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## Series: Molecular Medicine Institutions

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### Roswell Park Cancer Institute

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Roswell Park Cancer Institute (RPCI), founded in 1898, is America's first and among its larger cancer research, treatment, and education centers. It is the only National Cancer Institute (NCI)-designated comprehensive cancer center in western New York State. RPCI is dedicated to providing total care to the cancer patient, conducting research into the causes, treatment, and prevention of cancer, and to providing public and professional education and graduate education in conjunction with the State University of New York at Buffalo. RPCI was one of the first cancer centers named by the NCI as a model for comprehensive cancer centers to be developed throughout the country following adoption of the National Cancer Act in 1971. There are now 35 comprehensive cancer centers in the United States.

The campus in Buffalo, New York spans 25 acres and consists of 19 research and clinical buildings with about one million square feet of space. With the completion of the \$241.5 million Major Modernization Project this year, RPCI has a new 133-bed hospital, a diagnostic and treatment center (Fig. 1), outpatient clinics, a medical research complex, and renovated education, research, and support space. RPCI brings together on one campus a staff of 1900, including 100 physicians and 100 senior scientists, plus oncology nurses, allied health professionals, and lab technicians. In 1998, RPCI treated 19,500 patients from 44 states and 28 foreign countries. There were over 4000 hospital admissions and 101,000 outpatient visits.

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RPCI also manages over \$34.8 million in grants and contracts and has approximately 200 funded research projects.

RPCI was founded by Dr. Roswell Park, a noted surgeon. In 1898, he wrote to the New York State legislature of the need to have a center dedicated to cancer research. The monies approved by the state, in the first merger of government funding and scientific research, launched the Institute and brought together some of the finest minds to bear on the problem of cancer.

RPCI has a long-standing tradition emphasizing translational studies in which basic and clinical investigators collaborate to introduce basic findings into clinical practice. Some of the Institute's most notable achievements have resulted from these collaborations, including the following:

- Researchers discovered and licensed prostate-specific antigen (PSA), which is used for the early detection of prostate cancer.
- Investigators identified DNA sequence elements responsible for the proper expression of *PAX3*, an important developmental gene.
- The stress protein HSP110 has been cloned and shown to protect directly other proteins from heat-induced denaturation and aggregation.
- Investigators succeeded in introducing the HbsAG protein into the genome of potato plants, offering the promise of developing a direct delivery system for oral vaccines that could have practical and economic applications in third world countries where hepatitis B virus is epidemic.
- After two decades of laboratory studies and international clinical trials originating at



**Fig. 1. Roswell Park Cancer Institute, Buffalo, New York.**

RPCI, photodynamic therapy (PDT) received approval from the United States Food and Drug Administration (FDA) as a treatment for certain cancers including bladder, lung, and esophagus. Photofrin, the PDT photosensitizing agent, has been recently licensed by the FDA for the treatment of lung cancer. This innovative therapy is the direct result of collaborative interactions among radiation biologists, dermatologists, and medical and surgical oncologists.

- Researchers have developed new drugs to treat colorectal cancer including orally bioavailable 5-fluorouracil (5-FU) prodrugs and new topoisomerase I inhibitors.
- Investigators have discovered that interleukin-15 is an effective selective protector for drug-induced gastrointestinal toxicity in pre-clinical model systems.

## **Immunology**

The newly created Department for Immunology, headed by Soldano Ferrone, M.D., Ph.D., focuses

on molecular, biological, and immunological approaches to investigating the mechanisms underlying human diseases. Emphasis is on the immune system, including cell surface receptors, signaling systems, and the basic mechanisms by which cytokines regulate immunity. Growth and differentiation of normal and malignant leukocytes and other cell types are being explored as well. Cellular, genetic, and molecular elements of immune regulation of leukocyte and solid tumor malignancies are also investigated to improve therapeutic strategies. Programs from selected laboratories in the department are discussed below.

The primary objective of this laboratory (Thomas B. Tomasi M.D., Ph.D.) is to enhance understanding of the molecular mechanisms of regulation of major histocompatibility (MHC) class II and other molecules involved in antigen presentation.

