 1984).

Monoclonal antibodies, alloantisera and functional assays have been used to define at least three groups of human HLA Class II antigens: HLA-DR, SB, and DC. Structural studies show that the HLA-DR molecules are homologous to murine I-E and that DC antigens appear structurally homologous to the murine I-A. Recently, anti-DC-1 monoclonal antibodies were demonstrated to inhibit the generation of CTL, but not ADCC or NK cytotoxicity assays. In an extension of these studies, Steven Burakoff (CA 34129) and colleagues have shown that HLA-DR and SB antigens can function as targets for human CTL. As previously discussed, earlier observations have led to the hypothesis that the T8 molecule recognizes Class I molecules, whereas the T4 molecule is receptive for Class II molecules. In addition to the contradictions to this hypothesis discussed



above from the laboratory of Bo Dupont, Dr. Burakoff described another exception to this general classification: an OKT8<sup>+</sup> CTL whose target recognition is totally inhibitable by anti-DC monoclonal antibody. This CTL line was not inhibitable by a panel of anti-HLA-DR monoclonal antibodies. It is noteworthy that these CTL were also inhibitable by monoclonal antibodies OKT3 and anti-LFA-2, but none of eleven OKT8 monoclonal antibodies or seven OKT4 monoclonal antibodies inhibited cytolysis by these CTL, suggesting that these CTL do not utilize either the T4 or T8 molecules for target cell lysis. It is possible that an as yet undefined T cell surface molecule is involved in HLA-DC recognition (Krensky et al., 1983b).

Chris Platsoukas (CA 32070) has been studying the effect of monoclonal antibodies which recognize human T cell differentiation antigens to analyze the role of these antigens on T cell-mediated cytotoxicity against chemically-modified target cells, both allogeneic and autologous. The OKT3/anti-Leu-4 and OKT8/anti-Leu-2a monoclonal antibodies inhibited T cell-mediated cytotoxicity against autologous or unrelated trinitrophenyl (TNP)-modified targets in the absence of complement and at the effector cell level. These cytotoxic effector cells were OKT3<sup>+</sup>, OKT4<sup>-</sup>, OKT8<sup>+</sup>, OKT11<sup>+</sup>. To analyze the role of these antigens in the cytolytic process, the stages of cytotoxicity that were inhibited by these monoclonal antibodies were investigated. OKT3/anti-Leu-4 did not inhibit binding of effector cells to targets, whereas OKT8/anti-Leu-2a monoclonal antibodies blocked cytotoxicity by inhibiting binding of the effector cells to the chemically-modified target cells. OKT8/anti-Leu-2a did not affect post-adhesion stages of the cytolytic process, whereas in contrast, the OKT3/anti-Leu-4 monoclonal antibody inhibited a post-adhesion step of the cytolytic process that occurs before irreversible events of the programming for lysis stage take place. These results suggest that the T3/Leu-4 antigen may be associated with the triggering of lethal hit (and triggering of T cell functions in general) and signal transfer or transduction. In contrast, the T8/Leu-2a antigen appears to be involved in binding of effector to target cells and may contribute to the affinity of the effector cells for the target (Platsoukas, 1984).

### T Cell Receptor for Antigen

While work has been in progress for several years on lymphocyte function antigens and lymphocyte differentiation antigens described above, it has been only recently that progress has been made to define the cell surface structure by which T cells recognize specific antigens, the elusive T cell receptor. As is often the case in science, the discovery came essentially by accident. James Allison (CA 26321) and colleagues published the first report in which a monoclonal antibody was used to identify a candidate for the T cell receptor (Allison et al., 1982). In studies to develop a panel of monoclonal antibodies directed against tumor-specific cell surface antigens of a murine T-cell lymphoma, these investigators found that one of the monoclonal antibodies, 124-40, was highly specific for an epitope expressed by the lymphoma cells and not reactive with other normal or malignant lymphoid cells. This antigen was isolated and found to be a glycoprotein composed of disulfide linked subunits of 39,000 and 41,000 dalton molecular weights. A cell surface component with the same subunit composition was detected in extracts of normal T cells and T-cell

lymphomas which were unreactive with the specific monoclonal antibody, but this component was not found in extracts of B cells or bone marrow cells. Their observations suggested that the tumor-specific antigen reactive with monoclonal antibody 124-40 may be a clonally expressed epitope of a normal T cell-specific cell surface molecule. These findings made this molecule a likely candidate for the T cell receptor.

Ellis Reinherz, Stuart Schlossman (CA 25369) and colleagues, in studies of human T cells, obtained monoclonal antibodies to two clones of normal cytotoxic T cell lines which were obtained from the same individual but had different antigen specificities, one recognizing a Class I antigen and the other recognizing a Class II antigen. The monoclonal antibodies raised against these cell lines blocked both specific cell killing and proliferation in response to the antigens. (Meuer et al., 1983). These investigators have identified a clonally unique antigen recognition structure comprised of a 49,000 dalton and a 43,000 dalton molecular weight chain. This antigen, Ti-1, is linked to the T3 antigen and was identified on a Class I specific T8<sup>+</sup> T cell clone. To determine whether analogous receptor molecules could be found on other T cell clones with differing specificity, they produced monoclonal antibodies against a clonal structure, Ti-2, on a Class II specific T4<sup>+</sup> lymphocyte derived from the same donor. This structure was shown to be a disulfide-linked heterodimer like Ti-1 and is composed of subunits of similar molecular weight. Monoclonal antibodies against these antigen-specific functions do not cross react. The findings suggest that each T lymphocyte, regardless of subset derivation or specificity, uses an analogous Ti heterodimer for antigen-specific function. Ti is linked to T3 and is expressed on the cell surface at an identical density (30,000-40,000 sites per cell). Further studies with additional monoclonal antibodies indicated that the anti-clonotypic antibodies react with an epitope on this heterodimer and this structure is associated with T3 in the cell membrane. Similar molecules were detected on eight additional clones which did not express the determinant defined by the monoclonal antibodies. Biochemical studies indicated that this heterodimer contains the variable region of the T cell antigen receptor structure (Reinherz et al., 1983).

Michael Bevan (CA 25803) and colleagues have produced four independent monoclonal antibodies which may recognize the antigen receptor of a mouse CTL clone. All four monoclonal antibodies precipitate a 90,000 dalton surface molecule which reduces to two chains of approximately 42,000 daltons each and all four antibodies block antigen-specific cytolysis (Staerz et al., 1984). These cell surface components probably bear idiotypic T cell determinants, and are candidates for the T cell receptor.

Since the T cell receptor for antigen is primarily responsible for antigen recognition and specificity by the cytotoxic T cell, a system of dual recognition is emerging, which a) involves antigen recognition by the T cell receptor, but, in addition, b) requires Class I or II MHC recognition, or rather restriction, by the Leu-2a/T8 or Leu-3a/T4 T cell differentiation antigens for optimum killing. In view of current data and results, there has been speculation that the T3 antigen may be involved in triggering of T cell functions and in signal transfer or transduction (Platsoucas, 1984). Binding of the effector cells to the targets may be accomplished through: a) the T cell receptor, which provides specificity



(antigen recognition) and affinity; b) the Leu-2a/T8 antigen (primarily for Class I determinants) or the Leu-3a/T4 antigen (for Class II determinants) which may contribute to the affinity of the effector cells for the targets; and c) possibly other cell surface antigens (such as LFA-1). This binding may result in conformational or other changes on the cell surface involving the T cell receptor and the Leu-2a/T8 or Leu-3a/T4 antigens, which may cause the initiation of signal(s) to the T3 molecule which, in turn, is responsible for triggering of the lethal hit (programming for lysis). The T3 molecule may be physically located in proximity to the T cell receptor, and transfers or transduces these signals to other structures (possibly inside the cell, such as cytoskeleton components) responsible for delivery of the lethal hit. Evidence cited from the laboratories of Drs. Reinherz and Schlossman is compatible with Dr. Platsoucas' hypothesis that the T3 molecule is involved in triggering of T cell functions. Because these processes (recognition and triggering) require a sequence of events, it is reasonable to expect a supramolecular organization of these molecules on the cell surface. However, it appears that although the T cell receptor and the T3 antigen may be physically near on the cell surface, they perform distinct biological functions.

Ian Trowbridge (CA 17733), in collaboration with James Allison (CA 26321) and others, has studied variability of the MHC-restricted receptor on murine T cells by comparing tryptic peptide fingerprints of the receptor isolated from three T cell hybridomas and a T cell tumor. Both variable and constant peptides were seen. Constant peptides were most apparent when comparing receptors from this same mouse strain. Peptide fingerprints of receptors from two independent T cell hybridomas with the same idiootype and specificity were identical. These investigators also described a molecule detected on the surface of a human T cell leukemia, whose properties were identical to those reported for the MHC receptor on normal human T cells. The molecule was a dimer of 85,000 to 90,000 dalton molecular weight, containing two subchains of 46,000 and 40,000 daltons respectively. These studies were facilitated by the development of a monoclonal antibody designated T40/25 that detected a molecule on a human T cell tumor with properties identical to those reported for the MHC-specific receptor on normal human T cell clones (Kappler et al., 1983).

Charles Janeway (CA 29606) and colleagues have been able to prepare cloned helper T cell lines and have also prepared antisera and monoclonal antibodies specific for a receptor molecule on one such cloned T cell line. This antibody has allowed them to analyze the nature of the receptor structure. Studies show that the receptor molecule recognizes both self and non-self Ia molecules. It is a glycosylated 80-90,000 dalton molecular weight glycoprotein made up of an acid and base subunit. In the absence of glycosylation, each chain has an apparent molecular weight of 31,000 daltons, which agrees well with molecular analysis of the T cell receptor published by others (Kaye et al., 1984).

#### NATURAL KILLER CELLS

At the Second International Workshop on Natural Killer Cells held in May 1984 and partially funded by the Immunology Program (CA 37226), scientists in the natural killer (NK) cell field were given an opportunity to exchange

their most recent data, air controversies and initiate collaborations. The case for the biological relevance of these large granular lymphocytes continues to strengthen and was summarized by Sylvia Pollack (CA 37006) in a recent review (Pollack, 1983). In vivo manipulations of the NK compartment, by either passive transfer of NK-enriched cell populations or selective depletion of NK cells, provide direct evidence for the role of NK cells in vivo. Despite inherent experimental limitations due to the complexity of in vivo interactions, a compelling case is built for the biologic importance of the NK cell, such as its capacity to exert anti-tumor effects.

Much of the work in isolating and characterizing NK cells relies on monoclonal antibodies to NK surface markers. Bice Perussia (CA 37115), Giorgio Trinchieri (CA 20833) and co-workers have developed a number of monoclonal antibodies that react preferentially with NK cells. One of these antibodies, designated B73.1, is highly reactive with a subset of lymphocytes with NK and K (antibody-dependent killer) activity, and shows limited reactivity with neutrophilic polymorphonuclear leukocytes (PMN). Dr. Perussia and colleagues have characterized the lymphocyte subpopulation reacting with B73.1, including its cytotoxic functions. Virtually all lymphocytes with K/NK cytotoxic activity reside in the B73.1<sup>+</sup> subpopulation; this subset of cells bears the combination of antigens known to be present on K/NK cells; and the level of cytotoxicity shows a positive correlation with the actual number of B73.1<sup>+</sup> lymphocytes in individual donors (Perussia et al., 1983b). These investigators went on to characterize the cellular antigen recognized by B73.1, and described a protein of 50,000 to 72,000 daltons sensitive to pronase but not trypsin treatment. B73.1 inhibits ADCC mediated by K cells and neutrophils, but does not affect spontaneous cytotoxicity by NK cells (Perussia et al., 1983c). These results imply that the antigen defined by B73.1 might be the Fc receptor, or a structure closely related to it, on K/NK cells. Recently another antibody reactive with Fc receptors on human neutrophils, designated 3G8 (first described by Fleit et al., 1982, CA 30198) was analyzed by Drs. Perussia and Trinchieri. They discovered that 3G8 reacts with the same subset of lymphocytes detected by B73.1, which is responsible for almost all NK cytotoxic activity (Perussia and Trinchieri, 1984). These cells are large granular lymphocytes and are neither T nor B cells. Their results indicate that NK cells and PMN express the same Fc receptor for immune complexes, and that B73.1 and 3G8 recognize two distinct epitopes on the same receptor, which are preferentially expressed by either NK cells (shown by B73.1) or PMN (shown by 3G8). Utilization of these antibodies allows a more careful structural and functional analysis of molecules exposed in a differential way on the membranes of cells of different lineages. Drs. Perussia and Trinchieri's studies of the binding of OKT8 antibody to NK cells, as well as to CTL, was previously discussed (Perussia et al., 1983a).

Employing a combination of E-rosette techniques and monoclonal antibodies, Arthur Bankhurst (CA 24873) and co-workers have described the NK effector cell phenotype. They found that both OKM1<sup>+</sup>, E-rosette<sup>+</sup> and OKM1<sup>+</sup>, E-rosette<sup>-</sup> cells mediated NK activity. However, cells with a phenotype of OKM1<sup>+</sup>, E-rosette<sup>+</sup> (which also mediated ADCC) mediated more NK activity on a per-cell basis than those that were OKM1<sup>+</sup>, E-rosette<sup>-</sup> (Froelich and Bankhurst, 1983).

Many workers are dissecting the components of the NK cytotoxic process. These stages are generally thought to include binding, triggering of the lytic mechanism, and lysis of the target cell; each stage is further subdivided by some laboratories into various effector-target interactions and programming events, including the possible formation of complexes involved in the lytic event. Walter Newman (CA 36643) and Stephan Targan (CA 37205) have defined a trigger stage in the NK cytotoxic process. They utilized a murine monoclonal antibody 13.1, developed by Dr. Newman, which identifies a crucial cell surface structure involved in NK lysis (Newman, 1982). This antibody recognizes an epitope on the T-200 glycoprotein of NK cells and blocks NK lysis of the classic NK-target cell K-562 but not MOLT-4, a T-cell target (Targan and Newman, 1983). Their data suggest that the inhibiting effect of 13.1 is at the effector cell level and not the target cell level, so it may be identifying an NK receptor. Further tests were done to specify at what stage 13.1 interferes with lysis in the NK cytolytic reaction sequence. The antibody did not block initial NK-target cell interaction nor the events occurring during  $Ca^{++}$ -dependent programming for lysis. These and other data imply that 13.1 defines a distinct stage in the NK reaction sequence that links target binding to the initiation of calcium-dependent programming events. They further hypothesize that NK specificity may exist at a post-binding stage rather than at the initial NK-target binding interaction. It appears that the T-200 glycoprotein on NK cells triggers the initiation of the lytic events. Using additional monoclonal antibodies that immunoregulate T-200 molecules, Dr. Newman and co-workers were able to identify two separate regions, designated A and B, on the T-200 molecule. They demonstrated that the inhibition of NK lysis by anti-T-200 antibodies is a function of the site on that molecule to which these antibodies bind (Newman et al., 1983).

Antibody 60.3, developed by John Hansen (CA 39548) and colleagues, which recognizes a cell surface molecular complex Lp95-150 common to most leukocytes, inhibits the lytic function of both NK cells and alloreactive CTL (Beatty et al., 1983). Studies of this antibody have been previously discussed and data suggest that antibody 60.3 blocks lysis either by inhibiting activation of the lytic process or interfering with delivery of the lytic hit.

Eckhard Podack (CA 39201) and Gunther Dennert (CA 19334) have studied the membrane lesions arising during NK cytolysis. They describe two types of lesions with inner diameters of approximately 16 nm and 5 nm, which arise by membrane insertion of tubular complexes which may be assembled from subunits during cytolytic reaction. These tubules are seen on target cell membranes by immune electron microscopy, apparently forming transmembrane channels which appear to be at least partially responsible for cytolysis (Podack and Dennert, 1983).

Dorothea Zucker-Franklin (CA 34378) and her colleagues have demonstrated that NK cells bear a spectrum of enzymes on their surface which have diverse substrate specificities. While some of these enzymes mediate cytotoxicity, others apparently are involved in proteolysis (Zucker-Franklin et al., 1984). Dorothy Hudig (CA 38942), studying the proteinases associated with human NK cells that are necessary for lysis of tumor cells, has provided data suggesting a role for serine-dependent proteinase in the post-binding events of human NK cells (Hudig et al., 1984). She has evidence of a role for a chymotrypsin-like enzyme during the initiation of the "lethal hit" stage of killing but not for



the final stages of cell death. Additional data have led to the hypothesis that this enzyme is more likely to have a catalytic rather than directly cytotoxic function in NK-mediated killing.

#### LEUKEMIA-ASSOCIATED ANTIGENS

Developments in monoclonal antibody technology have greatly facilitated the study of surface determinants on tumor cells and have led to studies to exploit these antigens for therapeutic and diagnostic purposes, as well as to research on their biological and functional relevance.

John Pesando (CA 34206), in collaboration with Stuart Schlossman (CA 25369) and other investigators, has utilized a panel of monoclonal antibodies to acute lymphoblastic leukemia (ALL) cells to screen human hematopoietic cell lines, non-hematopoietic tumor cell lines, and human and non-human primate tissue for reactivity. Several monoclonal antibodies to the common acute lymphoblastic leukemia antigen (CALLA) were shown to identify a 100,000 dalton surface glycoprotein on CALLA-positive cells and similar results were obtained when leukemic cells from patients with ALL or hematopoietic cell lines were tested (Pesando et al., 1983). Dr. Pesando's studies on non-human primates demonstrated CALLA on cells comparable to those cells reactive in humans (Pesando et al., 1984). These results, coupled with other recent demonstrations of cross-reactivity of monoclonal antibodies to myeloid antigens with corresponding primate hematopoietic cells by Robert Todd (CA 39064), James Griffin (CA 36167) and colleagues, indicate the potential of primate models for evaluation of toxicity of serologic reagents to various human tumor-associated antigens (Letvin et al., 1983).

Dr. Pesando's group has also found that incubation of human leukemic cells with one CALLA monoclonal antibody (designated J-5) causes rapid and selective internalization of CALLA (antigenic modulation). This antibody also modulates surface expression of CALLA, having the effect of making target cells antigen-negative and resistant to the cytotoxic effects of the antibody. In an effort to increase the therapeutic potential of CALLA antibodies, Victor Raso (CA 29039), Stuart Schlossman and others have coupled the toxic subunit of ricin to antibody J-5. The conjugate proved to be an effective cytotoxin for CALLA-positive cultured cells and demonstrated no toxic effects in vivo in preliminary studies in rabbits (Raso et al., 1982). These results are of considerable therapeutic interest and are being actively pursued.

Richard Metzgar (CA 08975), Barton Haynes (CA 28936) and their colleagues have collaborated on phenotyping leukemia and lymphoma cells. This group studied a patient with adult T-cell leukemia whose T cells produce human T cell leukemia virus (HTLV) in vitro (Haynes et al., 1983a). Prior to culture, the patient's malignant T cells from peripheral blood, skin and joints did not express HTLV. The cultured malignant T cells expressed p19 antigen which is HTLV-associated and is a 19,000 dalton structural protein of the virus. In a screening of normal human tissues, Drs. Haynes, Metzgar and co-workers observed that anti-p19 reacted strongly with the epithelial component of normal human

thymus (Haynes et al., 1983b). The extent of reactivity of the anti-p19 monoclonal antibody was found to be a function of age of the donor of the thymus. It was demonstrated that all thymic epithelial cells positive for p19 contain thymosin alpha-1 and thymopoietin, but no other HTLV proteins or proviral HTLV DNA were demonstrated in these cells. Therefore, the p19 antigen identified is specific for the neuroendocrine component of thymic epithelium and is acquired during ontogeny. To explain this phenomenon, the group has proposed two alternative hypotheses: either there is a cross-reactivity between HTLV and thymic epithelial antigens, or HTLV p19 is a host-encoded protein selectively expressed in normal thymus and also induced in HTLV-infected T cells and incorporated into the virus. Because the anti-p19 antibody has also been found to cross-react with other normal tissues as well, Dr. Haynes is pursuing the biologic significance of this finding in ongoing studies. One area of interest is the potential usefulness of the anti-p19 antibody as a diagnostic probe for AIDS.

With all the attention to the sensitivity and specificity of monoclonal antibodies, the fact that conventional antisera still have a valid role in research can be overlooked. An example of the usefulness of such antisera is found in the work of Thalachallour Mohanakumar (CA 27416), who has presented evidence for a unique T-cell acute lymphoblastic leukemia (T-ALL)-associated antigen with a molecular weight of 100,000 daltons, using non-human primate antisera (Mohanakumar et al., 1983). Dr. Mohanakumar distinguishes this antigen from CALLA (described above), and since absorption of one of these primate antisera (designated OST) with normal human thymocytes did not diminish its activity to T-ALL cells, it does not appear to be a normal thymocyte-associated antigen either. Interestingly, OST shares some of the serologic and biochemical characteristics of monoclonal antibody OKT9 (anti-human transferrin receptor), since it fails to react with normal thymocytes and blood T lymphocytes, binds only to leukemic T lymphoblasts, and immunoprecipitates a glycoprotein of approximately 100,000 daltons. In contrast to OKT9, OST does not react with PHA- and ConA-activated normal T lymphoblasts. The relationship between structures identified by these two sources of antibodies needs to be pursued. Further studies have to be done to determine whether the 100,000 dalton glycoprotein expressed by T-ALL cells is structurally similar to CALLA with minor differences or whether the antigens are distinct from one another.

Robert Humphreys (CA 25873) and colleagues have also characterized membrane-associated leukemic cell molecules in an effort to explore how the immune system might control chronic leukemias. Using cells from hairy cell leukemia (HCL) patients, Dr. Humphreys identified 35,000 and 15,000 dalton proteins (p35 and p15) several years ago and recently demonstrated that the 35,000 dalton HCL subset-defining protein is the human analogue of murine Ii, a structurally invariant protein which is linked non-covalently with Ia alpha and beta chains, analogous to HLA-DR in humans (Spiro et al., 1984). In addition, another molecule of 41,000 daltons was isolated and resembled another Ii-associated molecule. In conjunction with earlier findings by other investigators, Dr. Humphreys' data tend to support the hypothesis that HLA-DR associated proteins could modulate function of leukemic cell HLA-DR determinants and might thereby influence the anti-leukemic response.



Related to the above hypothesis, Nobuyuki Tanigaki (CA 17276) and his co-workers have analyzed the human Ia system with alloantisera and mouse anti-human monoclonal antibodies. These investigators conclude that there appear to be three loci for genetic control of human Ia antigens (Tanigaki et al., 1983a; Sorrentino et al., 1983). In related findings, Dr. Tanigaki describes three human Ia subsets isolated from an HLA-homozygous lymphoid cell line of DR5 specificity (Tanigaki et al., 1983b). These subsets carried the specificities DR5 and MT2, MT2 alone, and MB3 alone. The subset carrying only MT2 was similar, in terms of alpha and beta chains, to the DR5/MT2 subset, while the MB3 subset had both chains distinct from the other two subsets. This further substantiates homology between human and mouse Ia systems.

### ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

Several grants have been funded in the last two fiscal years specifically for the study of AIDS, while additional research has been conducted within the scope of ongoing research grants. Many of the grants specifically to study AIDS have been awarded as cooperative agreements (U01 grants) to facilitate exchange of information, and many are large multi-faceted studies. Much of the basic research on the immunology of AIDS is covered within the scope of grants administered by the Divisions of Cancer Treatment and Cancer Etiology, and are not included in this report. Therefore, this report is not intended to be comprehensive, but will serve to highlight some of the AIDS research supported by the Immunology Program, Division of Cancer Biology and Diagnosis.

Within the last two years, many studies have been initiated to search for the etiologic agent of AIDS, as well as to clarify the nature of the interrelated immune deficiencies seen in this syndrome. It had been established early on that one of the hallmarks of this disease was the profound depletion of T lymphocytes, particularly those of the  $T4^+$  subset of helper/inducer lymphocytes. While a healthy individual has a ratio of  $T4^+$  to  $T8^+$  cells of approximately 2.0, this ratio is profoundly reduced in AIDS, and is also somewhat reduced in the AIDS Related Complex (ARC) which may be the prodromal phase of this disease. The recent reports of the isolation of human T cell leukemia virus-III (HTLV-III) from cells of AIDS patients, and additional serologic data, have implicated this virus as the likely etiologic agent of AIDS (Popovic et al., 1984; Gallo et al., 1984; Schupbach et al., 1984; Sarngadharan et al., 1984). These data indicate a strong association of the presence of this virus and/or anti-viral antibodies in patients with clinically-documented AIDS. The proclivity of this virus to replicate in and kill the  $T4^+$  subset of lymphocytes is consistent with the depletion of this cell subset seen in AIDS patients. However, much work remains to be done to determine why only some of those individuals exposed to this agent develop the disease. It is necessary to determine what, if any, other factors are involved in the etiology and pathogenesis of AIDS and to develop strategies for prevention and treatment.

Evan Hersh (CA 34674) and colleagues have registered 305 patients into this project during its first year: 20% were symptom-free (SF); 60% had the AIDS Related Complex (ARC); and 25% had AIDS defined as Kaposi's sarcoma (KS),

opportunistic infection or both. Of these, 135 have been studied in detail, and the data indicate a characteristic pattern of immunodeficiency in the symptom-free patients which worsened as they progressed into the ARC and AIDS status. These immune defects include low helper cells, an inverted helper/suppressor ratio, impaired lymphocyte proliferative responses, impaired delayed hypersensitivity, and elevated serum thymosin alpha-1. In the more advanced patients, serum lysozyme is elevated, there is circulating serum interferon, and NK cell activity becomes impaired in the most advanced cases. Suppressor cell activity was found in a co-culture system, and it correlated with an inverted helper/suppressor ratio. Serial studies of surface marker expression after mitogen stimulation showed that AIDS patients' cells failed to develop T10, HLA-DR and Tac (IL-2 receptor) increases after stimulation, indicating at least one set of factors related to failure of lymphocyte proliferation and effector cell function. After stimulation of leukocytes *in vitro* with herpes simplex virus, AIDS patients' cells could proliferate somewhat, but could not mount an NK cell response or produce alpha-interferon. In vitro addition of interleukin-2 (IL-2) partially improved these latter two functions. Dr. Hersh has also studied nucleic acid metabolism in AIDS patients' cells, and has found that the level of 5' nucleotidase was significantly low in SF, ARC and AIDS patients. A variety of immune modulators were studied for their effects on these immune functions in vitro. IL-2, Azimexon (an aziridine dye), and Thymosin fraction 5 all were capable of restoring certain immune functions. In vivo studies have been done with Azimexon and isoprinosine which have both restored immune functions (PHA and pokeweed mitogen (PWM) responses) and have resulted in diminished symptomatology of the ARC status. For the treatment of KS, partially purified alpha-interferon has been used. A dose of  $3 \times 10^6$  units/M<sup>2</sup> lead to responses in only 1/8 patients while a dose of  $20 \times 10^6$  units/M<sup>2</sup> lead to complete or partial remissions in 8/12 patients. Responses were best in those with minimal disease (Hersh et al., 1983a,b; Murray et al., 1984).

Michael Gottlieb (CA 12800) and colleagues are also evaluating the functional properties of the OKT8<sup>+</sup>/OKT4<sup>+</sup> T cell subpopulations in AIDS patients. Despite polyclonal-gammaglobulinemia in the sera of these patients, their peripheral blood lymphocytes produced negligible quantities of immunoglobulin when cultured in vitro for eight days in the presence of PWM. Patients' B cells, however, synthesized normal quantities of immunoglobulin when co-cultured with T cells from healthy donors, indicating preservation of B cell function. Unfractionated PBL or T cells of patient origin mediated marked suppression of PWM-driven immunoglobulin production by T and B cells from healthy donors. The suppressive activity was contained within the population of OKT8<sup>+</sup> cells and was sensitive to in vitro irradiation. On a per-cell basis, OKT8<sup>+</sup> cells of patients appear to have greater suppressive activity than normal control OKT8<sup>+</sup> cells. In addition, OKT4<sup>+</sup> cells from patients had less helper activity for induction of immunoglobulin synthesis than control OKT4<sup>+</sup> cells. Increased T suppression and reduced T help are probably a consequence of one or more viral infections and may contribute to progressive immune deficiency and susceptibility to malignancy in patients with AIDS (Benveniste et al., 1983).

Albert Donnenberg (CA 31687) and colleagues have been studying the cell-mediated immune response of AIDS patients' lymphocytes to cytomegalovirus (CMV) and herpes simplex virus (HSV). Modulation of virus-specific immunity was

assayed in vitro by measuring the lymphocyte blastogenic response and the production of lymphokines (leukocyte inhibition factor, LIF) by peripheral blood lymphocytes (PBL) stimulated with HSV or CMV antigens in the presence or absence of IL-1 (interleukin-1) and IL-2. PBL from the control and lymphadenopathy subjects responded to both antigens in the lymphocyte transformation assay measured on day 7 and the responses were significantly enhanced in cultures grown in the presence of antigen and IL-2. PBL from the AIDS patients were unresponsive, but responsiveness was restored by the addition of IL-2. Addition of IL-1 to antigen-stimulated PBL cultures failed to enhance the proliferative responses in all three study groups. LIF production was assayed in the supernatants from day-one PBL cultures. LIF was not produced by PBL from AIDS patients grown in the presence of viral antigens, whereas three of five patients from the lymphadenopathy group and three of five control subjects gave rise to positive responses. Addition of IL-1 to the antigen-stimulated cultures enhanced LIF production in the control and lymphadenopathy groups, but not in the AIDS patients. Addition of IL-2 did not modulate LIF production by antigen-stimulated PBL from the control or AIDS patients, while suppressing the LIF response of the similarly stimulated PBL from the lymphadenopathy patients (Sheridan et al., 1984).

Fred Valentine (CA 34976) has succeeded in establishing long-term cell lines from blood mononuclear cells of 21 of 36 patients with AIDS or ARC. Normal donors whose cells have been cultured in parallel have not yielded such lines. Cell lines have been established from homosexual males, female and male addicts, female Haitian, a transfusion recipient and from an infant born to an addict mother, all of whom have had AIDS or ARC. These lines have a common surface phenotype: negative for T-cell markers, NK markers, monocyte markers and surface immunoglobulin; positive for Class II antigens DR and DS. Some lines have detectable amounts of intra-cellular immunoglobulin. Lines tested to date are Epstein-Barr Virus Nuclear Antigen (EBNA) positive. DNA from three of the lines has been examined for rearrangement of heavy chain genes by Southern blotting and hybridization with a J-region probe. All were rearranged in the B cell configuration. Therefore, the presence of precursors of these lines in a majority of patients represents a disease-related finding; the cells would seem to represent an unusual type of B cell line (manuscript in preparation). Many additional experiments have been performed with lymphocytes from normal donors in which subpopulations of T cells are separated and reassembled in artificial mixtures with low numbers of cells of helper surface phenotype, and great excesses of cells with suppressor surface phenotype. When stimulated with microbial antigens and autologous monocytes, a vigorous proliferative response is obtained even at helper/suppressor cell ratios as low as 0.2. The response is proportional to the number of helper cells present, but even small numbers of helper cells respond normally in the presence of a great excess of cells of suppressor surface phenotype. In marked contrast, the maximum response of lymphocytes from 35 patients with AIDS to standard recall antigens was negligible even when substantial numbers of helper cells were present and ratios were near one, indicating that the failure of lymphocytes from AIDS patients to proliferate in response to microbial antigens is not explained solely by the low numbers of helper cells circulating in these patients. In studies of ARC patients, decreased helper/suppressor ratios were also found. In only one of eight patients did the lymphocytes proliferate to anywhere near a normal degree when stimulated, even though near normal numbers of lymphocytes



with helper cell surface phenotype were present. These observations support the concept that the failure of lymphocytes from these patients with ARC to respond normally does not result solely from low numbers of lymphocytes with the helper cell surface phenotype. Immunological studies on clinically healthy long-term sexual contacts of patients with proven AIDS indicated a decreased helper/suppressor cell ratio and a decreased response to mitogens. It is not yet clear whether this group with a known exposure to patients with AIDS will differ in immunological parameters from healthy homosexuals without a known exposure to a patient harboring the putative agent of AIDS (manuscript in preparation).

Olivia Preble (CA 34994) and colleagues, studying the role of endogenous acid-labile alpha-interferon in AIDS, have shown that the appearance of endogenous circulating interferon may precede clinical symptoms of AIDS in both hemophiliacs and homosexual men and, therefore, may be useful a prognostic indicator in members of high risk groups. Intravenous (IV) drug users and transfusion recipients with AIDS also have endogenous circulating alpha-interferon, but African patients with the traditional endemic forms of Kaposi's sarcoma do not (Eyster et al., 1983). Other potentially useful markers for the early diagnosis of AIDS have been proposed. Susan Zolla-Pazner (CA 15585) and colleagues have been studying the use of beta-2 microglobulin as a diagnostic marker and have demonstrated its value as a non-specific marker whose elevation is characteristic of confirmed AIDS, of early or milder forms of AIDS, and of a pre-clinical stage of AIDS (Zolla-Pazner et al., 1984). Allan Goldstein (CA 24974) and colleagues have demonstrated the utility of thymosin alpha-1 as an early assay for identifying individuals at high risk of AIDS (Goldstein and Naylor, 1984). Dr. Preble has also demonstrated that peripheral blood mononuclear cells from homosexual men with AIDS and from apparently healthy homosexual men may have significantly increased levels of 2',5'-oligo-adenylate (2-5A) synthetase, an enzyme specifically induced by interferon, but the enzyme activity may reflect locally high concentrations of interferon in lymphoid organs rather than serum interferon titers. Peripheral blood mononuclear cells from both homosexual men with AIDS and asymptomatic homosexual men are markedly deficient in their ability to respond biochemically to incubation with interferon *in vitro* by induction of 2-5A synthetase. Only eight of nineteen homosexual AIDS patients who received interferon therapy developed increased levels of 2-5A synthetase despite high titers of circulating interferon during therapy. The presence of endogenous circulating interferon prior to therapy was associated with poor clinical response in the patients who received interferon therapy. Endogenous circulating alpha-interferon was correlated with disseminated CMV infection, as evidenced by CMV viremia but not with CMV isolation from throat washings or urine (Preble et al., 1984).

Roland Mertelsmann (CA 33873) and colleagues have tested male homosexuals, twelve with Kaposi's sarcoma, four with reactive lymphadenopathy and five with opportunistic infections, for their lymphocyte proliferative responses to OKT3, PHA and another T cell monoclonal antibody. Also measured were the IL-2 production and proliferation in the presence and absence of exogenous highly purified human IL-2. All patients had significantly lower proliferative responses, as compared to normal controls, which was more pronounced when OKT3 was used as a mitogen rather than PHA. The decreased response was associated with decreased IL-2 production and was significantly lower in all diagnostic groups. The addition of highly purified peripheral blood lymphocyte IL-2 or recombinant



IL-2 enhanced the proliferative responses significantly in the vast majority of patients. Thus, not only patients with manifest disease, but also patients who were relatively asymptomatic except for lymphadenopathy, had significant defects in mitogen responses and IL-2 production, and it appears that the defective mitogen response is secondary to defective IL-2 production. Dr. Mertelsmann is studying whether the IL-2 receptor expression might be defective. A complete restoration within the normal range by exogenous IL-2 was seen only in a few patients. Normal and recombinant IL-2 showed essentially identical results (Ciobanu et al., 1983; Welte et al., 1983).

Stephen Hauptman (CA 34981) and colleagues have found that T lymphocytes from both lymphadenopathy and AIDS patients had a markedly depressed proliferative response in the autologous (auto) and allogeneic (allo) mixed lymphocyte reaction (MLR) compared to healthy homosexual or heterosexual controls. Since proliferation in the MLR depends on IL-2, they studied the production of and response to IL-2 in various groups of homosexual and heterosexual controls. IL-2 production was markedly depressed in the lymphadenopathy and AIDS patients, compared to the healthy homosexual or heterosexual controls. Although there was a significant increase in the auto-MLR of the lymphadenopathy patients with the addition of exogenous IL-2, there was no response in the auto-MLR of the AIDS patients. Comparison of isolated OKT8<sup>+</sup> T cells from controls and AIDS patients demonstrated markedly defective OKT8<sup>+</sup> T cells in the AIDS patients compared to the controls, i.e. extremely poor responsiveness in the auto-MLR, absent IL-2 production and absent response to exogenous IL-2. The diminished T cell proliferation in the auto-MLR in the lymphadenopathy group is associated with one defect, low IL-2 production, while the depressed proliferation in the AIDS group is associated with two defects, a low IL-2 production and the lack of response to IL-2. This lack of response to IL-2 was found to be due to an inability of AIDS patients' cells to generate IL-2 receptors, as determined by failure of activated T cells to absorb IL-2 or to bind anti-Tac (IL-2 receptor) antibody (Ebert et al., 1984). These studies demonstrate that IL-2 responsiveness helps distinguish homosexuals with lymphadenopathy from those with AIDS, and in addition, might help to identify lymphadenopathy patients who are at high risk of developing AIDS. Recent studies of five patients with the lymphadenopathy syndrome demonstrated a lack of response of the auto-MLR to IL-2. All five patients have since developed AIDS, suggesting the potential predictability of IL-2 responsiveness in the auto-MLR.

Stanley Schwartz (CA 35922) and colleagues have drawn several clinical and laboratory similarities between the complications associated with IV drug abuse and AIDS. They have recently examined the peripheral blood lymphocytes of a group of patients with a history of IV drug abuse who were hospitalized for treatment of various infections. Peripheral blood lymphocytes from these patients were observed to have decreased levels of NK cells and ADCC activities when compared to normal controls. Although incubation of patients' lymphocytes with PHA resulted in increased cytotoxicity against NK-resistant tumor target cells, cells from normal donors showed significantly greater enhancement. Alpha-interferon and IL-2 were also observed to enhance the NK activity of lymphocytes from both IV drug abusers and normal donors. Preliminary studies suggest that the extent of enhancement is inversely proportional to levels of endogenous interferon in serum. Patients' lymphocytes treated with either alpha-interferon

or IL-2 generally did not demonstrate final levels of cytotoxicity comparable to normal donor cells. However, the relative percent enhancement of NK activity induced by interferon or IL-2 was consistently greater for patients' lymphocytes. Most significantly, serum from patients with a history of IV drug abuse was particularly active in suppressing the NK and ADCC activities of allogeneic lymphocytes when compared with sera from healthy donors. These studies suggest that many of the complications associated with IV drug abuse may represent a prodromal phase of AIDS. Moreover, an analysis of serum interferon levels may indicate those patients likely to benefit from immunotherapy with exogenous interferon. These data are consistent with a role for a serum soluble suppressor factor in the etiology/pathogenesis of AIDS (Nair et al., 1984).

Richard Olsen (CA 31547) and colleagues are studying the immunosuppression commonly associated with retrovirus-induced animal tumors. Studies in the murine and feline retrovirus systems suggest that the 15,000 dalton envelope protein (p15E) of the virion may contribute to immunosuppression by interfering with normal lymphocyte function. They have examined the effect of inactivated feline leukemia virus (UV-FeLV) and p15 derived from this virus on ConA driven human T cell proliferation. Virus and p15E markedly suppressed mononuclear cell proliferative response to ConA. Suppression was not due to inhibition of monocyte accessory function or IL-1 secretion. In fact, the presence of monocytes partially protected T cells from UV-FeLV suppression. UV-FeLV, however, suppressed T cell secretion of and response to IL-2, indicating that UV-FeLV and derived p15E inhibit T cell proliferation by direct inhibition of T cell function. These findings, extended to the in vivo situation, suggest that retrovirus-associated suppression of the immune response involves induction of T cell, but not monocyte, dysfunction (Copelan et al., 1983).

George Cianciolo (CA 34671) and colleagues have also studied the immunosuppressive retroviral protein p15E and have demonstrated that ascites fluids from mice injected with a variety of monoclonal antibody hybridoma cell lines, as well as commonly used myeloma fusion lines, contain detectable amounts of p15E. Furthermore, pre-treatment of normal human monocytes with these ascites fluids inhibited their responses to chemotactic stimuli, suggesting that use of murine ascites fluids for monoclonal antibody production for immunotherapy protocols must be carefully evaluated. Examination of the human T cell leukemia-lymphoma virus, HTLV, for the presence of p15E-related antigens, using high-titered polyclonal rabbit antiserum to p15E, indicated the presence of both a 46,000 and a 61,000 - 67,000 dalton protein corresponding to the putative envelope glycoprotein of HTLV. They are now studying the p15E-reactive proteins of HTLV to determine if they, like p15E, have immunosuppressive properties, since an association between HTLV and AIDS has been proposed by others (Cianciolo et al., 1983, 1984).

Susan Zolla-Pazner (CA 15585) and colleagues have recently reviewed and summarized their experiences with AIDS, presenting the laboratory findings and clinical features of a large group of patients, and have discussed the leading hypotheses regarding this disease (El-Sadr et al., 1984). These investigators provided data in support of a role for a primary B cell defect in the pathogenesis of AIDS. In addition to their finding of elevated levels of beta-2 microglobulin in the serum of AIDS patients, and polyclonal hypergammaglobulinemia, AIDS

patients in their studies had elevated spontaneous IgM production from cultured peripheral blood lymphocytes as compared to cells from a healthy heterosexual control population. However, in vitro PWM stimulation of peripheral blood lymphocytes did not result in an increase of IgM production by lymphocytes from AIDS patients. These studies of spontaneous and induced IgM production suggest that B cells of AIDS patients are maximally stimulated in vivo and cannot be further stimulated in vitro with polyclonal activators. Thus, in addition to the well-described defects in cell-mediated immunity in AIDS, there is also evidence for a dysregulation of B cell function as manifested by elevated beta-2 microglobulin, hypergammaglobulinemia, increased spontaneous IgM production and defective responses to PWM. B cell dysfunction is also reflected by an increased frequency of infections by classical pyogenic organisms usually handled by humoral immune mechanisms. In addition, the lymphomas seen in AIDS patients are primarily B cell lymphomas. Dr. Zolla-Pazner has previously presented the hypothesis that the underlying dysfunction that gives rise to the immunologic abnormalities characteristic of AIDS is a hyperactivation of B lymphocytes, perhaps induced by infectious event(s) (Zolla-Pazner and Sidhu, 1983). The activated B cells would stimulate suppressor T cells, which would themselves be incapable of down-regulating the effect of B cells. However, the suppressor T cells would effectively regulate the helper T cells leading to depressed cellular immunity and the consequent development of opportunistic infection and various malignancies. Dr. Zolla-Pazner discusses the alternative hypothesis that AIDS is caused by a T cell-tropic agent (such as HTLV-III), but claims that this hypothesis does not explain the documented hyperactivity of B cells in AIDS patients. These findings are of interest, and may relate to the data from Dr. Valentine's laboratory regarding the establishment of long-term cell lines from blood mononuclear cells of AIDS patients which appear to represent an unusual type of B cell. These anomalies remain to be resolved and research is ongoing in these areas.

### Conference Support

The Immunology Program provided funds for partial support of the following conferences in FY 1984:

"Tenth International Congress of Transplantation Society"  
August 26-31, 1984      Minnesota

"Second International Workshop on Leucocyte Differentiation Antigens"  
September 17-20, 1984      Massachusetts

"Gordon Conference on Immunochemistry and Immunobiology"  
July 2-6, 1984      New Hampshire

"Second International Workshop on Natural Killer Cells"  
May 29-June 1, 1984      Michigan

"Workshop in Cell-Mediated Cytotoxicity"  
June 14-17, 1984      Maryland

"Lymphocyte and Antibody Networks: Impact of Infectious Agents"  
August 5-10, 1984 Vermont

"Fourth International Lymphokine Workshop"  
October 17-21, 1984 Germany



FISCAL YEAR 1984

IMMUNOLOGY PROGRAM

SUMMARY OF GRANTS BY SUBCATEGORY

(Includes P01, R01, R23, U01, R13 Grants)

Dollars in Thousands

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Subcategory	No. of Grants	Total Costs Awarded
Myeloma Proteins	14	\$ 1,866
Cell Surface Antigens	56	7,412
Cell Surface Determinants of Lymphocytes & Macrophages	55	7,587
Humoral Factors Other Than Antibody	43	5,516
Tumor-Related Antibodies	10	938
Immunobiology of Sarcomas, Carcinomas & Melanomas	6	673
Host/Tumor Immunopathology	11	1,560
Effects of Disease on Immune Function	21	2,033
Immunotherapy: Mechanisms Rather Than Therapeutic Result	16	2,186
Lymphocytes	104	15,359
Monocytes & Macrophages	33	5,900
Malignancies of the Immune System (Lymphoma/Leukemia)	23	2,575
Immune Surveillance	27	2,885
Immunotherapy in Animal Models	11	1,159
Bone Marrow Transplantation	7	1,793
Conference Grants	<u>7</u>	<u>34</u>
	444	59,476

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## MYELOMA PROTEINS

- R01 CA08497 Abnormal Proteins in Multiple Myeloma  
Putnam Indiana University, Bloomington
- R01 CA10056 Proteins in Multiple Myeloma and Related Blood Diseases  
Solomon University of Tennessee, Knoxville
- R01 CA12421 Structure of Immunoglobulin Messenger RNAs and Genes  
Adams Walter and Eliza Hall Inst. of Medical Research
- R01 CA13014 Studies on Proteins of Plasma Cell Cancers  
Beychok Columbia University
- R01 CA16858 Genetics and Biochemistry of Myeloma IG Production  
Morrison Columbia University
- R01 CA19616 Immunoglobulins in Multiple Myeloma and Amyloidosis  
Edmundson University of Utah
- R01 CA24432 Sequence, Shape and Specificity of Antibodies  
Haber Massachusetts General Hospital
- R01 CA25754 Control of Immunoglobulin Synthesis  
Storb University of Washington
- R01 CA31013 Immunoglobulin Genes of Normal and Leukemic Human DNA  
Blattner University of Wisconsin, Madison
- R01 CA32044 Human Monoclonal Antibodies from EBV-Transformed Cells  
Robinson Yale University
- R01 CA32582 Studies on Secretory Immunoglobulin  
Lamm Case Western Reserve University
- R01 CA34012 Differentiation Defects in Malignancies of the B Cell  
Vogler Vanderbilt University
- R01 CA34778 Analysis of Nonsmall Cell Lung Carcinoma Antigens  
Walker Scripps Clinic and Research Foundation
- R01 CA36606 Immunoglobulin Gene Expression in Myeloma Mutants  
Milcarek University of Pittsburgh

## CELL SURFACE ANTIGENS

- R01 CA13287 Genetic Basis of Antigenic Variation  
Hyman Salk Institute for Biological Studies
- R01 CA18470 Antigenicity and Tumorigenicity of Somatic Cell Hybrids  
Knowles Wistar Institute of Anatomy and Biology

R01	CA18600	Masking of Antigens at Cancer Cell Surfaces Codington	Massachusetts General Hospital
R01	CA19224	Relation of Blood Group and Human Tumor Antigen Hakomori	Fred Hutchinson Cancer Research Center
R01	CA21223	Antitumor Antibodies Generated In Vitro Levy	Stanford University
R01	CA21445	Antigens of Malignant Melanoma and Other Human Tumors Lloyd	Sloan-Kettering Institute for Cancer Research
R01	CA22540	Nature of T-Specific Human Carcinoma Antigens Springer	Evanston Hospital
R01	CA22674	Characterization of Fetal Antigens in Tumors Coggin	University of South Alabama
R01	CA22794	Human Neuroblastoma Antigens Seeger	University of California, Los Angeles
R01	CA23568	Immunoresponse to Human Surface Antigens Croce	Wistar Institute of Anatomy and Biology
R01	CA23770	Antigen Induced Lymphoma Haughton	University of North Carolina, Chapel Hill
R01	CA24263	Hybridomas: Production and Genetic Application Kennett	University of Pennsylvania
R01	CA24358	Leukemia Associated Antigens Billing	University of California, Los Angeles
R01	CA25134	Lymphocyte Surface Glycoconjugates Poretz	Rutgers--The State University of New Brunswick
R01	CA25139	Study of Group 5 Antigens in Hematologic Malignancies Lum	Fred Hutchinson Cancer Research Center
P01	CA25874	Human Melanoma and Tumor Specific Monoclonal Antibodies Koprowski	Wistar Institute of Anatomy and Biology
R01	CA26321	Cell-Surface Antigens of Murine Tumors Allison	University of Texas System Cancer Center
R01	CA27124	Molecular Approaches to Human Colon Cancer Kahan	University of Texas Health Science Center, Houston
R01	CA27416	Characterization of New Human IA and Leukemia Antigen Mohanakumar	Virginia Commonwealth University
R01	CA27534	Nucleolar Antigens of Human Cancer Cells Busch	Baylor College of Medicine