Initial studies focused on trophoblast cells, which are of fetal origin and are one of a very few types of normal cells that cannot be induced to express MHC antigens. In fact, the absence of MHC antigens on trophoblast cells is

believed to be critical in protecting the fetus against rejection by the maternal immune system and some spontaneous abortions are thought to be due to the aberrant expression of MHC antigens, particularly class II. Several novel mechanisms have been described that regulate MHC class II expression in trophoblast cell lines from human, rat, and mouse species. One mechanism involves a silencer in the upstream region of the promoter for the class II gene that binds two single-stranded DNA binding proteins. These have been cloned and found to be restricted to trophoblast cells. A second, particularly striking finding, was the complete absence of a protein, the class II transactivator (CIITA), in trophoblast cells. CIITA can be induced in almost every other cell in the body by cytokines such as interferon gamma (IFN- $\gamma$ ) and has been called the "master regulator" of MHC class II genes. If trophoblast cells are transfected with high enough levels of CIITA, the silencing mechanisms can be overcome and class II proteins appear on the surface of the trophoblast cell. Interestingly, many tumor cells were found to have a "trophoblast" phenotype that cannot be induced to express class II by any known cytokine or combination of cytokines. Again, transfection of most tumor cells with CIITA allows essentially normal transcription of class II, suggesting that all of the other factors involved in class II expression are intact.

The next step was to determine why the CIITA gene is so tightly turned off in trophoblast and certain tumor cells. Two common mechanisms of silencing genes are by methylation of CpG islands in the vicinity of the promoter and compacting chromatin by deacetylation of histone proteins in nucleosomes. First, it was determined that methylation of the promoter of either CIITA or the class II gene was probably not involved. However, interestingly, acetylation of histones induced by agents such as trichostatin A (TSA) that inhibit deacetylase enzymes elicits class II mRNA and class II cell surface protein on trophoblast cells and tumor cells in humans and mice. Unexpected was the finding that class II expression induced by deacetylase inhibitors appeared to be independent of CIITA. A very sensitive real-time quantitative polymerase chain reaction (PCR) technique was used and CIITA transcripts could not be detected after TSA treatment despite a 10- to 20-fold increase in class II. Moreover, a costimulating molecule, CD40, which is key to eliciting an immune response,

was significantly induced by this treatment. Thus, molecules whose expression is critically important to the immune response may depend on the status of histone acetylation which, in turn, by remodeling nucleosomes, may determine whether transcriptional factors have access to the promoters of these genes.

Mouse tumor cells transfected with class II and costimulatory genes have been shown by other researchers to be strongly immunogenic and effective vaccines against the unaltered wild-type cell. Therefore, the effects of treating tumor cells with deacetylase inhibitors are being studied in mouse tumor models.

The possibility that trophoblast and tumor cells lacking class II expression produce an inhibitor of CIITA has been explored. Thus far, three cytokines produced by trophoblast and tumor cells that inhibit class II and antigen presentation have been identified. One of these, transforming growth factor (TGF)- $\beta$ 1, is the focus of research because it is produced in substantial quantities by every tumor examined. Interestingly, TGF- $\beta$  has been shown at RPCI and elsewhere to inhibit CIITA transcription. Whether TGF- $\beta$  is an autocrine and/or paracrine inhibitor of class II expression and antigen presentation by tumor cells or by host antigen-presenting cells in the tumor bed is being studied. Other studies have determined that the host cells infiltrating the tumor bed present antigen very poorly and this can be reversed by several treatments that block TGF- $\beta$ . Because there is a known defect in antigen presentation in AIDS patients, studies have been extended to this disease. Dendritic cells isolated from the peripheral blood of AIDS patients, but not normal individuals, have large amounts of TGF- $\beta$ 1 on their surface bound to the type III TGF- $\beta$  receptor. These cells are deficient in the T cell allostimulatory activity. One cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF) has been found to reverse the TGF- $\beta$  suppression in vitro and in phase I trials. Preliminary data on about 12 patients have shown that GM-CSF can reverse the defect in antigen presentation. This is accompanied in some, but not all, patients by a rise in their CD4 T cell count. However, whether GM-CSF is clinically beneficial to AIDS patients is unknown.