R01	CA27628 Milgrom	Tumor-Specific and Tumor-Associated Antigens State University of New York at Buffalo
P01	CA28166 Edgington	Molecular Immunology and Pathobiology of Neoplasia Scripps Clinic and Research Foundation
R01	CA28420 Reisfeld	Molecular Profile of Human Melanoma Antigens Scripps Clinic and Research Foundation
R01	CA28461 De Leo	Cell Surface Antigens of Sarcomas Sloan-Kettering Institute for Cancer Research
R01	CA28564 Carey	Human Squamous Cell Carcinoma: Culture and Serology University of Michigan at Ann Arbor
R01	CA29909 Dreyer	Molecular Characterization of Human Tumor Markers California Institute of Technology
R01	CA29964 Haughton	UNC-CH Immunocytomas University of North Carolina, Chapel Hill
R01	CA30266 Gooding	Membrane Antigen Organization in Tumor Immunity Emory University
R01	CA30647 Irie	Human Monoclonal Antibodies to OFA-I University of California, Davis
R01	CA31378 Fishman	Immunochemical Studies of Placental Alkaline Phosphatase La Jolla Cancer Research Foundation
R01	CA31620 Bonavida	Inappropriate H-2 (K/D) and IE/C Antigens on Tumors University of California, Los Angeles
R01	CA31828 Ricardo	Immune Response to Syngeneic Leukemic B-Cell Antigens Wake Forest University
R23	CA32047 Glassy	Monoclonal Antibodies to Human Tumor-Associated Antigens University of California
R01	CA32132 Anderson	Autoimmunity to Testicular Germ Cell Oncofetal Antigens Dana-Farber Cancer Institute
R01	CA32578 Garver	Characterization of Tumor Antigen on Leukemia Cells Medical College of Georgia
R01	CA32925 Quaranta	Pancreatic Tumor Antigens Defined by Monoclonal Antibody Scripps Clinic and Research Foundation
R01	CA33693 Martin	MHC Coded Alloantigens on Lung Tumors U.S. Uniformed Services Univ. of Health Sciences
R01	CA34031 Ng	Analysis of Human Prostate Carcinoma Associated Antigens Columbia University

R01	CA34206	Human Leukemia-Associated Antigens
	Pesando	Fred Hutchinson Cancer Research Center
R01	CA34232	Expression of H-2 Antigens on SJL/J Tumors
	Beisel	Johns Hopkins University
R01	CA34342	Selecting Expressed Tumor Immune RNA with Hybridomas
	Paque	University of Texas
R01	CA34368	MHC Antigen Expression on Teratocarcinoma Cells
	Ostrand-Rosenberg	University of Maryland, Baltimore County
R01	CA34378	Tumor Cytolysis by Mononuclear Leukocytes
	Zucker-Franklin	New York University
R01	CA34913	Genetics of Response to Histocompatibility Antigens
	Howard	Institute of Animal Physiology
R01	CA35592	Structure and Function of Cell Surface Hyaluronate
	Underhill	Georgetown University
R01	CA35857	Immune Regulation to Fetal Antigens in Pregnancy-Cancer
	Rohrer	University of South Alabama
R01	CA35929	Monoclonal Antibodies to Human Squamous Cancer Antigens
	Carey	University of Michigan at Ann Arbor
R01	CA37099	Immunogenetics of Unique Tumor-Specific MHC Antigens
	Goodenow	University of California, Berkeley
R01	CA37156	Immunobiology of Unique Tumor-Specific Antigens
	Schreiber	University of Chicago
R01	CA37169	Tumor-Specific CTL Recognition of Transfected Cells
	Faller	Dana-Farber Cancer Institute
R01	CA37303	Structure-Function Studies of SV40 TSTA and H-2
	Pan	Wistar Institute of Anatomy and Biology
R01	CA37410	Organ Specific Antigens of Gastrointestinal Tissues
	Gold	Center for Molecular Medicine and Immunology
R01	CA37440	Molecular Studies of Human Melanoma Antigen
	Brown	Oncogen
R01	CA37645	Function and Structure of Leukemic Cell II
	Humphreys	University of Massachusetts
R01	CA37888	HLA Alloantigens on Cultured Human Tumor Cell Lines
	Pollack	Baylor College of Medicine
R01	CA38407	Antigenic Profile of Human Leukemic Cells
	Russo	Cornell University Medical Center



- R01 CA38500 Membrane Antigens Which Mediate Metastatic Phenotype  
Le Grue University of Texas System Cancer Center
- R01 CA39054 Cell Surface Antigens of Murine Tumors  
Callahan Colorado State University
- R01 CA39212 Transplantation Antigenicity of Virus Induced Tumors  
Hellstrom Oncogen

CELL SURFACE DETERMINANTS OF LYMPHOCYTES AND MACROPHAGES

- R01 CA04681 Genetic Studies with Mammalian Cells  
Herzenberg Stanford University
- R01 CA18640 Behavior of Weak Transplantation Antigens  
Silvers University of Pennsylvania
- R01 CA18659 Chemical Genetic and Cellular Aspects of Immunogenicity  
Gill University of Pittsburgh
- R01 CA18734 Immunologic Studies Related to Malignancy  
Jones University of Colorado Health Sciences Center
- R01 CA20473 Immunogenetics of the TLA Region of Chromosome 17  
Boyse Sloan-Kettering Institute for Cancer Research
- R01 CA20500 Structural and Serological Studies on IA Antigens  
Cullen Washington University
- P01 CA21112 Clinical and Basic Studies of Plasma Cell Dyscrasias  
Osserman Columbia University
- R01 CA21651 Teratocarcinoma and Embryonal Tumors: Surface Antigens  
Artzt Sloan-Kettering Institute for Cancer Research
- R01 CA22131 Immunogenetics of Ly Systems  
Boyse Sloan-Kettering Institute for Cancer Research
- P01 CA22507 Immunogenetics of the Major Histocompatibility Complex  
Dupont Sloan-Kettering Institute for Cancer Research
- R01 CA23469 Cells Involved in Spontaneous Regression of Tumors  
Yang University of Connecticut, Storrs
- R01 CA24067 FC Receptor Structure and Function  
Anderson University of Rochester
- R01 CA24433 Antigen Recognition by Cytotoxic Killer Cells  
Sears University of California, Santa Barbara
- R01 CA24473 Genetics and Functions of (H-2 Linked) I Region  
David Mayo Foundation

R01 CA25044 Hickman	Surface IgM of Malignant Lymphocytes and Plasma Cells Jewish Hospital of St. Louis
R01 CA25056 Misshell	Immunoregulatory Effects of Bacterial Substances University of California, Berkeley
R01 CA25532 Schwartzing	Glycolipids of Murine and Human Lymphocytes Eunice Kennedy Shriver Center Mental Retardation
R01 CA25613 Ross	Membrane Components of the Leukocyte Complement System University of North Carolina, Chapel Hill
R01 CA25893 Hyman	Cell Surface Molecules: Hematopoietic Differentiation Salk Institute for Biological Studies
R01 CA26891 Allison	Surface Antigens of Hepatocellular Carcinomas University of Texas System Cancer Center
R01 CA29194 Rajan	Somatic Cell Genetics of Cell Surface Antigens Yeshiva University
R01 CA29548 Hansen	Differentiation Antigens on Human Lymphocytes Pacific Northwest Research Foundation
R01 CA29679 Sibley	Genetic Analysis of Membrane Immunoglobulin University of Washington
R01 CA30147 Gottlieb	Genetic Markers, Leukemogenesis and Thymic Function University of Texas, Austin
R01 CA30654 Morgan	Regulation of Immune Responses by FC Portion of Antibody Scripps Clinic and Research Foundation
R01 CA31638 McKean	Characterization of MHC Restricted Antigen Presentation Mayo Foundation
R01 CA31798 Springer	Murine T-Lymphocyte Cell Surface Antigens Dana-Farber Cancer Institute
R01 CA31799 Springer	Chemistry of Tumoricidal Macrophage Surface Antigens Dana-Farber Cancer Institute
R01 CA32634 Ferrone	Heterogeneity of HLA-A, B Antigens on Tumor Cells Scripps Clinic and Research Foundation
R01 CA33555 Todd	Cell Surface Antigens on Human Macrophages Dana-Farber Cancer Institute
R01 CA34077 Mendelsohn	IL-2 Action on Normal and Malignant Lymphocyte Receptors University of California, San Diego
R01 CA34108 Kimura	Structures Related to Function on Cytotoxic T Cells University of Florida

R01	CA34110 Twomey	Functions of Clonally Derived Human Monocytes Baylor College of Medicine
R01	CA34787 Trowbridge	Human Cell Surface Antigens: Transferrin Receptors Salk Institute for Biological Studies
R01	CA34900 Wang	Chemical Analysis of Human and Murine T-Cell Antigens Medical University of South Carolina
R01	CA34965 Klein	Polymorphism of the Major Histocompatibility Complex Max Planck Institute for Biology
R01	CA35055 Flaherty	QA and TL Antigens Expressions and Function New York State Department of Health
R01	CA35638 Ware	Molecular Pathway of Human T-Cell-Mediated Cytotoxicity University of California
R23	CA35976 Hunter	Characterization of the Cytotoxic T-Cell Receptor University of Alabama in Birmingham
R01	CA35977 Suzuki	FC-Gamma Receptor-Mediated Regulation of Macrophage Univ of Kansas College Health Science and Hospital
R23	CA35986 Heagy	Monoclonal Antibody Analysis of Cloned/Mutant Killers Dana-Farber Cancer Institute
R01	CA36137 Oettgen	RADLV Leukemia Antigens Recognized by Cytotoxic T Cells Sloan-Kettering Institute for Cancer Research
R01	CA36167 Griffin	Surface Antigens of Human Myeloid Progenitor Cells Dana-Farber Cancer Institute
R01	CA36700 Freed	Structural Studies of the Products of the H-2 Complex National Jewish Hospital and Research Center
R01	CA37155 Perussia	Receptors for Immunoglobulin G on Leukocytes Wistar Institute of Anatomy and Biology
R23	CA37199 Wright	Mechanisms of NK Resistance in Tumor Cell Variants University of California, Los Angeles
R23	CA37439 Sitkovsky	Cell-Cell Contact Proteins of Cytotoxic T Lymphocytes Massachusetts Institute of Technology
R01	CA37827 Kornbluth	Analysis of Human NK Function University of Pennsylvania
R23	CA38055 Fleit	FC Receptor Expression During Myeloid Differentiation State University of New York at Stony Brook
R01	CA38396 Redelman	The Molecular Basis of T-Cell-Mediated Cytolysis University of Nevada, Reno

- R01 CA38404      Structural Studies of IA Alloantigens  
Silver                      Hospital for Special Surgery
- R01 CA38469      Heterogeneity of HLA-A, B Antigens on Tumor Cells  
Ferrone                      New York Medical College
- R01 CA39055      Regulation of Membrane Antigen Expression by Tumor Cell  
Callahan                      Scripps Clinic and Research Foundation
- R01 CA39069      Cloning H-22 and HLA-D Region Coding Sequences  
McDevitt                      Stanford University

HUMORAL FACTORS OTHER THAN ANTIBODY

- R01 CA12779      Leukocyte Regulatory Mechanisms  
Nowell                      University of Pennsylvania
- R01 CA15129      A Serum Immunosuppressive Factor in Cancer  
Oh                              Boston University
- R01 CA15585      A Soluble Mediator of Tumor-Induced Immunosuppression  
Zolla-Pazner                      New York University
- R01 CA17643      Regulation of T Cell Proliferation and Differentiation  
Smith                              Dartmouth College
- R01 CA17673      Regulation of the Humoral Immune Response by B Cells  
Hoffmann                      Sloan-Kettering Institute
- R01 CA19529      Cell-Mediated Immunity in Humans: Mechanisms and Uses  
Valentine                      New York University
- R01 CA22126      Ultraviolet Light Radiation and Immunoregulation  
Daynes                              University of Utah
- R01 CA24474      Generation of Cytotoxic T Cells by Helper Factors  
Finke                              Cleveland Clinic Foundation
- R01 CA24974      Chemical and Immunological Characteristics of Thymosin  
Goldstein                      George Washington University
- R01 CA26143      Control of Complement-Mediated Tumor Cell Cytolysis  
Lint                              Rush University
- R01 CA26504      Regulation of Granulocyte and Macrophage Production  
Stanley                              Yeshiva University
- R01 CA27903      The Biology of the Antitumor Actions of Interferons  
Epstein                              University of California, San Francisco
- R01 CA29062      Vasoactive Intestinal Peptide in Diagnosis of Leukemia  
O'Dorisio                      Ohio State University



R01	CA29991 Gupta	Interferon Action: Studies on Interferon Receptor System Sloan-Kettering Institute for Cancer Research
R01	CA30015 Mortensen	Acute Phase Reactants: Induction and Host Resistance Ohio State University
R01	CA30515 Sidell	Immunological Aspects of Retinoids in Human Cancer University of California, Los Angeles
R23	CA30894 Mier	Binding Studies with Purified Human T-Cell Growth Factor New England Medical Center Hospital
R01	CA31394 Lotzova	Effect of Interferon Inducers on NK Cell Cytotoxicity University of Texas System Cancer Center
R23	CA32319 Cohen	Immunologic Control of Tumor Cell Migration University of Connecticut Health Center
R01	CA33168 Incefy	Thymic Peptides, Monoclonal Antibodies and Cancer Sloan-Kettering Institute for Cancer Research
R01	CA33557 Plate	Transplantation Antigen Specific Immunosuppression Rush-Presbyterian-St. Luke's Medical Center
R01	CA33956 Colton	Physiochemical Studies of Immune Complexes Massachusetts Institute of Technology
R01	CA33994 Korngold	Interferon-Induced Immunosuppression and Tumor Rejection Wistar Institute
R01	CA34103 Day	Complement and Immune Complexes in Lymphosarcoma Oklahoma Medical Research Foundation
R01	CA34120 Schreiber	Molecular Regulation of Macrophage Cytocidal Activity Scripps Clinic and Research Foundation
R01	CA34121 Kind	Effect of Thymosin on Interferon Induction George Washington University
R01	CA34141 Godfrey	Isolation of Macrophage Agglutination Factor New York Medical College
R01	CA34344 Michael	Tumorigenesis: Immunoendocrine Systems Interactions State University of New York at Albany
R01	CA34573 Gifford	Studies of Cytolytic Factors from Macrophages University of Florida
R01	CA34805 Patek	Natural Cytotoxic Activity and Tumorigenesis Salk Institute for Biological Studies
R01	CA34951 Boyle	Nerve Growth Factor and Complement Pathway University of Florida

- U01 CA34976 Etiology and Immunological Basis of the Aid Syndrome  
Valentine New York University
- R01 CA35152 Cytolytic T-Lymphocyte Helper Factor  
Fan University of Minnesota of Minneapolis, St. Paul
- R01 CA35761 Mechanism of Melphalan-Mediated Tumor Eradication  
Mokyr University of Illinois at Chicago
- R23 CA35975 Biochemistry and Biological Role of Lymphotoxins  
Klostergaard University of Texas System Cancer Center
- R01 CA37385 Structure-Function of Interferon-Gamma and Its Receptors  
Yip New York University
- R01 CA37670 A B-Cell Growth Factor Produced by a Neoplastic B Cell  
Vitetta University of Texas Health Science Center, Dallas
- R23 CA37683 Variants of T-Cell Growth Factor From Malignant Cells  
Gootenberg Georgetown University
- R01 CA37925 The Production and Action of Lymphocyte Interferons  
Kamin Medical Research Institute
- R01 CA37932 Human Lymphokine that Blocks Growth of IA+ Target Cells  
Trucco Wistar Institute of Anatomy and Biology
- R01 CA37943 Cyclooxygenase Products and Mammary Cancer  
Fulton Michigan Cancer Foundation
- R01 CA39048 Production and Purification Mouse Immune Interferon  
Johnson University of Florida
- R01 CA39210 Lymphocyte Allogeneic Inhibition and Tumor Immunology  
Hellstrom Oncogen

TUMOR RELATED ANTIBODIES

- R01 CA15064 Immunochemical Studies on Carcinogenic Mycotoxin  
Chu University of Wisconsin, Madison
- R01 CA28149 Immunotherapy of a B-Cell Leukemia (BCLI)  
Vitetta University of Texas Health Science Center, Dallas
- R01 CA29889 Targeting Antibody-Toxin Conjugates to Leukemia Cells  
Houston University of Kansas, Lawrence
- R01 CA30663 Antibody-Directed Tumor Specific Chimeric Toxins  
Collier University of California, Los Angeles
- R01 CA34079 Specificity of Monoclonal Antibodies to Human Cancer  
Houghton Sloan-Kettering Institute for Cancer Research



- R01 CA28139      Immunobiology of Tumor Metastases  
Feldman                      Weizmann Institute of Science
- R01 CA28332      In Situ Antitumor Immunity and Effects of Radiation  
Lord                              University of Rochester
- R01 CA30196      Immunopathology of X-Linked Lymphoproliferative Syndrome  
Purtilo                              University of Nebraska Medical Center
- R01 CA31837      Mechanisms of Carcinogenesis  
Prenn                              Institute for Medical Research, Santa Clara County
- R01 CA33119      Human Tumor Host Relationships In Vivo  
Warnke                              Stanford University
- R23 CA36109      The Role of the Thymic Epithelium in Leukemogenesis  
Tempelis                              Mount Sinai Medical Center (Milwaukee)
- R01 CA36243      Immunopathologic Studies of Hodgkin's Disease  
Ford                              University of Texas System Cancer Center
- R01 CA37343      Autoimmune Paraneoplastic Syndromes  
Lennon                              Mayo Foundation

EFFECTS OF DISEASE ON IMMUNE FUNCTION

- R01 CA16885      Propagation of Thymus-Derived Lymphocyte Lines  
Ruddle                              Yale University
- R01 CA18234      Immunobiology of Primary Intracranial Tumors  
Roszman                              University of Kentucky
- R01 CA20543      Antigen-Antibody Complexes in Cancer Patients' Sera  
Rossen                              Baylor College of Medicine
- R01 CA24429      Immunosuppressants and Lymphocyte Function  
Winkelstein                              Montefiore Hospital
- R01 CA24873      Immunosuppression in Cancer Patients  
Bankhurst                              University of New Mexico, Albuquerque
- R01 CA26169      Immunosuppression by Avian Acute Leukemia Viruses  
Bose                              University of Texas, Austin
- R01 CA30461      Clonal Analysis of Cellular Immune Response in Melanoma  
Mukherji                              University of Connecticut Health Center
- R01 CA31547      Immunosuppressive Properties of Retrovirus Protein  
Olsen                              Ohio State University
- R01 CA32070      Cell Interactions in Leukemia  
Platsoucas                              Sloan-Kettering Institute for Cancer Research





- R01 CA31787 Irradiation and Marrow Transplantation in Large Animals  
Thomas Fred Hutchinson Cancer Research Center
- R01 CA33084 Mechanisms of Murine Tumor Eradication by Immunotherapy  
Greenberg University of Washington
- R01 CA33387 Passive Immunotherapy of Spontaneous AKR Leukemia  
Weinhold Duke University
- R01 CA33677 Immunologic Parameters of BCG-Induced Tumor Regression  
Kleinschuster Utah State University
- R01 CA34060 Elimination of Neuroblasts from Bone Marrow with AB + C  
Boyle University of Florida
- R01 CA34358 Immunogenicity of a Polyvalent Melanoma Antigen Vaccine  
Bystryn New York University Medical Center
- R01 CA34587 Mechanism of Action of Thiabendazole (TBZ)  
Goldschneider University of Connecticut Health Center
- R01 CA34751 Treatment of Suppressor Cell Activity in Melanoma  
Livingston Sloan-Kettering Institute for Cancer Research
- R01 CA36678 Removal and Analysis of Immune Complexes from Tumor Cats  
Jones Pacific Northwest Research Foundation
- R01 CA36996 Cellular Immunity to Tumors and Herpes Viruses  
Zarling Oncogen
- R01 CA38677 Augmentation of Human Immunity by Cyclophosphamide  
Mastrangelo Thomas Jefferson University

LYMPHOCYTES

- R01 CA04946 Severe Combined Immunodeficiency  
Bosma Institute for Cancer Research
- P01 CA12800 Immune Functions and Cancer  
Fahey University of California, Los Angeles
- R01 CA12844 Controls of Proliferation Specific for Leukemias  
Nakamura State University of New York at Buffalo
- R01 CA13339 The Syngeneic MLR and Host Defense Against Cancer  
Weksler Cornell University Medical Center
- R01 CA14049 Cellular Immunity and Regulatory Factors in Cancer  
Amos Duke University
- R01 CA14216 Characterization of Lymphoid Populations in Cancer  
Cone Yale University

R01 CA14462 Properties of Lymphoid Tumor Cells In Vivo and In Vitro  
Thorbecke New York University

R01 CA15334 Cellular Mechanisms in Tumor-Specific Immunity  
Smith University of Florida

P01 CA15822 Immunobiology of Normal and Neoplastic Lymphocytes  
Wilson University of Pennsylvania

P01 CA16673 Cell Differentiation Studies in Cancer Immunobiology  
Cooper University of Alabama in Birmingham

R01 CA16885 Propagation of Thymus-Derived Lymphocyte Lines  
Ruddle Yale University

R01 CA17733 Lymphocyte Antigens: Structure, Function and Synthesis  
Trowbridge Salk Institute for Biological Studies

R01 CA19170 Mechanisms of T-Cell Mediated Suppression of Tumor Growth  
Bernstein Fred Hutchinson Cancer Research Center

R01 CA19529 Cell-Mediated Immunity in Humans: Mechanisms and Uses  
Valentine New York University

R01 CA20531 Genetic Analysis of Normal and Malignant Lymphocytes  
Yunis Dana-Farber Cancer Institute

R01 CA20819 Phagocytic Cells: Regulation, Dysfunction and Disease  
Van Epps University of New Mexico, Albuquerque

R01 CA20823 Lymphocyte Production and Traffic in the Bone Marrow  
Rosse University of Washington

R01 CA22241 T-Cell Development: Immunogenetics, Defects, Therapy  
Scheid Sloan-Kettering Institute for Cancer Research

R01 CA22677 Pathobiology of Myeloma and Anti-Idiotypic Immunity  
Schreiber University of Chicago

R01 CA22786 Receptor Dynamics and Normal/Tumor Cell Function  
Bankert Roswell Park Memorial Institute

R01 CA23262 Terminal Transferase in Normal and Leukemic Lymphoid Cells  
Bollum U.S. Uniformed Services Univ. of Health Sciences

R01 CA23354 Human Natural Killing: Regulation and Recognition  
Koren Duke University

R01 CA24436 Lymphocyte Receptor Function  
Wofsy University of California, Berkeley

R01 CA24442 Chemical Basis for Receptor Recognition of Lysozymes  
Sercarz University of California, Los Angeles

R01	CA24472	The Development of Thymocytes and Their Progeny
	Basch	New York University
R01	CA24607	Suppressor T Cells of Mixed Leukocyte Reaction in Man
	Engleman	Stanford University
R01	CA25054	Cellular Mechanisms Regulating Antibody Production
	Mullen	University of Missouri, Columbia
R01	CA25253	Immunoregulatory Network Probed by Cell Hybridization
	Bankert	Roswell Park Memorial Institute
R01	CA25369	Human Leukemia Antigens: Isolation and Characterization
	Schlossman	Dana-Farber Cancer Institute
R01	CA25416	Immunogenetics of NK-1+ Natural Killer Cells
	Koo	Sloan-Kettering Institute for Cancer Research
R01	CA25583	Cell-Mediated Immunity in Mammary Tumor Models
	Lopez	University of Miami
R01	CA25612	Immunologic Effects on Tumor Growth and Rejection
	Plate	Rush University
R01	CA25738	T-Cell Differentiation: Molecular Mechanisms
	Scheid	Sloan-Kettering Institute for Cancer Research
R01	CA26284	Regulation of Adenosine Deaminase in Human Cells
	Daddona	University of Michigan at Ann Arbor
R01	CA26297	Primary Structure of MHC I Region Associated Antigens
	McKean	Mayo Foundation
R01	CA26695	Antigen-Specific T Cell Clones: Generation and Analysis
	Cantor	Dana-Farber Cancer Institute
R01	CA28196	Proteinases of Human Natural Killer Cells
	Hudig	University of California, San Diego
R01	CA28533	Mechanisms of Tumor Destruction by Immune Effectors
	Russell	Washington University
R01	CA28708	Immunoregulation of Myeloma Cell Differentiation
	Rohrer	University of South Alabama
P01	CA28900	Control of Antigen-Specific T-Cell Responses
	Eisen	Massachusetts Institute of Technology
R01	CA28936	Immunoregulation in Autoimmunity and Malignant Disease
	Haynes	Duke University
P01	CA29606	Immunoregulation--T Cells and Their Products
	Janeway	Yale University



R01	CA30280 Weisbart	T-Lymphocyte Regulated Tumor Cell Killing by Neutrophils University of California, Los Angeles
R01	CA30972 Bockman	Marrow Prostaglandins and T Cell Differentiation Sloan-Kettering Institute for Cancer Research
R01	CA31534 Tucker	Isotype Switching in a Neoplastic B Cell Model, BCL1 University of Texas Health Science Center, Dallas
R01	CA31536 Spitzer	Investigation of Human Mitogen Induced T-Cell Colonies University of Texas System Cancer Center
R01	CA31564 Yates	Role of Glycolipids in Glioma Resistance to Cytolysis Ohio State University
R01	CA31687 Donnenberg	Mechanisms of Lymphocyte Colony Formation Johns Hopkins University
R01	CA31918 Fanger	Antibody Dependent Cell Cytotoxicity Reaction Mechanism Dartmouth College
R01	CA31982 Ballas	Cytotoxic T Lymphocytes: Mechanisms of Generation University of Iowa
R01	CA32018 Perry	T Subset Interactions in Specific Tumor Immunotherapy Emory University
R01	CA32277 Lynch	FC Receptor-Bearing T Lymphocytes in Murine Myeloma University of Iowa
R01	CA32685 Sondel	The Immunobiology of Human Antileukemic Lymphocytes University of Wisconsin, Madison
R01	CA32739 Levy	Human T Lymphocyte Antigens and Their Genes Stanford University
R23	CA32757 Hamilton	Mechanisms of Minor-H Antigen GVHD University of Washington
R01	CA32801 Thorbecke	Immune Responses to Chemically-Induced Tumors New York University
R01	CA32841 Gooding	Effector Mechanisms in Rejection of SV40-Induced Tumors Emory University
R23	CA32969 Johnson	Functional Studies of Transformed Natural Killer Cells University of Nebraska Medical Center
R01	CA33005 Oeltmann	Molecular Mechanism of Natural Cell-Mediated Cytolysis Vanderbilt University
R01	CA33104 Basch	Somatic Cell Genetic Analysis of T-Cell Differentiation New York University

R01	CA33529	Processing of IA Molecules in B Cells and Macrophages Cullen Washington University
R01	CA33556	Autologous Mixed Lymphocyte Interactions Smith Thomas Jefferson University
R01	CA33589	Mechanism of NK Mediated Cytolysis Forbes Vanderbilt University
R01	CA33939	Lymphoid Cell Treatment of Leukemia Furmanski AMC Cancer Research Center and Hospital
R01	CA34105	Immunoregulation: Idiotype Networks and Clonal Dominance Brown St. Jude Children's Research Hospital
R01	CA34106	Murine T-Cell IA Antigens Hayes University of Wisconsin, Madison
R01	CA34107	Mineral Elements in the Generation of Cytotoxic T Cells Flynn Cleveland Clinic Foundation
R01	CA34109	Helper Cells/Factors from Nonresponders Waltenbaugh Northwestern University
R01	CA34112	Molecular Mechanisms in Cellular Immunology Callewaert Oakland University
R01	CA34127	Antibody Variable Genes: Development and Diversity Gearhart Johns Hopkins University
R01	CA34129	Regulation of Human and Murine Cytolytic T Lymphocytes Burakoff Dana-Farber Cancer Institute
R01	CA34189	Post-Thymic T-Cell Lineage Analysis Miller Boston University
R01	CA34442	In Vitro Induction of NK Cytotoxicity Golub University of California, Los Angeles
R01	CA34546	Studies of Normal and Neoplastic Lymphocytes Fu Oklahoma Medical Research Foundation
R23	CA34670	Role of C-Reactive Protein in the NK Response Baum Univ. of Health Sciences/Chicago Medical School
R01	CA34817	Regulation of Activity in Cloned Anti-Tumor Lymphocytes Russell Washington University
R01	CA34899	In Vitro Analysis of Antibody Regulation in Humans Stevens University of California, Los Angeles
U01	CA34981	Aids--Mechanism of Defective Immunoregulation Hauptman Thomas Jefferson University

R01 CA35457 Natural Killer Cell Heterogeneity and Differentiation  
Tai University of New Mexico, Albuquerque

R23 CA35496 Regulatory Mechanisms in Cell-Mediated Immunity  
Susskind Virginia Commonwealth University

R01 CA35654 Monoclonal T-Lymphocyte Factor Regulation of Myeloma  
Rohrer University of South Alabama

R01 CA35704 Mechanisms of Antigen Processing of Hemoglobin  
Kazim University of New Mexico

R01 CA35730 Requirements for B-Cell IA-Alloantigen Presentation  
Kubo National Jewish Hospital and Research Center

R01 CA35791 Role of NK Cytotoxic Factor NKCF in NK Cytotoxicity  
Bonavida University of California, Los Angeles

R01 CA35793 Natural Kill of HSV-1 Infected Targets: Basic Biology  
Fitzgerald Sloan-Kettering Institute for Cancer Research

R01 CA35978 The Role of Gangliosides in Modulation of Mitogenesis  
Merritt George Washington University

R01 CA36107 Immune Response to Modified Self and Tumor Antigens  
Scott University of Rochester

R01 CA36302 T-Cell Help in B-Cell Activation, Division, and Maturation  
Corley Duke University

R23 CA36403 Isotype Suppressor T Cells With FC Receptors in Myeloma  
Hoover University of Pennsylvania

R01 CA36642 Helper T Cells: Comparison of T-T and T-B Interaction  
Corley Duke University

R01 CA37006 Regulatory Interactions of NK Cells with B Cells  
Pollack University of Washington

R01 CA37252 Myeloma Cell Heterogeneity and Immunoregulation  
Kemp University of Iowa

R01 CA37344 Molecular Analysis--B Lymphocyte Activation  
Raschke La Jolla Cancer Research Foundation

R01 CA37372 Tyrosine Protein Kinases and Lymphocyte Activation  
Geahlen Purdue University, West Lafayette

R01 CA37374 Immunochemical Genetics of Murine Alloantigens  
Michaelson Center for Blood Research

R01 CA37388 Ontogeny, Regulation, and Characterization of NK/K Cells  
Kim Chicago Medical School





R01	CA22090	Antitumor Action of Phagocytes and Lymphocytes Nathan Rockefeller University
R01	CA25052	Immune Response In Vitro H-2 (IR) Locus Function Niederhuber University of Michigan at Ann Arbor
R01	CA26467	Effector and Suppressor Mechanisms in Tumor Immunity Stout Brandeis University
R01	26824	Mononuclear Phagocytes in Human Ovarian Carcinoma Mantovani Mario Negri Institute Pharmacologiche
R01	CA27523	Macrophages and Tumor Growth Evans Jackson Laboratory
R01	CA29266	Characterization of Monocyte Subsets in Blood Weiner University of Florida
P01	CA29589	Macrophage Activation: Development and Regulation Adams Duke University
P01	CA30198	Human Mononuclear Leukocytes in Cancer Silverstein Rockefeller University
R01	CA31199	Macrophage-Mediated Injury Causing Tumor Regression Russell University of Florida
R01	CA31447	Prostaglandin Control of Macrophage Antitumor Activity Zwilling Ohio State University
R01	CA32551	Hemopoietic Stem Cell Differentiation to Macrophages Stanley Yeshiva University
R01	CA32898	Differentiation and Function of Human Monocytes Trinchieri Wistar Institute of Anatomy and Biology
R23	CA33003	Characterization of Human Macrophage Heterogeneity Becker University of North Carolina, Chapel Hill
R01	CA33188	Macrophage Control of Normal and Leukemic Erythropoiesis Furmanski AMC Cancer Research Center and Hospital
R01	CA33225	Regulation of Myeloid Progenitor Cell Differentiation Pelus Sloan-Kettering Institute for Cancer Research
R01	CA33629	Differentiation and Anti-Tumor Activity of Macrophages Kaplan University of Kentucky
R01	CA34071	Macrophage Procoagulants Shands University of Florida
R01	CA35893	A Mechanism by Which Macrophages Injure Cancer Cells Granger Duke University

- R01 CA35961      Macrophage Resistance Versus Viruses and Tumors  
Morahan                      Medical College of Pennsylvania
- R23 CA36643      Role of Complement in the Immunopathology of Macrophages  
Newman                      University of North Carolina, Chapel Hill
- R23 CA36646      Study of Fluid Pinocytosis in Stimulated Neutrophils  
Davis                      Upstate Medical Center
- R01 CA36722      Calcium Transport in Activated Macrophages  
Gorecka-Tisera              University of Pittsburgh
- R01 CA38354      Development and Function of Pulmonary Macrophages  
Kim                      Columbia University
- R01 CA38408      Effector and Suppressor Mechanisms in Tumor Immunity  
Stout                      East Tennessee State University
- R01 CA39070      Regulation of IA Gene Expression  
Woodward                      University of Kentucky
- R01 CA39205      Mechanism of Human Monocyte Maturation  
Musson                      Children's Hospital, Philadelphia

MALIGNANCIES OF THE IMMUNE SYSTEM (LYMPHOMA/LEUKEMIA)

- R01 CA08975      Human Leukemia Associated Antigens  
Metzgar                      Duke University
- R01 CA15472      Immunity and Myeloma Tumors  
Eisen                      Massachusetts Institute of Technology
- R01 CA20499      Immunobiology of Cutaneous T-Cell Lymphomas  
Edelson                      Columbia University
- R01 CA25097      Differentiation of Immune System: Cell Surface Antigens  
Kersey                      University of Minnesota of Minneapolis-St. Paul
- R01 CA26479      Immune Functions of Tumor Cell Variants  
Fuji                      Roswell Park Memorial Institute
- R01 CA31479      Proliferation and Differentiation in Human Lymphoma  
Ford                      University of Texas System Cancer Center
- R01 CA31685      Differentiative Programs of Lymphoid Progenitor Cells  
Lebien                      University of Minnesota of Minneapolis-St. Paul
- R01 CA31789      Genetic-Viral-Immunologic Studies  
Datta                      Tufts University
- R23 CA31888      Monoclonal Antibodies Reactive with Human Leukemia Cells  
Ball                      Dartmouth College

- R01 CA32563 Pathogenesis of Preleukemic Aplastic Anemia  
Hoover Colorado State University
- R01 CA32577 Studies on Tumor Dormancy and Emergence  
Wheelock Hahnemann University
- R23 CA32800 Monocytes and the Immunodeficiency of Hodgkin's Disease  
Zamkoff Upstate Medical Center
- R01 CA32826 Glycosphingolipids in Oncogenesis and Differentiation  
Macher University of California, San Francisco
- R23 CA33127 Regulation of Erythropoiesis in B-Lymphocyte Neoplasms  
Mangan Montefiore Hospital
- R01 CA34052 T-Cell Interactions with Cloned IA+ Accessory Cells  
Kaplan University of Kentucky
- R23 CA34313 Antigen Expression on Fetal and Malignant Leukocytes  
Hofman University of Southern California
- R01 CA34549 Role of Natural Cytotoxic Cells in Experimental Lymphoma  
Ponzio University of Medicine and Dentistry of New Jersey
- R01 CA34654 Immune Response and Progressive Tumor Growth  
Manson Wistar Institute of Anatomy and Biology
- R23 CA35463 Monoclonal Antibodies Specific for Hairy Cell Leukemia  
Posnett Rockefeller University
- R01 CA36040 Defects of AML Leukemia In Replication and Maturation  
Chiao New York Medical College
- R01 CA36776 Lymphoblastoid Receptors for Epstein-Barr Virus  
Fowler University of South Alabama
- R01 CA37097 Nuclear Antigen Markers in Human Blood Cells  
Briggs Vanderbilt University
- R01 CA38325 White Blood Cell Oxidase in Leukemia and Normal Cells  
Newburger University of Massachusetts Medical School

#### IMMUNE SURVEILLANCE

- R01 CA03367 Natural Resistance to Lymphoma and Marrow Transplantation  
Trentin Baylor College of Medicine
- R01 CA15988 Immune Surveillance and Cancer  
Stutman Sloan-Kettering Institute for Cancer Research
- R01 CA19754 Immunoselection and Cancer: A Problem in Evolution  
Cohn Salk Institute for Biological Studies

RO1 CA20408	Immunodeficiency and Tumorigenesis
Shultz	Jackson Laboratory
RO1 CA20816	Pathogenesis of Autoimmunity
Gershwin	University of California, Davis
RO1 CA20833	Cell-Mediated Cytotoxicity in Humans
Trinchieri	Wistar Institute of Anatomy and Biology
RO1 CA22517	Monocyte Function in Neoplasia
Normann	University of Florida
RO1 CA23809	Natural and Tissue-Specific Immunity to Human Neoplasms
Saksela	University of Helsinki
RO1 CA25250	Natural Killer Cells Target Sites, Genetic Control, and Role
Klein	Caroline Institute
RO1 CA25641	Effect of Cancer on Human Monocyte Cytotoxic Mechanisms
Huard	University of Michigan at Ann Arbor
RO1 CA25917	Cellular and Genetic Aspects of Antitumor Immunity
Daynes	University of Utah
RO1 CA26344	Autologous Lymphocyte Reactions and Immune Surveillance
Weksler	Cornell University Medical Center
RO1 CA26782	Regulation by Natural Killer Cells
Kiessling	Caroline Institute
RO1 CA28231	H-2 Associated Natural Resistance
Carlson	Jackson Laboratory
RO1 CA28834	Basophil/Mast Cell Function in the Control of Cancer
Dvorak	Beth Israel Hospital
RO1 CA29355	T-Cell Nonresponsiveness in Gross Virus-Infected Mice
Blank	University of Pennsylvania
RO1 CA32553	Specific Anti-Tumor Activity by Armed Lymphoid Cells
Pollack	University of Washington
RO1 CA34199	Genetics and Regulation of Cell Mediated Cytotoxicity
Clark	Genetic Systems Corporation
R23 CA34302	Regulation of UV-Tumor Immunity by Cloned TS-Cell Lines
Roberts	University of Utah
RO1 CA34461	Regulation of Natural Killer Cells
Welsh	University of Massachusetts Medical School
RO1 CA34529	Oxidative Regulation of Human Natural Killer Cells
Seaman	University of California, San Francisco



- R01 CA34674 Study of Acquired Immunodeficiency and Kaposi's Sarcoma  
Hersh University of Texas System Cancer Center
- R01 CA35979 T-Lymphocyte Specific Genes  
Storb University of Washington
- R01 CA36033 Regulation of Human Natural Killer Lymphocyte Activity  
Kay University of Nebraska Medical Center
- R01 CA36860 Cellular Immunity to Endogenous AKR Leukemia Viruses  
Green Dartmouth
- R01 CA36921 Immunogenetics of Hybrid Resistance  
Bennett University of Texas Health Science Center, Dallas
- R01 CA36922 Immunobiology of Hybrid Resistance  
Bennett University of Texas Health Science Center, Dallas
- R01 CA37205 Mechanism of the NK Lethal Hit in Programmed Tumor Cells  
Targan University of California, Los Angeles

#### IMMUNOTHERAPY IN ANIMAL MODELS

- R01 CA11898 Brain Tumors: Immunological and Biological Studies  
Bigner Duke University
- R01 CA16642 Immunological Basis of Tumor Regression  
North Trudeau Institute
- R01 CA17818 Tumor Immunity and Tumor-Host Interactions  
Stutman Sloan-Kettering Institute for Cancer Research
- R01 CA27794 Mechanisms of Endotoxin-Induced Tumor Regression  
North Trudeau Institute
- R01 CA29992 Prevention of Oncogenesis Via Marrow Transplantation  
Pierpaoli Foundation for Basic Biomedical Research
- R01 CA30303 Selective Stimulation of Cell Mediated Cancer Immunity  
Hunter Emory University
- R01 CA31859 Immunotherapy of Cancer with TCGF-Grown Cytotoxic Cells  
Kedar Hebrew University of Jerusalem
- R23 CA32109 Adsorbed Leukemic Sera Depress Cultured Blast Viability  
Miller University of Minnesota of Minneapolis-St. Paul
- R01 CA35299 T Cells and Their Lymphokines in Cancer Immunotherapy  
Altman Scripps Clinic and Research Foundation
- R01 CA37389 Monoclonal Antibody Analysis and Therapy of B16 Melanoma  
Herd Georgia State University

R01 CA38415 Evaluation of Human Melanoma Antigen as a Vaccine  
Brown Oncogen

BONE MARROW TRANSPLANTATION

R01 CA20044 Transplantation Immunology  
Winn Massachusetts General Hospital

R01 CA28701 Chronic Graft-Versus-Host Disease in Radiation Chimeras  
Beschorner Johns Hopkins University

R01 CA29592 Active Specific Immunotherapy in Man: A Murine Model  
Kahan University of Texas Health Science Center, Houston

R01 CA33794 Minor Alloantigens in Clinical Graft-Versus-Host Reaction  
Elkins Children's Hospital of Philadelphia

P01 CA35048 Bone Marrow Transplantation in Leukemia  
Beutler Scripps Clinic and Research Foundation

R01 CA36725 Immunotoxins in Human Bone Marrow Transplantation  
Vallera University of Minnesota of Minneapolis, St. Paul

R01 CA37706 NK Cells and Bone Marrow Rejection  
Dennert University of Southern California, Los Angeles

R01 CA38355 Lymphocyte Function in Normal and Chimeric Mice  
Sprent Scripps Clinic and Research Foundation

## CONTRACT RESEARCH SUMMARY

Title: Resource Bank and Distribution Center for Cell Lines Useful in Research in Tumor Immunology

Principal Investigator: Dr. Anita C. Weinblatt  
Performing Organization: American Type Culture Collection  
City and State: Rockville, MD

Contract Number: NOI-CB-15533  
Starting Date: 9/28/81  
Expiration Date: 9/27/86

Goal: To provide an efficient system for the acquisition, cataloging, storage and maintenance of cell lines which are capable of long term growth in vitro and are useful in tumor immunology research. To offer to recipients expert advice on culture and characteristics of all lines shipped.

Approach: The cell lines in the bank are listed in a catalog, which is updated annually. New acquisitions are also announced in quarterly newsletters. The cell lines in the bank include, but are not limited to: B and T cell lines; lines useful in the study of macrophage/monocyte development; myelomas and their variants; cell lines useful in the study of immune effector mechanisms; and hybridomas. Lines are shipped for a fee upon request. These lines are screened for contamination with bacteria, fungi and mycoplasma; detailed characterizations are performed. Relevant lines are actively sought and persons wishing to donate lines are encouraged to contact the principal investigator or the project officer.

Progress: Cell line shipments are averaging one hundred fifty per month. Some of the interesting hybridomas in the bank have the following specificities: Thy-1.1; Thy 1.2; various antigens on leukocytes, red blood cells, macrophages; brain clathrin; T- and B-lymphocyte antigens including alleles of Lyt-1 and Lyt-2; immunoglobulin fragments; I-A determinants; and sheep red blood cells. Other interesting lines are: HUT 78 (TIB-161), a human cutaneous T cell lymphoma with properties of a mature T cell line; and EL4.IL-2 (TIB-181), a high IL-2 (T-cell growth factor) producer. Another IL-2 producer, HUT 102 (TIB-162), also releases a unique type C retrovirus associated with T-cell lymphomas. YAC-1 (TIB-160) is a lymphoma line, which is often used as a target in NK assays. P388D<sub>1</sub> (TIB-63) secretes IL-1 and is a monocyte/macrophage line that is very popular. Two other high-demand lines are rat-mouse hybridomas M1/42.3.9.8 (TIB-126) and M1/70.15.11/2 (TIB-128). Some exciting new lines have been acquired recently, some of which are in the final stages of workup before dissemination, while others are being distributed. These include, for example, CEM-CM3 (TIB-195), a human acute lymphoblastic leukemia line, which is used as a T cell fusion line. Reactivities of some new hybridomas include: cytotoxicity for immature T cells (TIB-183), human NK and K cells (TIB-200), and B cells and precursors (TIB-164).

Project Officer: Judith M. Whalen  
Program: Immunology Section  
FY 84 Funds: \$ 118,208

## CANCER DIAGNOSIS RESEARCH PROGRAM

### Description and Introduction

The Cancer Diagnosis Research Program supports research directed toward developing the ability to identify populations at high risk, to detect cancer at earlier stages, to make more accurate diagnoses, to stage tumors more precisely for prognostic and therapeutic decisions and to monitor more effectively the changes during and following therapy. Identification of populations at high risk should result in more targeted screening programs; the development of better tests for detection and diagnosis should result in earlier detection and identification of tumors, before they become seriously invasive and/or metastatic. Improvements in detection and diagnosis are critical to the physician in choosing the most effective therapeutic approaches.

The emphasis in diagnosis related research is on the transfer of conceptual and technological developments from basic research to the clinic. Some of the questions addressed include: How can knowledge of the process of invasion be applied to staging of tumors? Can altered metabolic products be used as markers? Can more sensitive detection methods be developed using such technologies as monoclonal antibodies and recombinant DNA? How can advances in engineering technology be translated into more effective diagnostic instrumentation?

Although individual research projects usually concentrate on a particular type of tumor or organ site, the techniques they develop are often generally applicable. Because of this, the Program has been organized into five broad disciplinary categories: Biochemistry, Immunology, Cytology, Pathology and Genetics. Biochemical diagnosis includes studies of hormones, enzymes, other proteins and metabolic products which are found in the circulation, in other biological fluids or associated with cells and which can be used to characterize tumors. Studies to develop immunological techniques for identification and characterization of tumor cells and for quantitating and characterizing the host immune response are included in Immunology. Cytology research stresses the development and improvement of automated techniques for cytological evaluation. Pathology research emphasizes studies to improve methods of tumor classification, including staging and correlations with prognosis. Genetic research includes applications of the newest advances in cytogenetics and molecular genetics as they show potential for cancer diagnosis. The inclusion of genetics research as an emphasis area is a new addition to the Diagnosis Program. Classifying studies under Multidisciplinary Approaches, as was the practice in the past, no longer suits the needs of the program as research approaches have become more defined. A budget summary of the Program by category is provided in the adjoining table.

A review of the Diagnosis Program's portfolio indicates that there are currently three areas that are particularly exciting and important to diagnosis research: new strategies for application of monoclonal antibodies, exploitation of genetic techniques and further development of sophisticated analytical instrumentation. The following report describes the current state of research and the potential impact of future developments in these three areas.



## Monoclonal Antibodies and Cancer Diagnosis

The introduction of monoclonal antibody technology in 1975 has revolutionized human tumor serological studies and opened up a wide spectrum of new potential applications in all areas of biomedical sciences (Kohler et al., 1975). The production and use of monoclonal antibodies is rapidly expanding; laboratories throughout the world are exploiting this new biotechnology (Research Editor, 1981). It is being used to elucidate basic events in the cell biology of neoplasia, to resolve the questions of tumor antigen specificity in human cancers, and to clarify the nature of host immune responses. It has excited a renewed interest in the immunological approach to cancer diagnosis, prognosis and monitoring of therapy.

The foremost application of monoclonal antibodies (MAbs) to cancer diagnosis is in the continuing search for the elusive tumor-specific marker. Earlier methods relied on the use of absorbed heteroantisera, obtained by immunizing animals with whole cells or cell fractions of human tumors. Inevitably, most or all of the antigens detected on the cell membranes of malignant neoplasms could also be detected on a small number of normal cells, either from embryonic tissue or from tissues at specific stages of postnatal differentiation. It is now generally agreed, based on a substantial body of data that few, if any, tumor-associated antigens are truly tumor specific (Old et al., 1981). Hybridoma technology provides uniquely specific monoclonal antibodies to probe tumor cells. It has spurred a renewed effort to generate monospecific antibodies that may be able to detect new tumor associated antigens. Such research has already resulted in the identification and quantitation of a remarkable number of antigens. A few of the more promising antigens will be discussed here. Some excellent reviews are available in this rapidly evolving field (Lloyd, 1983, McMichael et al., 1982).