Ultimately, successful cancer immunotherapy will depend on the ability of immune effector cells to gain access to tumor tissues. This is another area of research in the Department of Immunology (Sharon S. Evans, Ph.D.). Lymphocyte migration out of the blood, across the vas-

cular endothelial cell barrier, and into tissues involves a tightly orchestrated, multistep adhesion cascade. Although many tumors are highly vascularized, there is frequently only limited lymphocyte infiltration in these tissues. This is due in part to the absence or low-level expression of adhesion molecules on tumor vessels. These observations have led to the hypothesis that tumor microenvironments preclude recruitment of immune effector cells. This laboratory is attempting to identify novel strategies to promote tumor immunity by stimulating adhesive interactions between immune effector cells and tumor vessels. Recent studies on normal lymphocyte-endothelial interactions in non-tumor settings have revealed a previously unrecognized mechanism, whereby fever-range hyperthermia acts at multiple, independent levels to enhance lymphocyte adhesion to vascular endothelium.

New data suggest that fever-range temperatures enhance the immune response by amplifying the highly efficient mechanisms of lymphocyte extravasation. Superficially, direct exposure of lymphocytes to fever-range temperatures (38°–41°C for 6–8 hr) *in vitro* results in a marked increase in L-selectin-dependent adhesion of these cells to specialized blood vessels, termed high endothelial venules (HEV), within lymph node and Peyer's patch tissues. The L-selectin adhesion molecule functions as a gatekeeper, controlling extravasation by mediating the initial contact between free flowing leukocytes and endothelium under the high physiological shear forces found in blood vessels. Culture of lymphocytes under hyperthermia conditions also enhances the ability of these cells to traffic in an L-selectin-dependent manner to peripheral lymph nodes, mesenteric lymph nodes, and Peyer's patches *in vivo*. Similar responses are observed following hyperthermia treatment of lymphocytes from various species (i.e., human, mouse, rat), indicating that L-selectin regulation by hyperthermia, like the endogenous fever response, is highly conserved in warm-blooded animals. In sharp contrast, fever-range temperatures do not increase the binding activity of leukocyte function-associated molecule-1 (LFA-1), a second leukocyte adhesion molecule required for firm adhesion of lymphocytes to the blood vessel wall and subsequent transendothelial cell migration. These data suggest that, at least in lymphocytes, hyperthermia plays a relatively restricted role in controlling the initial adhesion events required for attachment

and rolling on vascular endothelium during extravasation.

Intriguingly, fever-range whole-body hyperthermia was also found to increase the ability of HEV in tissues to support L-selectin-mediated adhesion of lymphocytes. Taken together, novel findings that hyperthermia dynamically regulates L-selectin adhesion at the lymphocyte and vascular endothelial levels strongly implicate the thermal component of fever as an important physiologic mechanism to amplify lymphocyte recirculation through peripheral tissues.

Whereas the mechanisms underlying hyperthermia control of L-selectin adhesion have not been fully elucidated, several important pieces of the puzzle have emerged. Recent studies further indicate that hyperthermia increases L-selectin avidity for sialo-mucin-like endothelial receptors through the release of autocrine factors, without affecting L-selectin surface density. The region of the L-selectin molecule required for hyperthermia responses has been mapped to the 11 amino acid C-terminal cytoplasmic domain. Notably, this domain contains an  $\alpha$ -actinin binding site required for stable interactions with the cytoskeletal matrix and for L-selectin adhesion. Thus, a functional consequence of hyperthermia-induced anchoring of L-selectin to the structural cytoskeleton may be to increase the tensile strength of L-selectin. Molecular mechanisms by which hyperthermia controls lymphocyte-endothelial interactions are being examined further. Studies are planned to test the hypothesis that fever-range hyperthermia can promote recruitment of cellular mediators of the innate and adaptive immune response to highly vascularized tumor sites and regional lymph nodes through combined effects on adhesion in both lymphocytes and the endothelium.

## **Molecular Genetics**

In the broadest terms, this program (Peter D. Aplan, M.D.) addresses genomic instability associated with the development of malignant disease. The general model system focuses on non-random chromosomal translocations associated with leukemogenesis. Research is pursued in two broad categories. The first involves evaluating genes disrupted by chromosomal translocations as candidate oncogenes and how their disruption, or dysregulation, may lead to malignant transformation. The second category is centered

on defining the molecular mechanisms responsible for these nonrandom translocations.