Bast and his co-workers have recently developed a monoclonal immunoglobulin (OC125) by immunization of mice with a human ovarian cancer cell line. The antigenic determinant, CA125, is common to most nonmucinous epithelial ovarian carcinomas. An immunoradiometric assay has been developed to detect CA125 in sera of patients with epithelial ovarian carcinomas. In a recent study 82 percent of patients with surgically demonstrable ovarian carcinoma showed elevated levels of the antigen (Bast et al., 1983). A significant correlation has been found between the antigen and the state of the disease; antigen decreases during regression and increases as the disease progresses.

Antibodies that react with human colon carcinoma and not with normal colonic mucosa have been reported (Sears et al., 1982). Two monoclonals, 1116-52a and 19-9, identified a monosialoganglioside that is not related to carcinoembryonic antigen (CEA), the most extensively used marker in the diagnosis of colon cancer, but which is present in a significant number of cancer patients with no detectable serum CEA. Levels in sera taken preoperatively and postoperatively from 85 patients with resected colorectal cancer suggest that these monoclonal antibodies detect a new antigen which may prove to be a valuable prognostic aid.

The melanomas are the human tumors that have been most intensively studied using mouse monoclonals. Since the initial study of Koprowski et al., 1978, over

60 different monoclonal antibodies which react with melanoma have been developed. Although no antigen has been defined that is exclusive to melanoma cells several have restricted specificities which warrant further investigation (Lloyd et al., 1983).

A number of studies have used the hybridoma approach to generate antibodies with specificity for the major histologic types of lung cancer: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (squamous, adenocarcinoma and large cell). Major treatment decisions are based on making an accurate distinction between these two forms of cancer. Two murine monoclonal antibodies (703D4, 704A1) have been produced which bind to most (11/13) human non-SCLC but not to the SCLC (0/11) tested. The epitopes recognized by these antibodies were not expressed on a panel of normal adult human tissues (Mulshine et al., 1983).

Seon and his group (CA19304) have developed an anti-human leukemia-lymphoma (HLL) antibody, SN-1, which reacts only with leukemia T-cell lines derived from patients with T-cell type acute lymphoblastic leukemia (T-ALL), but does not react with specimens derived from 41 patients with other types of cancer (Seon et al., 1983). They have also generated monoclonal antibodies, termed SN2, SN2a, and SN2b, which define a human T-cell leukemia-associated cell surface glycoprotein, GP37.

Starling (CA27623) has successfully derived two mouse monoclonals against antigens expressed on prostate adenocarcinoma cell lines using the immunoperoxidase technique on formalin-fixed paraffin-embedded human tissues. Mab D83.21 was reactive to 11/19 (58%) primary and 1/6 (17%) metastatic prostate adenocarcinomas. It also bound to 2/4 bladder tumors but did not bind to 9 benign prostatic hyperplastic (BPH) or 30 normal tissues. Biochemical characterization is now in progress. Mab P6.2 reacted with 14/19 (68%) primary and 4/6 (67%) metastatic prostate tumors but not with normal or BPH tissues. However, unlike D83.21, antibody P6.2 reacted with 6/15 of the non-prostate tumor tissues tested, suggesting that the P6.2 antigen has a wider tissue distribution. Both antibodies appear to be distinct from other monoclonal anti-prostate antibodies recently reported. Based on the results of these studies, D83.21 and P6.2 appear to be detecting two new membrane antigens present on prostate tumors. Studies are in progress to use D83.21 and P6.2 in a panel with antibodies to other prostate antigens to determine if a combination of markers would be useful in prognosis and in immunohistopathological classification of prostate tumors (Wright et al., 1983).

Several other interesting approaches are being pursued in a search for tumor-associated antigens. Ceriani and colleagues (CA33871) have developed monoclonal antibodies to normal milk fat globule membranes. Some of these antibodies detected antigens (HME-Ags) present on the epithelia of resting as well as lactating breast; others detected antigens preferentially expressed on lactating breast. These monoclonal antibodies reacted with a high proportion of breast carcinomas. The investigators plan to conduct clinical studies to further substantiate the value of circulating HME-Ags as a valuable tool in the diagnosis and follow-up of breast cancer patients (Ceriani et al., 1983). Sell (CA34635) is generating MAbs to epitopes on premalignant cell populations isolated from rat livers early after exposure to chemical carcinogens. North (CA19613) has developed monoclonal antibodies to neurohypophysial principles

(vasopressin, oxytocin, vasotocin and their associated neurophysins) and used them to develop radioimmunoassays to detect tumor cells bearing these antigens for the diagnosis of small cell lung carcinomas. Plasma vasopressin and oxytocin have been found to be elevated ( $> 3$  times) in two-thirds of patients with small cell carcinoma of the lung (North et al., 1983).

The use of these reagents has already provided much insight into tumor cell characteristics and tumor cell biology. However, careful examination of the studies to date reveals that few of the antigens detected thus far are proving to be specific to human tumor cells. Most can be characterized as differentiation antigens. Nevertheless one cannot conclude that some tumor restricted antigens will not eventually be found. Many promising systems are still under investigation and new antigens are being continuously discovered. Future applications to diagnosis may depend on how imaginatively these reagents are used alone or in combination to reveal characteristics of tumor cells which distinguish them from their normal counterparts (Lloyd et al., 1983).

The recent development of monoclonal antibodies reactive with human lymphocyte cell surface antigens has opened up another promising avenue for clinical application. It has provided a powerful tool for the diagnosis of lymphoid neoplasms and for studies of immunoregulatory processes both in normal and diseased individuals. It is now well substantiated that lymphocytes which appear homogeneous morphologically can be separated into phenotypic and functional subpopulations based on the expression of surface membrane antigens (Kung et al., 1979). In the past, analyses of T-cell surface antigens were made using spontaneous auto-antibodies, heteroantisera or functional assays of T-cells (e.g. heat-labile rosette formation or binding of Fc portions of immunoglobulins). B-lymphocytes were identified by their expression of intrinsic surface membrane immunoglobulins, Ia antigens, receptors for the third component of complement and the Fc portion of IgG, and by the ability to form rosettes with mouse erythrocytes. These techniques have been hampered by difficulties involved in performing the assays and by limited availability and specificity of standardized antibody reagents. Recently, attention has shifted to the use of monoclonal antibodies for detailed immunological assessment of lymphocytes. They have been used to classify differentiation and maturational stages of T-cells, B-cells, null cells and monocytes and to distinguish functional subsets of these cells and to assess their distribution in blood (Sallan et al., 1980; Goldstein et al., 1982). Further refinements have resulted in descriptions of malignant cells as characteristic of pre-B cells, mature B-cells or plasma cells (Greaves et al., 1979). A large number of murine monoclonal antibodies that are reactive with antigens associated with human leukemias are already available. They include antibodies to myeloid leukemias, acute lymphocytic leukemia, and chronic lymphocytic leukemia (Foon et al., 1982). Ball and Fanger, 1983, used a variety of cell surface antigens to subclassify lymphocytic and myeloid leukemias. They described the phenotype of cells from 50 patients with leukemias based on the results of testing for binding to four monoclonal antibodies reactive with myeloidcell-associated antigens and three monoclonals reactive with lymphocyte-associated antigens. Using a large number of criteria including reactivity of the monoclonals, antigen density of positive cells and morphologic characteristics, they have related these parameters to diagnoses based on morphology and histochemistry. Their aim is to identify that subgroup of patients with acute myeloid leukemia who achieve long-term survival. Aisenberg (CA30020) analyzed human lymphoma and lymphocytic



leukemia with hybridoma antibodies defining T-cell subsets in order to develop a better classification of human lymphomas based on the functional characteristics of the malignant cell. He has established that monoclonal antibodies can provide more certain diagnosis and superior resolution of cell lineage in these disorders than is possible by morphology alone (Aisenberg et al., 1983).

A group of monoclonal antibodies termed the OK series which react with blood elements permit the analysis of lymphocytes and monocytes into functional subsets and allow classification of their state of maturation and activation. Over 400 papers have been published using these reagents since they appeared in 1979. Although the majority of the studies are concerned primarily with the biology of human T-cell immunoregulation, more recent papers have increasingly concentrated on investigations of imbalances in circulating immunoregulatory T-cell subsets in disease states. Cells expressing these antigens are identified and the antigen levels are compared to those of normal cells (Burton et al., 1983). The effect of tumor size, node involvement and overall extent of disease on the circulating T-cell profile in patients with squamous cancer of the lung was assessed by Ginns using the OKT3, OKT4 and OKT8 series of monoclonals. The investigators report that monitoring of the T-cell profile in these patients may be useful in clinical staging and in assessing disease activity. Their data support the concept that progressive alterations in cell-mediated immunity accompany disease spread (Ginns et al., 1983).

Measurement of the T-cell profile may be a useful tool for designing and monitoring therapy. Although these studies remain largely exploratory, they may prove quite useful as screening techniques and have already provided new insights into host immunological responses to various diseases. They should also result in more precise classification of malignancies of T or B-cell lineage and provide an improved basis for treatment selection and prognostication.

Another potential diagnostic use of monoclonal antibodies is in the field of cancer radioimmunodetection. Despite almost three decades of research in this area, the use of radiolabeled antibodies for in vivo tumor detection and imaging by scintigraphy is still in its infancy (Goldenberg et al., 1982). However, the work in humans is now rapidly progressing and may be considered as well established (Hine et al., 1980). In most of these studies, hyperimmune sera have been prepared by immunizing goats or rabbits with the tumor marker of interest. Because antibodies produced in this way are a mixture of immunoglobulins, the tumor-specific antibodies must be purified. The tumor markers that have been studied thus far include carcinoembryonic antigen (CEA) and alphafetoprotein (AFP), colon-specific antigen p (CSAp), prostatic acid phosphatase (PAP), human chorionic gonadotropin (HCG), and ferritin. Greater accretion of the radio-labelled antibody to the marker in the tumor than to the adjacent tissue permits the tumor to be visualized by scintigraphy. Some of the parameters being investigated to improve this technology include: choice of target marker, preparation of a more specific antibody to the marker, choice of appropriate radiolabel ( $^{131}\text{I}$ ,  $^{123}\text{I}$ ,  $^{111}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), the dose and time between administration and imaging, the use of whole antibody vs. antibody fragments, tumor size, and choice of an effective imaging/processing system. In clinical studies by Goldenberg and colleagues using heterologous  $^{131}\text{I}$  labeled goat antisera raised to CEA, to CSAP, to AFP/hCG and to PAP, radioimmunodetection techniques were successfully applied to over 550 patients. In the CEA study (CA37408) it was found that the method



has a high sensitivity for detecting CEA in the major cancer types known to express CEA, such as colorectal, ovarian, lung and cervical carcinomas. The largest series was colorectal cancer showing a 91% overall true positive rate of cancer detection and localization for both primary and metastatic sites, with a false positive rate of less than 4%. In 11 of 51 patients, occult tumors were detected and these were confirmed by other methods. It was found that radioimmunodetection was not reliable in revealing CEA-expressing tumors of less than 2cm and that it failed to demonstrate lesions devoid of CEA. It is interesting that the presence of very high levels of circulating CEA did not appear to prevent successful imaging with anti-CEA antibody. No adverse reactions of immune hypersensitivity were seen in any of the subjects, including those patients who received multiple injections of the radionuclide (Goldenberg et al., 1983). Radioimmunodetection studies with antibodies to AFP and hCG (CA37407) have achieved quite similar results to those with anti-CEA. But, because neither antibody preparation was affinity purified, they had a lower percentage of immune reactivity (Goldenberg et al., 1980). Polyclonal antibodies to hCG (CA37407) have been used to localize germ cell and trophoblastic neoplasms. The technique revealed 10 proven testicular tumors in a series of 13 patients, while providing no evidence of tumor in 3 patients free of disease (Goldenberg et al., 1980). Studies were also conducted using CSAP and PAP. CSAP, which is elevated in gastrointestinal lesions, (CA37409) also can be used with CEA to complement the localization of colorectal neoplasms, emphasizing the fact that a combination of antibodies directed at different determinants on the tumor cells may achieve better localization by accretion to multiple epitopes (Pant et al., 1982). The results with PAP show that PAP radioimmunodetection can disclose both primary and metastatic prostate carcinomas (Goldenberg et al., 1983). Monoclonal antibodies are now being extensively applied to this imaging technique (Solter et al., 1982). They are being evaluated to determine their localization efficacy and suitability in comparison to polyclonal antibodies. The high specificity of monoclonals may prove to be a disadvantage since this would limit the number of radioactive antibodies bound by the tumor. The solution may be to use a mixture of suitable antibodies, each directed against a different tumor antigen epitope.

Studies pursued by our grantees using monoclonal antibodies for imaging are of interest: Nelp and his group (CA29639) are evaluating the use of monoclonal antibodies labelled with  $^{123}\text{I}$  to the human melanoma-associated antigen, p97. About 85% of lesions are detectable in melanoma patients with significant levels of p97 in their tumors (Larson et al., 1983). Buchsbaum (CA36553) is using BA-1 and BA-3 monoclonal antibodies labeled with  $^{131}\text{I}$  for in vivo tumor localization of non-Hodgkin's lymphomas (Buchsbaum et al., 1983). Cheng (CA36903) is evaluating the usefulness of oncofetal protein (OFP) for detecting pancreatic tumor cells in rats at an early stage of cancer. He will produce monoclonal antibodies to OFP, develop a radioimmunoassay and carry out an in vivo localization of cancer cells by scintigraphic imaging.

Since the use of monoclonal antibodies in radioimmunodetection has only recently begun, it is still too early to conclude that they will have any advantage over highly purified conventional antibodies. Concurrent research on the different types of antibodies used (e.g. polyclonal, monoclonal), the choice of radionuclides and improvements in instrumentation should lead to better resolution and to rapid advances in this currently very active field. Prospective clinical trials could then be undertaken to assess the value of this modality as standard

procedure in cancer detection and diagnosis.

This discussion thus far has presumed the use of murine monoclonal antibodies. However, the use of these reagents has been shown to induce human antimouse antibodies upon repeated injections since these antibodies are seen as foreign proteins by the human immune system (Miller et al., 1981). Thus, for *in vivo* diagnostic and therapeutic applications in man the production of human rather than mouse or other rodent antibodies would clearly be desirable. Much effort has already been applied to this undertaking but with limited success (Kozbor et al., 1983). An announcement "Development of Myeloma or Human B-Cell Lines Suitable for Somatic Cell Hybridization to Produce Human Monoclonal Antibodies" was issued by the Diagnosis Program in early 1984 to stimulate further research in this area. Four grantees, Teng (CA36422), Heitzmann (CA36310), Bernier (CA33425), and Volsky (CA36320) are now engaged in this endeavor.

It is clear that we are now seeing only the beginnings of the impact of monoclonal antibodies on diagnosis. But the findings to date are full of promise. As investigations are vigorously pursued in cell biology, human genetics and tumor immunology, we can expect a continued high rate of transfer of this knowledge to clinical medicine.

#### Automated Systems for Cancer Diagnosis

Pathology has provided the standard for cancer diagnosis against which all other methods must be evaluated. Despite its success, histopathologic evaluation methods are largely subjective and based on interpreting a complex combination of specific determinates and patterns. The development of fully automated systems for diagnosis holds the promise of providing rapid, objective and quantitative evaluation of cell and tissue samples with techniques that transcend the limitations of microscopy. The progress to date toward this goal will be summarized in the following discussion.

Serious attempts to automate cytology have been under way for more than two decades. The most fruitful approach has been in flow cytometry, in which specific properties of cells are measured as a stream of liquid carries cells one at a time past one or more sensors. An alternative approach, and one which is in many ways complementary to flow cytometry, is automatic image analysis. Image analysis systems extract quantitative data from digital images, and in the broadest sense, involve pattern recognition and specific feature analysis including analysis of relationships which may have little similarity to those perceived by eye. The applications of image analysis are as broad as the range of human vision, from the analysis of astronomical images to the analysis of microscopic images. This report will focus on flow cytometry and on two applications of image analysis, automated cytology and two-dimensional electrophoresis.

Flow cytometry provides a method for rapidly obtaining information on a wide variety of cell characteristics. In a typical system, cells are suspended in a liquid that carries them individually past one or more sensors. Typical sensors include those for changes in electrical resistance (Coulter Sensors) which measure cell size and number, as well as optical sensors which utilize conventional or laser light sources with measurement of absorption, scattering or

fluorescence. The optical sensors allow measurements of a number of morphological, biochemical, immunological, physical and functional cell features in individual cells at rates of 1000 cells per second or greater. Systems with two or more sensors can measure multiple parameters simultaneously (Wheless, CA33148). Some systems physically separate and collect cells with particular characteristics. The high sampling rate and multiple parameter capabilities of recent flow cytometers require computer assisted data acquisition, processing and storage in order to optimize their potential. Some applications of computers to data acquisition, display, storage and processing have been described (Voet et al., 1982).

The identification of specific cellular characteristics by flow cytometry relies to a large extent on pre-treatment of the cells with stains or other markers. Markers for DNA, RNA, cell surface antigens, hormone receptors, and cell phase have been reported. Advances in immunology and cell biology have greatly increased the number of potential markers and the range of characteristics which can be measured. Rapid progress should be expected as advances in these areas continue.

A large body of data exists relating aneuploidy to a variety of human malignancies. Measurements with high resolution flow cytometers show aneuploidy associated with more than 90% of all human solid tumors and associations with histologic type, tumor activity and patient survival in some neoplasms (Braylan, 1983). Barlogie et al. (CA28771) have demonstrated shorter patient survival associated with hypertriploid abnormality. They have also presented evidence that leukemic cells contain higher levels of double stranded RNA than normal hemopoetic cells. Darzynkiewicz (CA28704) has applied fluorescent probes to measure DNA and RNA simultaneously and to study cell kinetics in cells containing 5-bromodeoxyuridine.

The analysis of cell surface antigens and receptors has been widely applied in basic immunology and genetics. The use of hybridoma techniques allows measurement of the expression of human-specific surface antigens which can act as markers for specific genes (Kamarch et al., 1983). This technique combined with flow cytometry allows the monitoring and manipulation of specific genes in hybrid mouse cells and may eventually have application to studies of specific cell surface antigens associated with particular neoplasms. Monoclonal antibodies to specific cell surface antigens have been measured using flow cytometry in order to monitor use of these antibodies in therapy and to detect circulating tumor cells.

Hormone receptors are associated with a number of human tumors and provide another type of marker useful in diagnostic flow cytometry. Fluorescently labeled estradiol appears to be a promising tool for studies of estrogen receptor associated with breast cancer (Barlogie et al., 1983, CA28771).

A method for measuring intracellular antigens has been reported recently (Schroff et al., 1984). If this technique proves broadly applicable, it will open a whole new area of potential diagnostic applications of flow cytometry.

Dynamic studies of the uptake of stains by cells have been described (Beumer et al., 1983). These techniques may have applications to isoenzyme measurements and other systems of potential clinical or diagnostic importance.



Flow cytometry has also been used to measure grade and to determine prognosis of renal adenocarcinomas and adenomas in cells from paraffin-embedded thin sections (Bennington and Mayall, 1983). This study and earlier studies by others indicate that flow cytometric measurements of embedded tissues are feasible and produce reliable, quantitative information.

High resolution automated image analysis systems have applications in cytometry histopathology, microscopy, two dimensional electrophoresis and many other areas. Two areas in which considerable progress has been made are automated cytology and automated analysis of two dimensional electrophoresis patterns. The application of new high speed, high data storage capacity and multiprocessor computer systems and advances in on-line data storage and off-line data storage capabilities, including high density random access memory, packed format magnetic storage devices and laser encoded digital disk technology, should eliminate limited data storage as one of the major obstacles to the development of high resolution image analysis systems. Research in artificial intelligence and the development of new innovative approaches that do not emulate human analytical patterns should also facilitate advances in this area.

The application of automated image analysis in histopathology and cytology has advanced greatly during the past five years. A number of systems are being developed to allow images to be acquired, digitized (converted into a series of numbers representing the characteristics of the image) and analyzed using computers. The degree of automation of these systems varies considerably, but several cases of nearly total automation of specific applications have been reported (Wied, CA13271; Castleman, CA31718; Bacus CA36657). The major advantages of fully automated, high resolution cytology or histopathology systems are that they should provide accurate, consistent, and objective results and analysis of stored data using mathematical and statistical techniques which provide more information than would be directly available to a human observer. Cell image analysis involves the following steps: 1) sample preparation, 2) image acquisition, 3) image processing and 4) data analysis.

Careful sample preparation simplifies the analysis by decreasing the number of confounding elements on the slide (overlapping cells, artifacts, cell fragments, etc.) Ideally, cells should be deposited evenly without overlap over the surface of the slide and the preparations should be consistent from slide to slide. Cell sorting can be used to make cell preparations that emphasize particular characteristics. An innovative system for slide preparation is being developed which utilizes the opto-electronics of a flow cytometer under the control of a complex computer program to prepare highly ordered arrangements of fixed or living cells on a microscopic slide (Tyrer, CA28706). Various centrifugation techniques have been used to remove cellular debris and to produce slides in which cells are distributed evenly in a single layer. Another approach to this problem has been the development of computer programs sophisticated enough to eliminate fragments, cell clusters and other artifacts from diagnostic consideration. The feasibility of this approach has been demonstrated for studies of urinary sediments (Koss, CA32345). Once cells are prepared properly, they are stained with suitable stains, fluorescent dyes or other markers, to allow identification of particular cellular characteristics.



Image acquisition systems range from simple adaptations of conventional microscopes to sophisticated scanning microscopes operating at very high speeds and designed for automated scanning. Most early image acquisition systems have used scanning microphotometers (SMP) which provide good resolution but relatively slow scanning rates. Newer systems have used video digitizing systems where a videocon (TV) camera views slides through conventional microscope optics. An ultrafast scanner designed to acquire data at rates as high as 48 MHz has been developed at The University of Arizona (Bartels et al., 1981; Shack, CA24466). This system promises to allow automated scanning of cell preparations at rates comparable to those of flow cytometry.

The image processing step has been one of the most difficult to automate, and many current systems rely on human operators (Lea, 1983, Zajicek et al., 1983). While vision seems intuitively simple, experience in artificial intelligence research has demonstrated that it is extremely complex (Waldrop, 1984). Segmentation, the process of separating the image into its component parts, has been a major problem, even for relatively simple images such as well separated cells in a clear background. As more elements are added, the problem becomes increasingly difficult. Despite these difficulties, progress has been and continues to be made in the development of computer algorithms utilizing edge detection (thresholding), density mapping (differentiation) or combinations of these (Greenberg, CA27313; Swank, 1983). Other systems make no attempt to duplicate human perception of the image, but rely directly on analyses of the digital image data (Koss et al., 1982, CA32345). Stains for identification of specific cell characteristics are useful for automated cell imaging as they are for flow cytometry.

Data analysis, the final step in the process, is limited only by the amount of information available and the power of hardware and software systems to process it. One system (Swank et al., 1983, CA 27313) analyzes more than 200 individual cell features, both individually and using multivariate techniques. One system has been developed to use a microcomputer not dedicated to a particular instrument or to image analysis alone (Lea, 1983).

The ultimate aim of an automated system is the ability to screen cytology samples rapidly and with a minimum of human interaction and to identify those which are outside normal limits. Substantial progress has been made with the development of increasingly automated systems, and processing rates projected to exceed 1000 cells per second (Castleman, CA31718) are being projected for the future.