The *SCL* gene has been investigated most thoroughly in the evaluation of genes at translocation breakpoints. In collaboration with Dr. Kenneth Gross, a transgenic mouse model of T cell leukemia has been developed by crossing transgenic mice that overexpress *SCL* (also known as *TAL1* or *TCL5*) with transgenic mice that overexpress *LMO1* (also known as *TTG1* or *RBTN1*). All of these double transgenic mice develop aggressive T cell malignancies that are clinically, morphologically, and immunophenotypically similar to human T cell acute lymphoblastic leukemia (ALL). However, the *SCL* transactivation domain is not required for this oncogenic effect, implying that *SCL* might exert its oncogenic action through a dominant negative mechanism. Transient transfection experiments suggest that it may occur through sequestration of the *SCL* binding proteins E2A, ITF2, and HEB. Also, examination of thymocytes obtained from mice prior to the onset of a frank leukemia show clear differences between the double transgenic mice and control littermates in terms of thymocyte number, immunophenotype, proliferative index, and clonality.

Future plans are to refine the model, using cre-lox technology to control *SCL* expression in postnatal thymocytes, as well as using the double transgenic mice to evaluate chemotherapy directed to T cell malignancies. Experiments designed to investigate the biochemical basis for *SCL* action include a yeast two-hybrid screen that has demonstrated an interaction between *SCL* and the p44 subunit of the basal transcription factor TFIID, confirmed using glutathione-S-transferase (GST) fusion proteins and co-immunoprecipitation of the native proteins. This interaction, which is unique to *SCL* among the bHLH proteins tested, is not surprising given that *SCL* possesses a transcription activation domain. The functional relevance of this finding is being determined.

Recently, a novel t(14;21)(q11;q22) chromosome translocation has been cloned from a patient with T cell ALL. The translocation involves a V(D)J recombinase-mediated event between T cell receptor (TCR)- $\alpha$  and a region on 21q22, and presumably activates a transforming gene. Two transcripts have been found near this breakpoint; one encodes a novel bHLH protein that is not usually expressed in hematopoietic cells but is highly expressed in leukemic cells that have undergone a t(14;21)(q11;q22) transloca-

tion. Investigation of the second transcript continues.

Two novel translocations have been cloned from patients with myelodysplasia following multiagent chemotherapy for a primary malignancy. Both of these translocations generate therapy-related myelodysplastic syndromes (t-MDS) that result in in-frame fusions of the *NUP98* gene with two distinct partner genes. Stable transfection experiments and transgenic mice are being used to evaluate the oncogenic potential of the *NUP98* fusions.

The second general area of investigation is focused on the molecular mechanisms that cause chromosomal translocations. Translocations that can be induced by genotoxic chemotherapy are of particular interest, owing to the clinical relevance. *AML1* and *MLL* genes are frequently translocated in patients who develop acute myeloid leukemia following treatment with chemotherapeutic regimens that incorporate topoisomerase II poisons. Specific sites within the breakpoint cluster regions of the *AML1* and *MLL* genes have been identified that are uniquely sensitive to double-strand DNA cleavage induced by topoisomerase II poisons. The possibility that this cleavage event initiates translocations induced by topoisomerase II poisons is being investigated.

Numerous studies using reverse transcription PCR (RT-PCR) or fluorescence in situ hybridization (FISH) have demonstrated clearly that translocations leading to a fusion of the *AML1* and *TEL* genes are very common, present in 30% of patients with B cell precursor ALL. To gain clues as to the mechanism of these translocations, genomic breakpoints were cloned and sequenced. *TEL* breakpoints are clustered within a 4.0 kb segment, near a 240 bp purine/pyrimidine repeat region. In collaboration with Dr. William Burhans, neutral-neutral 2D gel electrophoresis is being used to determine if this purine/pyrimidine tract is a site for replication fork pausing, which might make the region more susceptible to DNA double-strand breaks.

## Molecular and Cellular Biology

A major program focus of this department (Kenneth W. Gross, Ph.D.) is the use of animal model systems to study a broad spectrum of human disease and biology. In particular, the faculty has strong and diverse expertise in mouse molecular genetics, which permits exploitation of the powerful manipulatable genetics of the mouse for

gene discovery, analysis, and disease modeling. Advances in molecular genetics, driven by the Human Genome Project, have led to construction of dense maps and development of facile methods for recovering genes of interest and studying complex gene interactions.