Two dimensional electrophoresis is a method for high resolution separation of proteins and peptides. The improved resolution is obtained by following an initial electrophoretic separation by a second separation using another electrophoretic technique applied at right angles to the first. While a variety of electrophoretic techniques may be utilized, the most powerful 2-D electrophoretic methods utilize combinations of isoelectric focusing (IEF) and polyacrylamide gel electrophoresis (PAGE). Isoelectric focusing separates proteins by charge as they migrate across a pH gradient under the influence of an electric field. Proteins will stop migrating when they reach their isoelectric point, the pH at which their charge drops to zero. The PAGE technique allows separation of proteins by molecular weight based on the slower migration of larger particles through the gel. Special "stacking gels" and gels with pores which progressively decrease in size are also used with PAGE to increase resolution. Sodium dodecyl

sulfate can be added to the gel (SDS PAGE) to remove charge effects and facilitate high resolution mapping of protein components. An excellent review of methodology, analysis and applications of 2-D electrophoresis has been presented (Dunn and Burghes, 1983). Theoretical estimates of the resolution of current systems are in the range of five to ten thousand spots. Practical experience indicates that 1000-2000 spots is a more realistic range (Dunn & Burghes, 1983; Taylor et al., 1983).

Once the protein separation is complete, the gels are fixed and the proteins stained to show their position on the gel. Several staining techniques are available, including protein stains such as Coomassie Blue, fluorescent compounds, silver stains and radionuclide labels. Silver stains are 100 to 200 times more sensitive than Coomassie Blue but may interfere with the detection of radioactivity and may produce streaks. A method of color staining using silver has been developed which is useful in the identification of specific proteins.

Radiolabeling is often an effective way to identify proteins. Introduction of radioactive precursors during protein synthesis results in labeled proteins with properties identical to those of their unlabeled counterparts. Labeling after synthesis may affect protein separation. The pattern of radioactivity on the gel can be measured by autoradiography, by imaging with spark chambers, proportional counters or by other imaging methods. Spots may also be cut out of the gel and measured by liquid scintillation counting.

Image analysis in 2-D PAGE requires spot detection, quantitation, pattern matching, image storage, identification and characterization. These steps involve many of the same problems discussed in the section on cell image analysis, locating spots on the gel, differentiating between spots and artifacts, quantitating proteins or peptides, matching patterns between gels and identifying specific proteins.

The complexity of patterns produced by 2-D PAGE, has increased the importance of computerized image analysis. The Data Acquisition Step is very similar to that utilized for cell image analysis, consisting of scanning densitometers and television cameras, as well as specialized detectors designed to scan patterns of radioactivity on the gel surface. The wide range of possible protein concentration over the gel surface creates a problem for both manual and automated quantitative analysis. The variation in spot intensity also interferes with the detection and analysis of spots on the pattern. The development of a video scanner based system for the quantitative analysis of 2-D gels has been described (Jansson et al., 1983). This system appears to have overcome some of the problems inherent in the use of TV cameras for data acquisition and may represent significant progress toward automation of this step. The ability of TV cameras to resolve protein spots has also been discussed both for conventional cameras (Schneider and Klose, 1983) and for a charge coupled device camera (Toda et al., 1984). These results suggest that systems based on relatively inexpensive video scanners can be reliably applied to data acquisition and optical density measurements of 2-D gels.

One image analysis problem unique to 2-D PAGE involves pattern matching, determining the relative positions of spots from one gel to another. Variations in gel composition, differences in equipment operation and dimensional changes

introduced by the handling of gels can affect the location of spots in both dimensions. Protein markers and digital image manipulation techniques are being investigated as means to resolve this problem (Gerstein et al., 1983).

Two dimensional electrophoresis has been widely applied to many areas of biological research. Applications utilizing body fluid analysis for the detection of cellular changes related to cancer may prove useful for screening, detection or diagnosis. For this to become a reality will require the further development of sophisticated computer systems for the analysis of 2-D PAGE patterns. Advances in computer technology, image acquisition technology and immunology will play major roles in the development of such systems.

### Genetic Approaches to Cancer Diagnosis

Although only about two percent of all human genes have been identified and only half of those have been assigned to specific chromosomes, most of the approximately 350 markers on the human gene map have been assigned in the last 7-8 years. This enormous accomplishment was made possible by the refinement of somatic cell hybridization technology, the development of methods for isolating single chromosomes, and the rapid application of DNA recombinant technology. Furthermore, there has been a tremendous increase in family studies which have established the genetic linkage of a variety of heritable traits and facilitated assignment to specific chromosomes.

Prior to the development of somatic cell hybridization, the ability to assign genes to particular chromosomes was limited. X-linked characteristics were established by pedigree analysis and other characteristics were mapped by association with observed chromosomal alterations such as translocations, trisomies, monosomies, etc. Progress was limited by the ability to obtain sufficient family information to establish pedigrees, the fact that only expressed characteristics could be evaluated, and the limited number of abnormalities associated with inherited visible chromosome alterations. In addition, interpretation of the data was complicated by the fact that expression of a particular gene might be controlled by products of other, physically unlinked genes, i.e. genes located at a considerable distance from the known gene, either on the same chromosome or on another. Function or expression of a structural gene can depend on processing genes, temporal genes or architectural genes; mutations in any of these genes could result in the same phenotype as a direct mutation of the structural gene.

A major technological breakthrough came in 1971 when enzymes from Hemophilus influenzae were used to produce specific DNA fragments of the monkey virus, SV40 (Danna and Nathans, 1971). These enzymes, called restriction endonucleases, recognize specific DNA sequences and catalyze cleavages at these sites. Subsequently, restriction endonucleases from many bacterial species were purified and each was shown to recognize a unique, specific sequence in DNA, resulting in a series of specific cleavages and yielding genomic fragments of defined lengths. Using these enzymes and nucleic acid hybridization it was possible to construct physical maps of various viruses and then of more complex genomes. The physical map differs from the genetic map in that it establishes the physical linkage between fragments of DNA that do not necessarily have any defined function. The genetic map, on the other hand, describes chromosomal locations of expressed



gene products, functions or phenotypes. The rapid advances in mapping the human genome have come as a result of the exploitation of physical techniques to explore regions of the chromosomes with no identified biological function as well as to define more precisely the locations of known markers.

Genetically undefined DNA segments representing single- or low-copy sequences have been assigned specific chromosomal locations based on somatic cell hybridization techniques. The undefined segments are obtained by cleavage with restriction endonucleases; the presence of these segments in the DNA of somatic cell hybrids with only a single human chromosome is determined by nucleic acid hybridization. Complementary DNA (cDNA) probes prepared from these segments can then be used to analyze chromosome breaks, deletions and translocations in inherited diseases and neoplasia. This approach was used extensively to dissect the globin gene region and to define the abnormalities in various hemoglobinopathies (Orkin et al., 1978; Orkin et al., 1979; Orkin et al., 1980). In this case, a gene region with known functions was analysed. But the approach can also be used to find abnormalities in undefined regions. Restriction endonucleases are used to cut extracted human DNA into defined lengths and these fragments are then copied to construct libraries of cDNA probes. Since these endonucleases recognize specific nucleotide sequences, any changes which alter recognition sequences, such as single base changes, deletions, or insertions, will change the size and/or number of DNA fragments obtained and result in a different restriction pattern. For example, if an enzyme normally cuts the DNA at sites A, B and C to create 2 segments of lengths AB and BC, a base change at site B could result in no cleavage at site B and only a single segment of length AC. A deletion or insertion in the segment AB would result in a change in the length of segment AB. Because the charge density of a DNA fragment is dependent upon its size, alterations in restriction fragment patterns can be evaluated by separating the different sized fragments electrophoretically on agarose gels, transferring onto nitrocellulose paper (i.e., blotting) and hybridizing with specific standard DNA probes from cDNA libraries by the method of Southern (i.e., Southern blot). The altered fragments are called restriction fragment length polymorphisms (RFLPs). RFLPs are now being used in attempts to map the entire human genome; they have been of great value in establishing chromosomal linkages between known markers that were too far apart to be related by pedigree analysis.

Recently, Cavenee and his colleagues (Cavenee et al., 1984) established a recombinant DNA library enriched in fragments from human chromosome 13. They described four probes which identified polymorphic loci on this chromosome; these are expected to allow subregional mapping and, when combined with family linkage data, to allow a comparison of physical and genetic distances. Hence, a more detailed map of chromosome 13 will result. This chromosome contains the gene that predisposes an individual to retinoblastoma and these authors predict that mapping the region of this gene should allow them to develop a system for pre-clinical diagnosis.

Dr. Richard Gatti (CA35966) is attempting to identify cancer susceptibility genes by performing linkage analyses on high-risk cancer pedigrees. He is studying twenty-one cancer families. He has identified thirteen polymorphisms in a cDNA library from HL-60 cells tested against a panel of DNA from nine donors. These probes are now being mapped to specific genomic chromosomes using mouse-man somatic cell hybrids. In addition, Dr. Gatti is collaborating with



Dr. Geoffrey Cooper to test whether DNA from members of his cancer-prone families will transform mouse NIH 3T3 cells in culture. If transformation occurs and the transforming DNA is cloned, these clones would be particularly useful markers in mapping the cancer susceptibility genes in these families.

Dr. Joseph Gertner (CA32066) is attempting to identify genetic markers for the syndrome of multiple endocrine neoplasia, type II (MEN II) by defining RFLPs in genomic DNA from members of affected families. He will determine whether any of the isolated RFLPs segregate with the MEN II trait. If this proves to be true, the RFLP could be used to identify individuals possessing the trait. He has already established cell lines from fifty-two individuals in a large kindred population; in addition, a second kindred population has been identified and fourteen of fifty members have been sampled. Extensive screening for DNA polymorphisms is now in progress, concentrating on chromosome regions with markers known to be segregating in one of the kindreds.

Rapid progress in DNA recombinant biology over the last few years has produced major breakthroughs in technology for detecting specific DNA sequences and for processing samples more rapidly and accurately with considerably less starting material. Because of these advances, clinical studies using DNA recombinant technology are now more feasible, and the Diagnosis Program has chosen to encourage collaborative studies between basic scientists and clinicians to test the value of these new technologies in cancer diagnosis. Although a few laboratories are working in this area, a broader, more intensified effort at this time is expected to explore the clinical utility of this research approach more rapidly. A recent Program initiative entitled "Application of Recombinant DNA Technology to Diagnosis of Cancer" resulted in the receipt of nineteen applications. Three of these applications were of high quality and represent research efforts which uniquely capitalize on the availability of clinical material and technological expertise. The Program plans to continue stimulating this area of research.

Another area of genetic research with important implications for cancer diagnosis is the identification of heritable sites on chromosomes that may be useful in determining an individual's predisposition to cancer. Although genetic alterations have been assumed to underlie the formation of tumors, the first clear connection between a specific genetic change and a particular malignancy was the consistent observation of the Philadelphia chromosome translocation in patients with chronic myelogenous leukemia (CML) (Nowell and Hungerford, 1960; Rowley, 1973). Since then, many other chromosomal aberrations, e.g. translocations, inversions, duplications and deletions, have been shown to occur nonrandomly and consistently with specific types of cancer (Sandberg, 1980; Wolman, 1983).

The nonrandomness of observed chromosome alterations in a number of specific types of cancer suggests that normal chromosomes may contain site-specific properties which increase the potential for karyotypic change and/or instability. Fragile sites appear to be examples of classes of sites which may predispose chromosomes to specific breakages. They have been defined as heritable points on human chromosomes which under certain culture conditions appear in metaphase as non-staining gaps. The term fragile has been used because these non-staining regions coincide with chromosome breakpoints and sites of rearrangements. Most of these sites have been identified in cells grown in medium deficient in folic

acid and thymidine (Sutherland, 1979). There are currently 21 fragile sites (18 rare and 3 common), as defined above; they occur on 13 chromosomes (Hecht and Sutherland, 1984). Aphidicolin, an inhibitor of DNA polymerase, has been shown to induce nonrandom chromosomal gaps and breaks in cultured human lymphocytes (Glover et al., 1984). Aphidicolin induced sites may represent another class of heritable chromosomal sites which may predispose these chromosomes to genetic alterations and perhaps to cancer.

Cytogeneticists have been observing chromosomal abnormalities for years and have been making correlations between these sites and particular diseases. The search for correlations between chromosomal markers and cancer has intensified recently. New techniques have been developed for high resolution banding of chromosomes and this has facilitated the identification of additional markers. There are several questions that need to be explored: Are there other classes of site-specific chromosome instabilities that might be useful for studying predisposition to cancer? How do these sites correlate with the occurrence of different human cancers and/or with known changes in chromosome structure regularly associated with specific types of human cancers?

A number of chromosome abnormalities observed in leukemia and lymphoma have been shown to be highly correlated to regions of chromosomes known to contain fragile sites (Yunis, 1983). Attempts are being made to determine whether there is a relationship between oncogenes and the chromosome aberrations associated with cancer.

Dr. Jorge Yunis (CA31024) is using methotrexate to synchronize cells from bone marrow aspirates. With this technique he can routinely obtain mitotic chromosomes with large numbers of reproducible bands; this high resolution banding consistently allows the identification of specific chromosome abnormalities in patients with acute nonlymphocytic leukemia (ANLL). He is using this technique in a large prospective study to evaluate clinical course, response to treatment and survival patterns of individuals with specific chromosomal defects. He will also examine normal cells from leukemia patients to determine whether a fragile site can be elicited and whether the presence of such a site predisposes the individual to develop cancer.

Dr. Frederick Hecht and his colleagues (CA25055) are also investigating the relationship between chromosomal fragile sites and specific cancers. They are addressing questions similar to those of Dr. Yunis but they are concentrating on different cancers. In addition, they are developing new techniques for eliciting other types of chromosome abnormalities which should open new areas of exploration, e.g., are these sites heritable?; do they correlate with specific cancers?; can they be used as indicators of predisposition to cancer?; are they the result of a similar defect to the one underlying fragile site formation?

The intriguing preliminary observations from the few laboratories working on these problems combined with the potential importance of fragile sites and/or other heritable chromosomal sites as predictors of cancer risk have prompted the Diagnosis Program to encourage additional research in this area. The concept for an RFA on this topic was cleared by The Board of Scientific Counselors for the Division of Cancer Biology and Diagnosis in June, 1984. Since only a few laboratories are studying this problem directly and consider-

able data will be required before a predictive relationship between chromosomal sites and any cancer can be established, it is reasonable to encourage new laboratories and laboratories working in peripheral areas to develop research programs focused on this problem. Clearly, this will remain an important area of research for many years. Techniques need to be improved and new ones developed; heritable patterns and incidence of fragile sites need to be determined; and more definitive correlations with different cancers and chromosome changes need to be established.

Because malignant cells from leukemias and lymphomas are relatively easy to obtain and culture, more research has been focused on the cytogenetics of these tumors than on solid tumors. In order to study the genetic changes which occur when a tumor begins and then progresses, single tumor cells must be examined. Solid tumors contain different populations of tumor cells as well as a variety of other cell types, including fibroblasts, endothelial cells, lymphocytes, macrophages, other blood cells, etc. Therefore, unambiguous evaluation of tumor cell characteristics first depends on the ability to separate the cancer cells from the normal cells. After this separation has been achieved, there are still other variables which may confuse interpretations. The method of tumor dissection may introduce changes in cell structure and biochemistry. If the tumor cells are cultured, it is difficult to know whether the population that grows is representative of the cells in the tumor in vivo or whether a natural selection takes place in vitro resulting in growth of only the most malignant cells.

Before significant progress can be made in the area of cytogenetics of solid tumors, better methods for both short term and long term culture of human tumor cells must be developed. Numerous advances in cell culture technology have been made, but most of the research has been with normal cells; the emphasis has been on development of media and on maintaining "normal" cell characteristics. These techniques are ready to be tested more extensively on human tumor cells. Criteria must be established to determine whether the population of cells in culture has maintained genetic properties characteristic of the original tumor. In order to facilitate the transfer of cell culture technology to the study of human solid tumor cytogenetics, the Diagnosis Program is planning to conduct a meeting between leaders in the cell culture field and cancer cytogeneticists.

Once the stumbling block of cell culture is overcome, a variety of techniques can be applied to solid tumor studies. In the past, cytogeneticists have counted the chromosomes of tumor cells and have determined that many solid tumors in the early stages of progression are diploid or very close to diploid. Quantitative measurements of DNA content can be made by cytophotometric analyses based on dye binding to DNA. However, these kinds of measurements provide limited information since they only determine DNA content and do not reveal the specific genetic alterations of individual chromosomes. For example, this type of measurement does not give information about chromosomal rearrangements which involve no net change in DNA content. Also, small deletions of DNA segments generally would not be detected. Since these types of changes may be the earliest events in tumor formation, and possibly the most informative for early diagnosis, direct examination of tumor cell chromosomes may provide significant additional information relevant to early detection and diagnosis.



Progress is being made in the study of solid tumors despite the technological difficulties. A non-random involvement of chromosomes #1, #6, and #7 has been reported from cytogenetic studies of direct preparations from melanocytic lesions, as well as from early passage cell cultures and/or cell lines derived from these same lesions (Balaban et al., 1984).

Also, a method for culturing human gynecologic carcinoma cells has been reported (Crickard et al., 1984). When cells were grown on an extracellular matrix of defined origin, sufficient material was obtained for histological and cytological examination, karyotype analysis and tumor marker analysis. The results indicated that the cultured tissue maintained many of the characteristics of the fresh tumor tissue.

All of the efforts to identify and characterize genetic alterations associated with particular neoplasms should lead to new methods for detection and diagnosis of cancer. They may also improve the ability to identify individuals predisposed to develop cancer. The new genetic approaches to cancer diagnosis are a direct result of the technological advances in the fields of molecular and cellular biology. The various techniques described are contributing to the rapid accretion of genetic data and encouraging clinicians and basic scientists to pool their resources to explore many problems which were previously unapproachable.

### Discussion

Both the quantity and diversity of diagnostic research has increased significantly in the past year. Some of this increase can be attributed to responses to the various Diagnosis Research initiatives aimed at encouraging research in specific areas of cancer diagnosis. More importantly, this increase is due to recognition by the biomedical research community that there are technologies and information now ready to be studied and redirected for clinical application.

Progress in hybridoma technology has made production of new monoclonal antibodies relatively straightforward. This has resulted in an explosion of research aimed at identifying new tumor markers and reevaluating the usefulness of known tumor antigens. Immunodiagnostic techniques allow quantitative measurement of a given antigen independent of its biological activity. Many previous methods for determining the presence of tumor markers depended on biochemical approaches, e.g. enzyme assays. A particular protein produced in excess by a tumor could have a labile biological activity; thus, an assay dependent on activity might be negative or artificially low while an antibody against the protein would demonstrate its presence more accurately and quantitatively. The use of hybridoma technology for cancer diagnosis has stimulated the development of more specific reagents.

The continuous rapid development of sophisticated computer hardware and software has had a tremendous impact on biotechnology. These developments allow rapid analysis of data which is critical for techniques based on input and processing of large quantities of information. Diagnostic techniques such as flow cytometry and image analysis are becoming more practical as the capacity for data storage and the speed of data analysis both increase. Research efforts to exploit



technological advances are very promising and it is expected that automated systems will become more available and more effective.

Mapping of the human genome and studies of the molecular genetics of eukaryotic cells are proceeding at an astounding pace. Investigators are beginning to use this information to study genetic aspects of human cancer, and more studies are being designed which apply this knowledge to diagnosis. The program initiative to encourage application of DNA recombinant technology to cancer diagnosis was very successful and it is expected that future initiatives in this and related areas will continue to exploit the latest advances in genetic approaches.

TABLE 1

CANCER DIAGNOSIS RESEARCH PROGRAM  
ALL PROJECTS EFFECTIVE DURING FISCAL YEAR 1984

Number	Category	Grants		Contracts	
		Number	Current Funding (in Thousands)	Number	Current Funding (in Thousands)
1	Biochemistry	20	1,809	0	0
2	Immunodiagnosis	47	4,041	0	0
3	Cytology	21	2,674	0	0
4	Pathology	9	727	0	0
5	Multiple Disciplines	5	620	1	734
6	Resource/Service	0	0	10	834
7	Small Business Innovative Research	1	56	0	0
TOTALS		102	\$9,927	11	\$1,568

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BIOCHEMISTRY

- R01 CA04486 Pathology of Digestive Tract and Other Mucous Membranes  
Zamcheck Boston City Hospital
- R01 CA14185 Modified Nucleosides in Cancer and Normal Urines  
Chheda Roswell Park Memorial Institute
- R01 CA22599 Programs of Normal and Malignant Lymphocytes  
Silverstone Sloan-Kettering Institute for Cancer Research
- R01 CA23945 Assessment of Malignancy in Human Chondrosarcomas  
Rosenberg Montefiore Hospital and Medical Center
- R01 CA25376 Development of Serum Nuclease Isozyme Test for Cancer  
Tsou University of Pennsylvania
- R01 CA29062 Vasoactive Intestinal Peptide in Diagnosis of Leukemia  
O'Dorisio Ohio State University
- R01 CA30627 In Vivo Release of Transformed Cell-Specific Proteins  
Webb Ohio State University
- R01 CA30667 A Study of Cancer Associated Colonic Mucin  
Boland University of California, San Francisco
- R01 CA30687 Progesterone-Specific Protein in Endometrial Secretions  
Richardson Vincent Memorial Hospital
- R01 CA31218 Thyrotropins from Tumors of Trophoblastic Origin  
Amir Beth Israel Hospital
- R01 CA32585 Creatine Kinase BB as a Tumor Marker  
Dermer University of North Carolina
- R01 CA33207 Calcitonin in Thyroid Carcinoma  
Sorenson Dartmouth Medical School
- R01 CA33615 Multiple Forms of HGH: Measurements and Actions  
Vander Laan Whittier Institute for Diabetes & Endocrinology
- R01 CA33739 Porphyrins in Cancer Treatment: Molecular-Level Studies  
Margalit Tel-Aviv University
- R01 CA34881 Biochemical and Clinical Application of Acid Phosphatase 5  
Lam University of Texas Health Sciences Center
- R01 CA35329 Systematic Study of Three Types of Glycosyltransferases  
Matta Roswell Park Memorial Institute
- R23 CA35602 Secretory Activity of C-Cells in Pathologic Conditions  
Halpern Massachusetts General Hospital

- R01 CA37506 GAG as Bladder Cancer Markers in High-Risk Population  
Hurst University of Oklahoma Health Science Center
- R23 CA38063 Protein Markers of Renal Cell Carcinoma  
Tracy University of Rochester
- R23 CA38483-01 Bombesin-like Peptides: Structure and Physiology  
Spindel Massachusetts General Hospital

#### IMMUNODIAGNOSIS

- R01 CA18404 Neuroendocrine Differentiation in Human Tumors  
Baylin Johns Hopkins Medical Institution
- R01 CA19304 Human Leukemia & Lymphoma Associated Antigens  
Seon Roswell Park Memorial Institute
- R01 CA19613 Ectopic Hormones in Small Cell Carcinoma of the Lung  
North Dartmouth College
- R01 CA20364 Immunodiagnosis of Mesothelioma  
Seigler Duke University
- R01 CA21399 Binding of Bacteria to Normal & Leukemic Lymphocytes  
Teodorescu University of Illinois Medical Center
- R01 CA22141 Protein SAA in Neoplastic Disease  
Benson Indiana University School of Medicine
- R01 CA22595 Detection of Medullary Thyroid Cancer in Families  
Jackson Henry Ford Hospital
- R01 CA25088 Human Ribonucleases and Cancer  
Glitz University of California, Los Angeles
- R01 CA25338 Thyroglobulin Radioimmunoassay in Patients with Thyroid Cancer  
Charles University of California, Irvine
- R01 CA26246 Assay of Human Tumor or Organ-Associated Antigens  
Saravis Boston City Hospital
- R01 CA27081 Immunodiagnosis of Mesothelioma  
Singh University of Pittsburgh
- R23 CA27623 Characterization of Prostate Cell Plasma Membranes  
Starling Eastern Virginia Medical School
- R01 CA29552 Differential Counting of Lymphocyte Subpopulations  
Teodorescu University of Illinois Medical Center