Gene targeting technology and modern transgenic approaches, which take advantage of the ability to perform gene modification of BAC constructs by homologous recombination in bacteria, provide outstanding opportunities to delineate, in rigorous and exquisite detail, the molecular regulatory mechanisms controlling specific genes and to examine the phenotypes exhibited by a diverse spectrum of specific mutations. Specialized genetic resources and expertise within the program include the following: gene targeting technology (Dr. Paul Soloway) and transgenic technology (Dr. Kenneth Gross); reservoirs of genetic variation in wild-derived mouse stocks, such as *Mus spretus* and *Mus musculus* (Dr. Rosemary Elliott, Dr. Richard Swank) and the use of such variation for high-resolution genetic mapping through interspecific backcrosses with *Mus domesticus* (Dr. Richard Swank, Dr. Rosemary Elliott); expertise in congenic construction (Dr. Rosemary Elliott); specialized physical mapping methodologies, such as radiation hybridization analysis (Dr. Rosemary Elliott) and restriction landmark genomic scanning (RLGS) (Dr. Williams Held); and sophisticated software development for genetic mapping (Dr. Kenneth Manly).

In addition, extensive resources are being developed by Dr. Pieter de Jong, Chair of the Cancer Genetics Department, for the Human and Mouse Genome Initiatives. These include construction of state-of-the-art BAC/PAC libraries for human, mouse, and other species and functional genomic resources, such as "expression chip" and genomic array technologies, that are being contemplated. Moreover, given the expanded state-of-the-art vivarium and the soon-to-be operational small-animal magnetic resonance imaging (MRI) facility at RPCI, which will permit high-resolution, noninvasive imaging of mice, considerable potential exists for exciting synergisms between these technologies and resources that can be brought to bear on problems of interest. Indeed, the convergence of these resources is particularly propitious given the increased interest and emphasis on development of the mouse as the surrogate for the study of humans.

A highly interactive group of investigators,

listed below, is using these resources in these research areas.

- Dr. Heinz Baumann is studying the physiological, cellular, and molecular actions of inflammatory and hematopoietic cytokines, along with the genetic regulation and biological role of major acute-phase plasma proteins.
- Dr. Kailash C. Chadha is investigating the biology of the interferons and identification of cofactors that play a significant role in the pathogenesis of AIDS.
- Dr. Rosemary W. Elliott is attempting to identify the genes involved in a mouse model for susceptibility to colon cancer and a hybrid infertility phenotype using a variety of genetic strategies.
- Dr. Kenneth W. Gross is undertaking delineation of transcriptional regulatory determinants for renin genes and identification of the gene responsible for an animal model of neuromotor dysfunction.
- Dr. William A. Held is using RLGS approaches to examine genetic correlates of liver and colon tumorigenesis in mouse models and to study genomic imprinting and identify genetic and epigenetic alterations in human tumors of diverse origins.
- Dr. Joseph T.Y. Lau is studying molecular regulation and functionality of carbohydrate structures, particularly sialic acid-containing glycans. He is also developing mouse models to examine the functionality of sialic acids by altering the expression of the cognate sialyltransferases.
- Dr. Kenneth F. Manly is adapting his highly popular and successful Map Manager program to other operating systems and developing microcomputer software to identify and map quantitative trait loci to assist identification and characterization of genes involved in human disease in association with the Human Genome Project.
- Dr. Steven C. Pruitt is investigating mechanisms responsible for regulation of Pax3 expression during neuroectoderm and mesoderm differentiation.
- Dr. Paul D. Soloway's field is extracellular matrix remodeling and immunity, using gene targeting approaches to modify expression of matrix metalloproteinase inhibitors.
- Dr. John R. Subjeck is attempting to characterize the molecular and biophysical properties of the heat shock protein hsp 110, and clarify

roles of hsp 110 and 170 in the immune response during fever-level hyperthermia.

- Dr. Richard T. Swank is investigating mouse pigment dilution mutants that serve as animal models for Hermansky-Pudlak Syndrome (HPS) and identification of genes responsible for several of the mouse HPS model using a variety of positional cloning strategies.
- Dr. Charles Wenner is elucidating the mechanisms in which  $TGF\beta 1$  maintains a growth advantage in progressively malignant cells.