R01 CA29639	Tumor Imaging with Radiolabeled Monoclonal Antibody
Nelp	University of Washington
R01 CA30019	Purification of Tumor Antigens of Defined Specificities
Gupta	UCLA Center for Health Sciences
R01 CA30020	The Cell Surface Phenotype of Malignant Lymphoma
Aisenberg	Massachusetts General Hospital
R01 CA30209	Immunochemical Studies of Gastrointestinal Cancer
Alpert	Baylor College of Medicine
R01 CA30255	Immunolocalization of Human Malignant Tumors
Alpert	Baylor College of Medicine
R01 CA31762	Immunologic Diagnosis of Myeloblastic Leukemia
Taub	Columbia University School of Medicine
R01 CA32245	Detection & Characterization of Mesenchymal Antigens
Hirshaut	Sloan-Kettering Institute for Cancer Research
R01 CA32302	Controlled Trial: CEA Prompted 2nd Look in Bowel Cancer
Slack	University of London
R01 CA33239	Tissue Blood Group Antigens in Urothelial Neoplasia
Limas	University of Minnesota
R01 CA33425	Human Myeloma Cell Line
Bernier	Dartmouth Medical School
R01 CA33767	Bombesin-Like Peptides in Oat Cell Carcinoma
Moody	George Washington University
R01 CA33871	Circulating Tumor Components
Ceriani	Children's Hospital Medical Center
R01 CA34039	Carbohydrate Determinants as Human Tumor Markers
Lloyd	Sloan-Kettering Institute Cancer Research
R01 CA34187	Monoclonal Antibodies in Diagnosis & Prognosis of Cancer
Chee	Scott Laboratories, Inc.
R01 CA34635	Preneoplastic Markers Detected by Monoclonal Antibodies
Sell	University of Texas Science Center at Houston
R01 CA34765	Specificity of Antitransforming Gene Product Antibody
Sklar	St. Jude Children's Research Hospital
R01 CA34782	Immunological Detection of Pyrimidine Dimers in Situ
Ley	Lovelace Medical Center
R01 CA34880	Clinical Application of Esterase, a Monocyte Marker
Lam	University of Texas Health Sciences Center

- R01 CA35227 Human and Murine Hybridoma Antibodies in Acute Leukemias  
Olsson State University Hospital, Copenhagen, Denmark
- R01 CA35354 Monoclonal Antibodies to Human Sarcoma Membrane Antigens  
Brown University of Illinois
- R01 CA35460 Monoclonal Anti-Ebna Antibodies  
Volsky University of Nebraska Medical Center
- R01 CA36310 Cell Lines & Methods for Human Hybridomas  
Heitzmann Salk Institute
- R01 CA36320 New Approach to Produce Human Monoclonal Antibodies  
Volsky University of Nebraska Medical Center
- R01 CA36422 Heteromyelomas for Human Monoclonal Antibody Production  
Teng Stanford University Medical Center
- R01 CA36450 Isolation of a Hodgkin-Related Antibody  
Taylor University of Southern California
- R01 CA36553 Radiolabeled Antibody Localization of B-Cell Lymphoma  
Buchsbaum University of Minnesota
- R01 CA36903 Radioimmuno-detection of Rat Pancreatic Tumors  
Cheng University of Iowa
- R01 CA36934 Immunocytochemical Studies of Prostatic Acid Phosphatase  
Lam University of Texas Health Scientist Center
- R01 CA37407 AFP/HCG Radioimmuno-detection in Testicular Cancer  
Goldenberg University of Medicine & Dentistry of NJ
- R01 CA37409 CSAP Radioimmuno-detection of Colorectal Cancer  
Goldenberg University of Medicine & Dentistry of NJ
- R01 CA37408 Clinical CEA-Tumor Radioimmuno-detection  
Goldenberg University of Medicine & Dentistry of NJ
- R01 CA37411 Immunological Heterogeneity of CEA  
Primus University of Medicine & Dentistry of NJ
- R01 CA37412 Radiological Localization of Human Tumors  
Goldenberg University of Medicine & Dentistry of NJ
- R01 CA38355 Viral Proteins: Possible Systemic Signals for Tumors  
Ritzi Texas Tech. University

CYTOLOGY

- R01 CA13271 Automated Cancer Cell Diagnosis by the TICAS Method  
Wied University of Chicago



R01	CA23393	Flow Analysis of Human Malignant Lymphoid Cells Braylan University of Florida
R01	CA24466	Ultrafast Scanner Microscope in Laboratory Automation Shack University of Arizona
R01	CA27283	Early Detection of Transformed Cells Hawkes Michigan Molecular Institute
R01	CA27313	A Search for Pre-neoplastic Cell Markers in Sputum Greenberg Baylor College of Medicine
R01	CA28704	Chromatin Probes for Distinguishing Malignant Cells Darzynkiewicz Sloan-Kettering Institute for Cancer Research
R01	CA28706	Cell Positioning System: Development and Use in Cancer Tyrer Cancer Research Center
R01	CA28770	Biophysical Probes for Automated Cytology Tsou University of Pennsylvania
R01	CA28771	Cytology Automation Barlogie M. D. Anderson Hospital and Tumor Insitute
R01	CA28921	Merocyanine Dyes as Leukemia-Specific Probes Schlegel Pennsylvania State University
R01	CA30148	Development of Lanthanide Fluorescent Stains Vallarino Virginia Commonwealth University
R01	CA31049	Clinical Test for Automated Prescreening Device Wied University of Chicago
R01	CA31718	Automated Cytology Prototype Development Castleman California Institute of Technology
R01	CA32314	Fluid Cell Sorter Wheless University of Rochester
R01	CA32345	Computer Image Analysis of Cells in Urothelial Cancer Koss Montefiore Hospital and Medical Center
R01	CA33148	Multidimensional Slit-Scan Detection of Bladder Cancer Wheless University of Rochester
R01	CA34870	Nuclear DNA Study of Gynecologic Cancers Fu University of California, Los Angeles
R01	CA35898	Quantitative Probes in Cancer Prevention & Diagnosis Hemstreet University of Oklahoma
R43	CA36657	Cervical Cancer Detection & Screening (Phase I) Bacus Cell Analysis Systems, Inc. Oakbrook, ILL

R01 CA37352 Tumor Diagnosis by Rapid DNA Ploidy Pattern Analysis  
Bibbo University of Chicago

R01 CA37368 Cell Morphology in Follicular & Diffuse Lymphomas  
Preston Carnegie-Mellon University

#### PATHOLOGY

R01 CA14264 Pathology of Cell Differentiation in Leukemia  
Bainton University of California, San Francisco

R01 CA22101 Study of Head and Neck Cancer by Serial Section  
Kirchner Yale University

R01 CA26422 Clinico-Biologic Correlation in Lymphoma and Leukemia  
Rappaport City of Hope National Medical Center

R01 CA29211 Immunohistologic Study of Uterine Cancer  
Taylor University of Southern California

R01 CA33717 Bronchioloalveolar Carcinoma: Diagnosis and Pathobiology  
Singh University of Pittsburgh

R01 CA36245 Intermediate Filament Proteins as Tumor Markers  
Trojanowski University of Pennsylvania

R01 CA36250 Cytoskeletal Hybridoma Antibodies as Diagnostic Reagents  
Gown University of California, Los Angeles

R01 CA37194 Monoclonal Antibodies in Classification of Tumors  
Battifora City of Hope National Medical Center

R01 CA37944 Immunohistochemical Classification of Human Breast Tumor  
Raam Tufts University

#### MULTIPLE DISCIPLINES

R01 CA25582 Fluorescence Endoscopy and Photoradiation Therapy  
Balchum University of Southern California

R01 CA32066 Genetic Linkage in Multiple Endocrine Neoplasia, Type II  
Gertner Yale University

R01 CA33618 Preclinical Training in Endoscopic Programs  
Rayl VA Medical Center Lake City, Florida

R01 CA35040 Early Detection of Medullary Thyroid Carcinoma  
Samaan University of Texas System Cancer Center

R01 CA35966 MHC and Cancer Susceptibility Genes in Man  
Gatti University of California, Los Angeles

CONTRACT RESEARCH SUMMARY

Title: Human Tumor Cell Line Bank for Diagnostic Studies

Principal Investigator: Dr. Robert J. Hay  
Performing Organization: American Type Culture Collection  
City and State: Rockville, MD

Contract Number: N01-CB-14351

Starting Date: 9/29/81

Expiration Date: 9/28/86

Goal: The objectives of the program are to acquire, characterize, catalog, store and distribute a variety of cell lines having special utility for research in tumor diagnosis. Well characterized lines from solid tumors as well as from normal tissues will be included. Information concerning properties and utility of these lines will be provided to all interested investigators.

Approach: Cell lines selected in consultation with the Government Project Officer (GPO) and advisors will be expanded from token holdings or new submissions to produce seed and distribution stocks. These will be characterized using published ATCC procedures. Initially, standard tests for the absence of microbial contamination will be applied and species verification will be accomplished by assay for the isoenzymes of glucose-6-phosphate dehydrogenase, lactic dehydrogenase and nucleoside phosphorylase. Distribution will begin following satisfactory completion of these tests. Lines in the existing bank are being characterized further with regard to isoenzyme profiles, karyology, surface antigens, etc. as time and funds permit.

Progress: The lines selected from the former cell bank by the advisory committee were partially recharacterized and catalogued for distribution. In addition, 15 human breast cancer lines were transferred to the ATCC from the EG & G Mason Institute and token stocks on a variety of other human cell lines (965) were transferred from the Naval Biosciences Laboratory (NBL). Fourteen of the former and 127 of the latter were initially selected for examination and possible addition to the bank after joint consultations between the GPO, advisors and ATCC scientists. Three of the breast cancer lines remain problematic and many of the NBL lines appear to be stromal in nature. In general, fibroblast-like lines have not been included unless a tumor line from the same patient is also available. To date, the Human Tumor Cell Line Bank consists of 115 lines. These are shipped for a fee upon request along with directions on reconstitution and culture. An average of 117 cell lines have been shipped per month during the past 12 month interim.

Project Officer: Bernice T. Radovich, Ph.D.  
Program: Diagnosis  
FY 84 Funds: \$147,340.

CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer: Patient Resource

Principal Investigator: Dr. Theodore Maycroft  
Performing Organization: Butterworth Hospital  
City and State: Grand Rapids, MI

Contract Number: N01-CB-23927

Starting Date: 9/15/77

Expiration Date: 9/14/86

Goal: To develop a specimen resource for blood from breast cancer patients and benign disease patients to be used in a search for and verification of new breast cancer markers.

Approach: Thirty milliliters of blood are collected prior to surgery from breast disease patients who are scheduled to undergo biopsy and/or primary surgery for breast lesions. Another specimen is collected, when feasible, 5-10 weeks postmastectomy from the same patient. Annual drawings are made on patients with malignant diagnoses. Patients with benign diagnoses are asked to complete annual questionnaires for a period of two years after biopsy. Serum specimens are stored at  $-70^{\circ}\text{C}$ , then shipped to an NCI designated blood bank facility. Appropriate clinical data is sent to a central data center.

Progress: Surgeons who perform 95% of all breast biopsies in any of the three participating institutions have signed letters of agreement allowing their patients to enter directly into the study. Since the inception of the program, 4,067 patients have become participants in the project; 926 of these patients have been found to have malignant breast disease. Approximately 62,000 vials containing serum specimens have been shipped to the central storage facility at Mayo Clinic. 1230 collections have been made on the annual anniversary of malignant disease patients and 3,309 follow-up questionnaires have been completed on benign disease patients. This information has been forwarded to the central data center. Over 17 shipments of specified panels of sera have been utilized by qualified researchers and reported assay findings are presently being analyzed for statistical significance.

Project Officers: Bernice T. Radovich, Ph.D. & Ihor J. Masnyk, Ph.D.  
Program: Diagnosis  
FY 84 Funds: \$70,013



CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer: Patient Resource

Principal Investigator: Dr. Charles W. Blackwell  
Performing Organization: Cancer Research Center  
City and State: Columbia, MO

Contract Number: N01 CB-23925

Starting Date: 9/01/77

Expiration Date: 8/31/86

Goal: To serve as a specimen resource for blood from breast cancer patients and controls to be used in a search for and verification of new breast cancer markers.

Approach: Since late 1977, blood samples have been drawn from volunteer Breast Cancer Demonstration Detection Project and Women's Cancer Control participants and, more recently, from patients in two local hospitals who are scheduled to have breast surgery. In addition, post-mastectomy (30 to 100 days) and annual samples are drawn from those women who have a diagnosis of breast cancer. After appropriate consent forms are signed, 30 ml of blood is collected and processed into 10-13 one ml aliquots of serum. The serum is stored at  $-70^{\circ}\text{C}$  and then shipped frozen to the Mayo Clinic for storage and dissemination. Appropriate clinical data is sent to the central data center, Information Management Services (IMS). Updated clinical histories are obtained for one year from patients with benign biopsies and for two years after the last blood from normal participants.

Progress: By March 31, 1984, 13,203 samples of blood had been drawn on 7,538 different participants. This included 6,704 control women, 307 pre-op benign, 188 pre-op malignant and 424 other malignant. On April 30, 1984, Mayo Clinic reported that they have in storage 141,561 vials submitted from Columbia. In addition, clinical update histories have been submitted to IMS on 5,773 participants. Because of special efforts on the part of the serum markers personnel and a cooperative effort with the Women's Cancer Control Program, we have only had to designate 16 participants as lost to followup.

Project Officers: Bernice T. Radovich, Ph.D. and Ihor J. Masnyk, Ph.D.  
Program: Diagnosis  
FY 84 Funds: \$ 87,240

CONTRACT RESEARCH SUMMARY

Title: Data Management System for NCI Serum Panels

Principal Investigators: Dr. Lee A Richman and  
Dr. C.M. Dayton  
Performing Organization: Ebon Research Systems  
City and State: Washington, DC

Contract Number: N01-CB-14359

Starting Date: 8/08/77

Expiration Date: 7/31/84

Goal: To provide data management and statistical programming support for research projects being conducted by the Diagnosis Branch, Division of Cancer Biology & Diagnosis, NCI. Specifically, to analyze data generated through the NCI Serum Bank in order to determine which assays show promise for the early detection and diagnosis of cancer.

Approach: To perform statistical analyses of laboratory and clinical data from NCI serum panels and to prepare summary reports of the results. Included are levels of sensitivity, specificity, percent of correct classifications, chi-square tests, histograms and Calcomp plots, ROC plots, logistic and discriminant analyses, and other statistical comparisons.

Progress: Since October 1, 1983 Ebon has completed three serum panel analyses: 190 sera where LDHk Activity was measured, 178 sera where Neuron Specific Enolase (NSE) was measured, and a triple panel of 180 sera where Lipid-bound Sialic Acid (LSA), Galactosyltransferase (GT), and Carcinoembryonic Antigen (CEA) were measured.

Project Officer: Bernice T. Radovich, Ph.D.  
Program: Diagnosis  
FY 84 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biomedical Computing Support of Breast Cancer Markers Program

Principal Investigator: Ms. Marlene Dunsmore  
Performing Organization: Information Management Services, Inc.  
City and State: Bethesda, MD

Contract Number: N01-CB-14339

Starting Date: 3/31/81

Expiration Date: 3/30/85

Goal: To increase the usefulness of the data generated in projects related to the diagnosis of human breast cancer.

Approach: A central clinical data file was developed by the contractor for the breast tumor biomarkers program. This file allows preparation of various serum panels for testing new biological markers for breast cancer, setting up studies on multiple markers involving multiple institutions, and comparisons of the results from various studies. It provides a data base from which material can be quickly and conveniently retrieved. This data file is also intended for testing new ideas, identifying groups of subjects suitable for more detailed study and for preparing reports to the medical community and the general public.

Progress: In support of the Biological Markers Program, background and clinical data have been gathered on 20,591 blood specimens from three collecting institutions, a total of 203,604 vials. The main file update system modified clinical history files with new information and continues to edit for data consistency. Benign tumor and metastatic cancer follow-up information continue to be submitted, as well as follow-up data on asymptomatic women. The availability of the sera was officially announced in JNCI in November, 1983. To date, 21 shipments of sera have been processed and shipped to investigators. The Data Center has prepared coded serum panels and statistically evaluated the assay results submitted by each investigator.

Project Officers: Bernice T. Radovich, Ph.D. & Ihor J. Masnyk, Ph.D.  
Program: Diagnosis  
FY 84 Funds: \$172,790.

CONTRACT RESEARCH SUMMARY

Title: NCI Sera Bank Facility: Biological Markers in Breast Cancer

Principal Investigator: Dr. Vay Liang Go  
 Performing Organization: Mayo Foundation  
 City and State: Rochester, MN

Contract Number: N01-CB-33931

Starting Date: 2/01/83

Expiration Date: 08/31/85

Goal: To establish and maintain a storage facility for serum specimens to be used in a program designed to search for biological markers in breast cancer.

Approach: Serum specimens are being secured from breast cancer patients, benign disease patients, normal controls, and a screening population under three separate collection contracts. The material is being shipped to Mayo and processed, recorded and stored in -70°C freezers under easily retrievable conditions. Clinical data are available in a central data center. The sera will be used in the search for and verification of new breast cancer markers.

Progress: Storage and inventory methods have been developed. A special vial has been designated and is being supplied to the collection areas. An operational shipping schedule has been established on a regular basis. Samples have been catalogued and systematically stored in 36 freezers. Inventory collected up to April 30, 1984 is listed below:

<u>Collection Centers</u>	<u>No. Patients</u>	<u>No. Vials</u>	<u>No. Shipments</u>
Wilmington, Delaware	595	6,132	50
Grand Rapids, Michigan	5,970	62,720	143
Columbia, Missouri	13,186	141,561	112
TOTALS	19,751	210,413	305

Since June 19, 1979, 18 coded serum panels have been shipped to individual investigators for evaluation of new breast cancer markers.

Project Officers: Bernice T. Radovich, Ph.D. & Ihor J. Masnyk, Ph.D.  
 Program: Diagnosis  
 FY 84 Funds: \$124,213.



CONTRACT RESEARCH SUMMARY

Title: Maintenance of the NCI Serum Diagnostic Bank

Principal Investigator: Dr. Vay Liang W. Go  
Performing Organization: Mayo Foundation  
City and State: Rochester, MN

Contract Number: N01-CB-84258  
Starting Date: 9/30/78

Expiration Date: 11/30/84

Goal: To establish and maintain a bank of frozen sera from patients with cancer, patients with benign diseases and from normal individuals, for the evaluation of immunodiagnostic and biochemical tests of potential clinical usefulness in the diagnosis of cancer.

Approach: Collect and make serum samples available for evaluation of biochemical and immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the Diagnosis Program.

Progress: A bank of sera are established and maintained from patients with histologically diagnosed malignancies, benign diseases and healthy individuals, together with a computerized clinical data and inventory system. Sera are stored at  $-75^{\circ}\text{C}$  for long-term storage with adequate continuous temperature monitoring and quality control. The sera are made available by the Project Director to investigators who request it for evaluation of immunodiagnostic, hormonal and enzymatic tests for cancer. The sera collected are adequate to determine the sensitivity and specificity of specific tumor markers and their comparative values with other tumor markers. The current inventory also includes blood collected from the University of Minnesota and the Philadelphia Geriatric Center, for long term storage under former contracts, and is stored in 51 freezers at  $-75^{\circ}\text{C}$ .

The availability of multiple serum aliquots on each patient has enabled a multi-institutional NCI collaborative multiple marker study for lung cancer diagnosis to be carried out. This involved shipping five identical panels to three participating laboratories where a total of 11 assays are being performed. The study will determine by multivariate discriminant analysis techniques to what extent the simultaneous assay of several unrelated tumor markers will improve the sensitivity and specificity of any of the markers used alone. In addition, 14 panels were shipped to individual investigators during this contract year.

Project Officer: Bernice T. Radovich, Ph.D.  
Program: Diagnosis  
FY 84 Funds: \$233,051

CONTRACT RESEARCH SUMMARY

Title: Use of Multiple Markers in Lung Cancer Diagnosis

Principal Investigator: Dr. Ada R. Wolfson  
Performing Organization: Research & Education Institute  
UCLA  
City and State: Torrance, CA

Contract Number: N01-CB-23929

Starting Date: 9/30/82

Expiration Date: 9/29/84

Goal: To evaluate the diagnostic utility of multiple simultaneous assays of serum markers in lung cancer patients and to determine by appropriate statistical techniques if there is a combination of markers that will provide a sensitive and specific clinical test for lung cancer diagnosis.

Approach: Approximately 1000 frozen coded sera from lung cancer patients, patients with benign disease and normals will be supplied from the NCI Serum Bank for assays on four peptide hormones: lipotropin, calcitonin, alpha glycopeptide subunit chorionic gonadotropin and its beta subunit. After receipt of assay data, NCI will supply clinical data for statistical analysis of performance of the four markers. These results will be used in combination with data from other collaborators in this study to ascertain by multiple discriminant analysis techniques whether a combination can be found which would be useful for diagnosis and/or prognosis of lung cancer.

Progress: Approximately 949 coded serum samples from the NCI Serum Bank have been assayed for lipotropin, calcitonin, alpha glycopeptide subunit and chorionic gonadotropin and its beta subunit. Serum panels have included samples from 320 patients with lung cancer of all histologic types, 150 healthy controls, 175 patients with benign respiratory disease, 154 patients with cancer other than lung, and 25 patients undergoing thoracotomy for non cancer diagnoses, sampled pre and post operatively. Assay results of these 4 peptide hormones have been combined with 7 other serum markers for multivariate analysis using 11 serum markers. Biostatistical decision rules have been developed, using selected serum markers, which best classify individual samples as originating from patients with lung cancer, benign respiratory disease, cancer other than lung, or from healthy controls. Decision rules have been tested for sensitivity and accuracy in predicting the diagnosis of localized or advanced lung cancer individuals of a population containing all diagnostic groups. These decision rules include recursive partitioning, linear discriminant and logistic regression.

Project Officer: Bernice T. Radovich, Ph.D.  
Program: Diagnosis  
FY 84 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Use of Multiple Markers in Lung Cancer Diagnosis

Principal Investigator: Dr. Morton K. Schwartz  
Performing Organization: Sloan-Kettering Institute  
City and State: New York, NY

Contract Number: N01-CB-23915

Starting Date: 9/30/82

Expiration Date: 9/29/84

Goal: To evaluate multiple simultaneous assays of markers in sera of lung cancer patients and to determine by appropriate statistical techniques whether there is a combination that will increase both sensitivity and specificity to the point of clinical utility.

Approach: Approximately 1000 frozen coded sera from lung cancer patients, patients with benign disease and normals will be supplied from the NCI Serum Bank for assays on lipid-bound sialic acid, total sialic acid, ferritin, Beta<sub>2</sub>-microglobulin and nucleosides and bases. After receipt of assay data, NCI will supply clinical data for statistical analysis of performance of the five markers. These results will be used in combination with data from other collaborators in this study to ascertain by multiple discriminant analysis techniques if a combination can be found which would be useful for diagnosis and/or prognosis of lung cancer.

Progress: In addition to completion of the assays on Panel A as reported previously, assays have now been completed on Panel B (315 specimens), Panel C (263 specimens) and Panel D (229 specimens). The biostatisticians are using the data from these panels in an effort to validate the multivariate model which was established from the data obtained with Panel A. Chemical analysis is currently under way on the last panel of specimens, Panel A' (307 specimens). Statistical analysis is continuing on Panels B, C and D.

Project Officer: Bernice T. Radovich, Ph.D.  
Program: Diagnosis  
FY 84 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Use of Multiple Markers in Lung Cancer Diagnosis

Principal Investigator: Dr. Leonard Deftos  
Performing Organization: University of California, San Diego  
City and State: La Jolla, CA

Contract Number: N01-CB-23930

Starting Date: 9/30/82

Expiration Date: 9/29/84

Goal: To evaluate multiple simultaneous assays of markers in sera of lung cancer patients and to determine by appropriate statistical techniques if there is a combination that will increase both sensitivity and specificity to the point of clinical utility.

Approach: Approximately 1000 frozen coded sera from lung cancer patients, patients with benign disease and normals will be supplied from the NCI Serum Bank for assays on parathyroid hormone (PTH) and calcitonin. After receipt of assay data, NCI will supply clinical data for statistical analysis of performance of the two markers. These results will be used in combination with data from other collaborators in this study to ascertain by multiple discriminant analysis techniques if a combination can be found which would be useful for diagnosis and/or prognosis of lung cancer.

Progress: As a prelude, a planning session of all contractors and biostatisticians in this multi-institutional study was held on November 8, 1982 to discuss experimental protocol and quality control and to expound on the statistical methodology to be used in analysis of the data. An additional review was held on site on October 6, 1983 while the study was in progress. All of the samples received to date have been assayed for PTH and calcitonin and the results forwarded to the NCI. Biostatisticians at the three institutions involved in this project are collaborating with NCI to perform multivariate analysis of the 11 serum markers to determine which of these assays in Panel A used in combination, best distinguish patients with advanced lung cancer from normals and from patients with benign disease. Analysis of the final panels will be performed and the results forwarded according to protocol.

Project Officer: Bernice T. Radovich, Ph.D.  
Program: Diagnosis  
FY 84 Funds: 0



CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool to Detect Early Cancer of Bowel

Principal Investigator: Dr. Victor A. Gilbertsen  
Performing Organization: University of Minnesota Health  
Sciences Center  
City and State: Minneapolis, MN

Contract Number: N01-CB-53862

Starting Date: 6/30/75

Expiration Date: 12/31/84

Goal: To demonstrate significant reduction in mortality from colorectal cancer between the screened and the control groups. The test groups will be screened employing the Hemocult (R) form of the guaiac test for occult blood in the stool in combination with a diagnostic protocol to locate the source of bleeding.

Approach: Forty-five thousand participants between 50 and 80 years of age with no prior history of colorectal cancer and residing in the state of Minnesota, were randomized into three groups (two experimental, one control) by age, sex and geographic region of the state. Guaiac slides were completed and returned to the University by mail to be developed. Test groups submitted slides annually or biennially; the control group did not submit slides. Participants submitting slides positive for blood received the diagnostic protocol at the University of Minnesota hospitals and clinics, including a complete history and physical examination, upper G.I. series x-ray (and gastroscopy if indicated), proctoscopy and colonoscopy.

Progress: The screening phase of the study was completed December 31, 1982. Follow-up procedures include an annual survey of all participants to determine vital status and monitor incidence of disease. Epidemiologists guide the tracing and determination of vital status on all non-respondents as well as the procurement of certification of death. The Deaths Review Process that has been used throughout the study is continuing; the protocol includes review of all pathology material, treatment and discharge summaries and hospital and other records relating to the death of a participant. This year the Deaths Review Committee was reconstituted; the new Committee has no voting members who are project participants. The project pathologist reviews all pathology material and submits a report to the Committee but the Committee now includes an external pathologist. All cancer deaths receive particular attention for colorectal involvement; all deaths involving colorectal malignancy are classified as from or with colorectal cancer. A Policy and Data Monitoring Group has been established to advise the Director, DCBD, on major policy issues related to this study. The Group will work with the investigators and the NCI to assure that appropriate data are secured and meaningfully analysed.

Project Officers: J. Masnyk, Ph.D., Philip Prorok, Ph.D. & Sheila E. Taube, Ph.D.  
Program: Diagnosis  
FY 84 Funds: \$733,540

CONTRACT RESEARCH SUMMARY

Title: Facility for Preparing and Housing Virus Infected Intact and Chimeric Mice

Principal Investigator: Mr. Brian Weatherly  
Performing Organization: Bioqual, Inc.  
City and State: Rockville, MD

Contract Number: N01-CB-25005  
Starting Date: 10/1/82                      Expiration Date: 9/30/85

Goal: Perform a variety of in vivo experiments in mice (up to a colony of 3000 animals) that cannot be performed on NIH campus as designated by the Project Officer. These experiments are to be performed in support of intramural research programs in the Immunology Branch, NCI.

Approach: Experiments are to be performed involving the transfer of normal and neoplastic cells, infection with virus, inoculations of combinations of cells and virus, irradiation with  $\gamma$ -rays or ultraviolet light, preparation of radiation chimeric mice, and thymus transplants. Protocols and details of experiments are to be carried as directed by the Project Officer.

Progress: Performance of this contract has been very satisfactory. A number of experiments involving virus infection, allogeneic lymphocyte transfer, and combinations of the above have been performed in several mouse strain combinations. For example, as of April 1, 1984, 6920 mice have been received into the contract facility. Of this number only 18 have died. 190 ml of cytomegalovirus has been prepared. 743 radiation chimeric mice have been made, 65 spleen cell preparations have been made, 1916 mice have been injected intravenously, 2152 mice have been injected intraperitoneally, 144 mice have injected subcutaneously, acities has been recovered from 35 mice, and 295 mice have been tail bled. The mice have been delivered to Immunology Branch laboratories on schedule as requested, and record keeping of stock mice and experimental protocols have been accurate. The Principal Investigator has ordered mice as requested by the Project Officer.

Significance to Cancer Research: This experimental mouse facility is required to support the intramural research programs of the Immunology Branch of NCI in that it provides research that cannot be performed on the NIH campus due to animal restrictions and use of infectious agents in NIH animal colonies. All of the protocols used in the facility relate to a variety of cancer-related issues including studies on radiation chimeras, induction of tumors, passage of tumors, immunological resistance to syngeneic tumors development of models of immune deficiency, and reconstitution of the immune system.

Project Officer: Dr. Gene M. Shearer  
Program: Immunology Support  
Technical Review Group: Intramural Support Contract Subcommittee A  
FY 84 Funds: \$265,162.00

CONTRACT RESEARCH SUMMARY

Title: Maintain an Animal Holding Facility and Provide Attendant Research Services

Principal Investigator: Ms. Leanne DeNenno  
Performing Organization: Bioqual, Inc.  
City and State: Rockville, MD

Contract Number: N01-CB-33876  
Starting Date: 1/11/83                      Expiration Date: 10/31/87

Goal: Maintain colonies of inbred mice (12,000 animals), inbred rats (500 animals), and rabbits (40 animals) and carry out selected breeding protocols with these animals as specified by the project officer. These animals are to be maintained in support of intramural research programs in the Immunology Branch, NCI.

Approach: Colonies of mice, rats, and rabbits are to be housed and fed according to National Research Council standards. Technical manipulations and breeding are to be carried out as directed by the project officer.

Progress: Performance on this contract has been highly satisfactory. The animal colonies have been established and are being maintained according to National Research Council standards. Animal health has, in general, been excellent, and breeding protocols have been satisfactory. Recordkeeping and transferring of animals to and from the NIH Campus have all been satisfactory. Maintenance of frozen products in appropriate freezers has been satisfactory.

Significance to Cancer Research: This animal colony is necessary in support of intramural research programs in the Immunology Branch of NCI. Many of these programs are concerned with the immune response to cancer.

Project Officer: Dr. David H. Sachs  
Program: Immunology Support  
Technical Review Group: Intramural Support Contract Subcommittee A  
FY 84 Funds: \$460,608

CONTRACT RESEARCH SUMMARY

Title: Characterization of HLA Antigens of Donors' Lymphocytes

Principal Investigator: Dr. Richard Aster  
Performing Organization: Blood Center of Southeastern Wisconsin  
City and State: Milwaukee, WI

Contract Number N01-CB-33935  
Starting Date 8/1/83 Expiration Date: 7/30/88

Goal: To analyze as carefully as possible the cell surface histocompatibility antigens in order to subsequently analyze the relationship between those antigens and the ability of those donors' cells to mount appropriate immune responses.

Approach: Analysis of cell surface antigens is performed by two different detection systems: serology and cellular typing. The serologic analysis is performed using carefully screened alloantisera in assays of complement dependent cytotoxicity. The cellular analysis is done by analyzing secondary restimulation of lymphocyte populations selectively immunized against allo-antigens in primary response (PLT), particularly against antigens of the SB locus.

Progress: Serotyping has been performed on 188 different cell samples. The quality of the serotyping has been of superb quality. Even where unexplained complexities have been noted in individual sera reactivities they have been reproducible in retesting of the same donor's cells. Improvement has been made in the sera used for testing. Improvement in quality control has been made by scrambling sera on the typing tray to assure objective scoring of reactions. Also, 7 sera samples were tested for anti-HLA reactivity.

The principal investigator is now Dr. Richard Aster. Cellular typing will be handled by a subcontract with the previous PI who has moved to Pittsburgh. That move has resulted in delays in implementation of routine cellular typing. The overall level of effort has not changed because of serotyping in excess of that predicted.

The support of this contract has been essential for many intramural studies including those described in Z01CB05067, 05100, 05101, and 05110

Significance to Cancer Research: Evidence from animal models and from epidemiologic studies in humans suggest that host cellular immune responses are crucial in determining the outcome of neoplastic diseases. Cellular immune responses are under control by genes of the major histocompatibility complex (HLA in man). In order to therapeutically manipulate these cellular immune responses, we must first understand their normal operation and genetic control.

Project officer: Dr. J. Stephen Shaw  
Program: Immunology Support  
Technical Review Group: Ad Hoc Intramural Technical Review Group  
FY 84 Funds: \$94,175.00 estimated



CONTRACT RESEARCH SUMMARY

Title: Radioimmunoassay and Enzyme Immunoassay of Immunoglobulin Molecules

Principal Investigator: Norman Beaudry  
Performing Organization: Hazelton Biotechnologies,  
City and State: Corp.  
Vienna, Virginia

Contract Number: N01-CB-44030

Starting Date: 6/30/84

Expiration Date: 6/29/87

Goal: To perform radioimmunoassays of immunoglobulin molecules as well as ELISA assays of antibody molecules in lymphocyte culture supernatants or in biological fluids.

Approach: The contractor is to quantitate human IgA, IgM, IgE, lambda and kappa light chains in various fluids using double antibody radioimmunoassay procedures and reagents defined and supplied by the project officer. Furthermore, the contractor is to measure antibodies produced by lymphocytes stimulated by antigens in vitro. This contract provides critically required research support for the study of immunodeficiencies that are associated with a high incidence of malignant transformation that causes human B and T cell leukemias.

Progress: New contract

Significance to Cancer Research: These studies helped elucidate the abnormalities of the immune system associated with the development of cancer. They have assisted in the categorization of malignancies of the lymphoid system and in defining lymphokines involved in suppression of humoral immunity.

Project Officer: Thomas Waldmann

Program: Cancer Biology Resource

Technical Review Group: Intramural Support Contract Subcommittee A

FY 84 Funds: \$190,516

A

CONTRACT RESEARCH SUMMARY

Title: Radioimmunoassay and Enzyme Immunoassay of Immunoglobulin Molecules and Antibodies

Principal Investigator: Norman Beaudry  
Performing Organization: Hazelton Laboratories, Inc.  
Vienna, Virginia

Contract Number: N01-CB1-4344

Starting Date: 6/30/81

Expiration Date: 6/29/84

Goal: To perform radioimmunoassays of immunoglobulin molecules as well as ELISA assays of antibody molecules in lymphocyte culture supernatants or in biological fluids.

Approach: The contractor is to quantitate human IgG, IgA, IgM, IgE, lambda and kappa light chains in various fluids using double antibody radioimmunoassay procedures and reagents defined and supplied by the project officer. Furthermore, the contractor is to measure antibodies produced by lymphocytes stimulated by antigens in vitro. This contract provides critically required research support for the study of immunodeficiencies that are associated with a high incidence of malignant transformation that causes human B and T cell leukemias.

Progress: The contractor has established radioimmunoassays for IgG, IgA, IgM and lambda and kappa light chains of man and ELISA assays for antibodies. These assays were used to quantitate immunoglobulin and antibody synthesis by human lymphocytes in in vitro cultures. Patients with the adult T cell leukemia associated human T cell leukemia/lymphoma virus were shown to have a malignant expansion of suppressor T cells that react with a mono-clonal antibody anti-Tac that identifies the inducible receptor for T-cell growth factor, whereas patients with the Sezary syndrome have a malignant expansion of helper T cells that are Tac antigen negative. The assays for immunoglobulin molecules have been an integral part of studies of a suppressor lymphokine produced by a T cell line derived using HTLV. The lymphokine suppresses lectin stimulated immunoglobulin synthesis without affecting T cell proliferation. These studies are defining the nature of disorders of the immune system related to cancer.

Significance to Cancer Research: These studies helped elucidate the abnormalities of the immune system associated with the development of cancer. They have assisted in the categorization of malignancies of the lymphoid system and in defining lymphokines involved in suppression of humoral immunity.

Project Officer: Thomas A. Waldmann, M.D.  
Program: Cancer Biology Resource  
Technical Review Group: Ad Hoc Review  
FY '84 Funds: -0-

CONTRACT RESEARCH SUMMARY

Title: Transplantation, Induction, and Preservation of Plasma Cell Tumors in Mice and the Maintenance of Special Strains

Principal Investigator: Martha J. McGowan, Judith Wax  
Performing Organization: Litton Bionetics, Inc.  
City and State: Bethesda, MD

Contract Number: N01-CB2-5584  
Starting Date: 02-01-82                      Expiration Date: 1-31-87

Goal: Induction, transplantation, preservation and shipping of plasmacytomas, T- and B-cell lymphomas in mice. Breeding of (congenic) strains of mice, to find genes controlling susceptibility and resistance to the induction of plasma cell tumors by pristane maintenance of wild mouse colony.

Approach: Maintain a closed conventional colony of inbred and congenic strains of mice, suitable for maintaining mice for long term plasmacytoma induction experiments. Develop BALB/c congenic strains carrying plasmacytomagenesis resistance (PCT-R) genes. Carry out procedures for identifying markers used in the construction of congenic strains. Maintain colonies of pedigreed wild mice. Harvesting and shipment of N<sub>2</sub>-frozen transplantable tumors, serum, ascites, tissues, high molecular weight DNA, pedigreed breeders to qualified investigators and collaborators.

Progress: The tumor induction studies and development of BALB/c.DBA/2 congenic strains has continued to progress well and has permitted enumeration of three dominant resistance genes and possible identification of one of these. Contractor now has developed the ability to provide high molecular weight DNA from tumors and mice and, in addition, is converting many of the commonly used lines to tissue culture, which potentially could reduce costs on shipments and in vivo transplantation. Contractor continues to perform excellently and deliver tumors, inbred and wild mice, and tumor products to the Laboratory of Genetics and other investigators upon request. The tumor reference bank proved invaluable to us by supplying the tumors for ongoing studies on the myc, abl and myb oncogenes. This colony is probably the only pedigreed source of many of the important representations of the genus Mus.

Significance to Cancer Research: Provides essential support for the study of plasmacytomagenesis (carcinogenesis) with the specific goal of determining the genetic basis of susceptibility to tumor induction by mineral oil. Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Dr. Michael Potter  
Program: Immunology Support  
Technical Review Group: Intramural Support Contract Proposal Review Committee  
FY 84 Funds: \$706,955.00

CONTRACT RESEARCH SUMMARY

Title: Maintenance and Development of Inbred and Congenic Resistant Mouse Strains

Principal Investigator: Ms. Martha McGowan  
Co-Principal Investigator: Mr. J. Scott Arn  
Performing Organization: Litton Bionetics, Inc.  
City and State: Rockville, MD

Contract Number: N01-CB-25585  
Starting Date: 3/1/82 Expiration Date: 1/31/87

Goal: To maintain a colony of inbred pedigreed strains of mice which are needed to support ongoing NCI intramural research in transplantation immunology.

Approach: The contractor maintains a colony of approximately 40 special inbred and congenic resistant strains of mice by pedigreed brother-sister mating. Quality control testing is carried out at each generation by cytotoxicity typing of animals from each strain. Alloantisera are raised between mouse strains to assist in this quality control typing, and sera and animals are shipped by the contractor to collaborating investigators at NIH and elsewhere.

Progress: The contractor has maintained all inbred and congenic resistant strains of mice in excellent condition. Breeding of each strain and of hybrid strains, recordkeeping, and quality control testing have all been highly satisfactory. A backcrossing program has been instituted for all congenic resistant strains in order to keep the backgrounds of these strains identical. This involves backcrossing of each congenic to the reference background line once every 6-10 generations. This program has also been very satisfactory to date. Twelve new recombinant H-2 haplotypes have been identified during the process of this backcrossing, and these have been bred to homozygosity and established as new valuable inbred congenic strains.

Hybridoma reagents have been produced, stored and shipped starting with cell lines developed by the Project Officer. Antisera for histocompatibility antigen typing have been prepared in a variety of combinations and have been found to be excellent reagents. A series of new strain-restricted typing sera have been produced in order to identify each strain in the colony and distinguish it from all other strains. Shipping of animals and sera to collaborating investigators at NIH and elsewhere has been very satisfactory. The animals shipped from these pedigreed colonies have generally been of excellent health and have provided breeding stock for the production of larger numbers of experimental animals in numerous laboratories. Computerization of records and reports has been performed and has led to better quality and efficiency of data handling.

Significance to Cancer Research: This animal facility is needed for the breeding and maintenance of these inbred congenic resistant strains of mice. These animals make possible research on individual histocompatibility antigens and, in particular, the role of the major histocompatibility complex in the transplantation of tissues and cells and in the immune response to cancer.

Project Officer: Dr. David H. Sachs  
Program: Immunology Support  
Technical Review Group: Intramural Support Contract Proposal Review Committee  
FY 84 Funds: \$511,880.00



CONTRACT RESEARCH SUMMARY

Title: Maintenance of a Feral Mouse Breeding Colony

Principal Investigator: Ms. Martha McGowen

Performing Organization: Litton Bionetics, Inc.  
City and State: Rockville, Maryland

Contract Number: N01-CB-33878

Starting Date: 12-01-82

Expiration Date: 09-30-85

Goal: Induction of mammary tumors with biological (hormones and mouse mammary tumor virus, MMTV) and chemical carcinogens in various feral strains of Mus musculus and other species of Mus. Breeding of (congenic) strains of feral Mus musculus which contain specific genetically transmitted MMTV genomes. Maintenance of a pedigreed feral mouse breeding colony.

Approach: Maintain a closed pedigreed colony of feral mice, suitable for long term mammary tumor induction experiments. Genetically introduce specific endogenous MMTV proviral genomes from inbred mouse strains, into the genetic background of the MMTV-germ line negative M. musculus CZII strain. Harvesting and shipment of N<sub>2</sub>-frozen primary and transplanted mammary tumors, tissues, and pedigreed feral mice to qualified investigators and collaborators.

Progress: The contractor has continued to maintain all of the colonies of feral mice in excellent condition as well as satisfactorily perform the breeding program, quality control, and maintenance of records. The first tumor induction study has been completed using the Mus musculus musculus Czech II strain of mice and the chemical carcinogen dimethylbenzanthracene (DMBA). This study has led to the identification of a new feral strain of MMTV and a common insertion locus associated with the development of mammary tumors. Two new sublines of the Czech II strain have been initiated, one injected with the exogenous feral MMTV and another subline which is free of the virus.

New tumor induction study has been initiated with each of these sublines. The development of Czech II mice; congenic for individual endogenous MMTV genomes from Balb/c and C3H/ouJ mice; has led to the identification of five sublines each containing a single unique proviral genome. The sublines are currently at the fourth backcross generation.

Significance to Cancer Research: Provides essential support for the study of mammary tumorigenesis with the specific goal of dissecting the genetic and molecular interaction between genetically transmitted MMTV genomes and exogenous carcinogens. Provides essential biological material for other investigators studying the biology of mouse mammary tumor virus as well as other classes of genetically transmitted retroviral genomes.

Project Officer: Dr. Robert Callahan

Program: Immunology Resource

Technical Review Group: DEA; Ad Hoc Intramural Technical Review Group

CONTRACT RESEARCH SUMMARY

Title: Support Services for the Laboratory of Tumor Immunology and Biology

Principle Investigator: Dr. Ronald Gillette

Performing Organization: Meloy Laboratories, Inc.  
City and State: Springfield, Virginia

Contract Number: N01-CP-01018

Starting Date: 11-20-80 Expiration Date: 11-19-84

Goal: To maintain athymic mice bearing human tumor transplants; to prepare purified monoclonal immunoglobulins and fragments and to radiolabel preparations of the aforementioned.

Approach: The contractor houses and maintains athymic mice bearing human tumor transplants for several months; these mice are used in radiolocalization experiments with monoclonal antibodies. The contractor prepares purified immunoglobulins of each of several monoclonal antibodies; immunoglobulins and their fragments are radiolabeled by the contractor. The contractor also maintains tumor and normal human cell lines.

Progress: The contractor has successfully maintained several hundred athymic mice, each for several months, bearing human breast, colon, and melanoma tumor transplants. These tumors are monitored for time to tumor appearance, tumor size, and response to monoclonal antibody therapy. The contractor has purified to homogeneity several monoclonal immunoglobulins. Numerous human tissue and normal cell lines have been successfully maintained. The contractor has successfully radiolabeled several monoclonal immunoglobulin and fragment preparations.

Significance to Cancer Research: This contract is necessary for the preparation of purified immunoglobulins to be used in monoclonal antibody research. The radiolabeled immunoglobulins and athymic mouse experiments are necessary preclinical investigations if any of the monoclonal antibodies developed are to be used for localization of tumors in carcinoma patients.

Project Officer: Dr. Jeffrey Schlom

Program: Immunology Resource

Technical Review Group: DEA; Intramural Support Contract Proposal  
Review Committee

FY 84 Funds: \$280,000

B

CONTRACT RESEARCH SUMMARY

Title: Hybridoma Assays and Related Laboratory Tests

Principle Investigator: Dr. Ronald Gillette

Performing Organization: Meloy Laboratories, Inc.  
City and State: Springfield, Virginia

Contract Number: NO1-CB-33872

Starting Date: 10-01-82 Expiration Date: 09-30-85

Goal: The contractor maintains a hybridoma production laboratory in which selected hybridomas are cloned and screened for specific monoclonal antibody production. Tissue sections are cut from paraffin embedded and fresh surgical specimens supplied by the project officer; these sections are stained using immunoperoxidase techniques.

Progress: The contractor screened hybridoma cell culture supernatants for relevant monoclonal antibody production using solid-phase radioimmunoassays. Hybridoma cell lines producing new useful monoclonal antibodies were double cloned and mass quantities of tissue culture supernatants and ascites fluids containing the antibodies were prepared. Quantities of previously existing antibodies were also prepared in this manner. Extracts of human tumor tissue and human tumor cell lines were prepared for use in characterizing assays for new and existing antibodies. Paraffin embedded and cryogenic specimens were sectioned at a 5 micron thickness. Immunoperoxidase assays and cytospin preparations were performed.

Significance to Cancer Research: This contract is needed to process the large quantity of tissue sections and perform radioimmunoassays needed to screen monoclonal antibodies for specificity. This contract is also needed to produce large quantities of cell culture supernatant fluids and ascities fluids needed for monoclonal antibody research in anticipation of clinical trials and to supply the numerous laboratories requesting these reagents.

Project Officer: Dr. Jeffrey Schlom

Program: Immunology Resource

Technical Review: DEA; Intramural Support Contract Proposal  
Review Committee

CONTRACT RESEARCH SUMMARY

Title: Construction and Characterization of Genomic DNA Libraries

Principal Investigator: Dr. J. Norman Hansen

Performing Organization: University of Maryland  
City and State: Baltimore, MD

Contract Number: N01-CB-3-3934

Starting Date: 08-01-83

Expiration Date: 07-31-86

Goal: Construction of recombinant bacteriophage (libraries) containing entire genomic representation of 5-10 different mouse species and sub-species. Screening of these libraries with a variety of immunoglobulin variable and constant region gene probes for selection of corresponding homologues. Characterization of the isolated clones containing the genes of interest.

Approach: Genomic DNA from species and sub-species selected will be introduced into appropriate bacteriophage following partial digestion with restriction endonuclease enzymes. These libraries will be assayed by filter hybridization to select genes homologous to immunoglobulin genes from inbred strains. Selected genes will then be characterized by restriction enzyme digestion (mapping), hybridization with radioactivity labeled DNA probes, and then introduced into plasmid vectors. Preparative amounts of subclones will then be supplied to P.O. for further analysis.

Progress: Restriction endonuclease digested genomic DNA from three wild mouse species has been prepared, ligated to bacteriophage DNA and packaged into viable phage to produce three libraries representing the respective species. These libraries have been screened with immunoglobulin C<sub>K</sub>, V<sub>K</sub>, C<sub>H</sub> and V<sub>H</sub> gene probes. Positive clones have been identified and purified. These clones have subsequently been analyzed by restriction endonuclease mapping and appropriate regions subcloned for nucleic acid sequence analysis. Two additional libraries are currently in preparation and will be subjected to similar studies.

Significance to Cancer Research: One of the possible mechanisms involved in the generation of neoplasia is mutations occurring in structural genes. The present contract supplies materials to permit an assessment of mutational events occurring in multi-gene families in the germline. This analysis will provide information on the occurrence of such diverse events as point mutation, recombination and gene interaction.

Project Officer: Dr. Stuart Rudikoff

Program: Cancer Biology Resource

Technical Review Group: Ad Hoc Intramural Support Contract Review Group

FY 84 Funds: \$95,537 (estimate)

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