FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ AUGUST 1993

BONE MARROW UNIT CHIEF OUTLINES NEW CHAPTERS IN TRANSPLANTATION

by Celia Hooper

Sometime this September, the head of NHLBI's new Bone Marrow Transplantation Unit hopes to open a new chapter in the history of bone marrow transplantation at NIH. In a recently approved experimental protocol, John Barrett will perform the first of 50 allogenic transplants, ablating the marrow of a patient with chronic myelogenous

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This just in. . . .

On Aug. 3, President Bill Clinton officially announced his intention to nominate **Harold Varmus** as the new director of NIH. Varmus shared the Nobel Prize in Medicine or Physiology in 1989 for his work on proto-oncogenes. He has been at the University of California Medical Center in San Francisco since 1970. A move to Bethesda would actually represent a return to NIH for Varmus, who was a Clinical Associate in the National Institute of Arthritis and Metobolic Diseases from 1974 to 1979.

Varmus has already demonstrated keen interest in the Intramural Research Program, requesting that all tenured IRP scientists send in one-page summaries of their research. Varmus also plans to maintain a small lab, possibly within NCI.

Stay tuned ... *The NIH Catalyst* hopes to present a detailed profile of Varmus and his plans in an upcoming issue. ■

Recalibration of Clinical Center Management Fund Assessment Policy Aims at Flexibility and Fairness

In June, the NIH Medical Board, the NIH Board of Scientific Directors and the NIH Institute Directors approved a new system to manage the cost of running the Clinical Center. The Clinical Center Management Fund Assessment policy is expected to provide a more fair and flexible structure to support intramural clinical research initiatives. John Gallin, Scientific Director of NIAID and Chair of the Clinical Center management fund assessment committee, reviews below the history of the Clinical Center cost management and details of the new system. ■

THE NEW CLINICAL CENTER MANAGEMENT FUND ASSESSMENT POLICY

by John I. Gallin

The Clinical Center is the single most expensive service component of the NIH Intramural Program, with a budget of about $220 million. When the Clinical Center was opened 40 years ago, the cost of support for the Center was divided among the Institutes based on the number of beds assigned to each institute. This approach worked well for about 30 years. But as NIH grew, with more Institutes and larger, more complex clinical research programs, it became clear that this was not a fair way to "tap" Institutes for support of the Clinical Center. Therefore, in 1988, then-NIH Deputy Director for Intramural Research Joseph E. Rall implemented a fundamental change from the single-bed-space cost factor to a multifactorial assessment based on a variety of different services.

The change was a major improvement; however, several problems remained. Large quarterly swings in the Clinical Center budget hit Institutes with unpredictable charges throughout the year, creating havoc for the scientific administrators and frequently disrupting the purchase of supplies for intramural research. The financial strain that the Clinical Center was exerting on the rest of the institution was becoming acute. As a result, in 1989, Dr. Rall asked me to chair a committee to review the process of Clinical Center management fund assessments.

THE COST MANAGEMENT COMMITEE RECOMMENDED MAJOR CHANGES IN THE MECHANISM FOR CLINICAL CENTER ASSESSMENTS.

The committee's foremost concern was fluctuations in the charges to the Institutes. We recommended establishing a basal bed-cost system based on minimum funds needed to keep the hospital open without any patients. The committee

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BUILDING A BETTER WORKPLACE AT NIH

Lance A. Liotta

Opening Lines of Communication

On August 10, the Task Force on Fairness in Employment Practices, chaired by John Diggs, former Deputy Director for Extramural Research, and Sandy Chamblee, Senior Policy Advisor and Counselor to the Director, held an Open Forum on Reprisal and Retaliation in Masur Auditorium. Ruth Kirschstein, Acting Director of NIH, opened with a statement of commitment to equal opportunity for all. Dr. Kirschstein, whose career at NIH has been devoted to bringing minorities and women into science careers said of her efforts: "It has been a challenge, and for the most part, gratifying, because we have had some measure of success. But nothing could be more important to me, as a member of NIH's workforce myself, than seeing that our own NIH campus becomes a more harmonious place in its diversity. By harmonious, I don't just mean no more complaints, no more negative newspaper stories, and no more Congressional hearings. By harmonious, I mean a workplace where there is equal opportunity for all in hiring, placement, career development, and promotion — in every facet of NIH activity ...

"I would like to see managers who aggressively recruit underrepresented minorities into jobs and who try to help employees improve their skills and climb higher. I would like to see an NIH where there is an atmosphere in every office and lab and clinic of harmony and good spirit, an absence of intolerance and of prejudice ... Where discrimination is a despised thing of the past ... Where decent human relations prevail — lab chief to lab technician, nurse to doctor, office supervisor to clerk, and guest worker to animal caretaker."

After Dr. Kirschstein spoke, a series of former and current NIH employees described their personal experiences and gave suggestions for improvement. The ground rules were that those offering testimony would not refer to other individuals by name in the Open Forum. Following the open session, the Task Force held a closed session. To continue this vitally important dialog, we have included a question on our FAX-BACK page. We would like to hear your insights to help us ensure a future in which all employees are uniformly treated with fairness and respect.

THE SECRETARY OF HEALTH AND HUMAN SERVICES
WASHINGTON D.C. 20201

AUG 4 1993

MEMORANDUM TO: Philip Lee, M.D.
Assistant Secretary for Health

SUBJECT: Allegations of Racial and Gender Discrimination and Sexual Harassment at NIH

I am extremely concerned about the continuing allegation of racial and gender discrimination and sexual harassment at the National Institutes of Health.

I know you will agree that discrimination and harassment of any sort are unacceptable and completely contrary to the values and mission of this Department. Our policy is to fully utilize the talents and capabilities of all employees and to create a work environment where no one is denied the opportunity to fully contribute. We must make clear our commitment to this policy and take all the necessary steps to effectuate it.

Although constructive steps have been taken at NIH -- including the establishment of the Task Force on Fairness in Employment Practices and the employee hotline for reporting instances of alleged misconduct -- the public repetition of old allegations, as well as the raising of new ones, leads me to believe that we have not succeeded either in changing the situation or in clearly communicating our commitment to change as quickly as possible.

I would like you, therefore, to personally oversee the initial steps that have been taken and to establish, in collaboration with the Acting Director of NIH, a more comprehensive approach to address the problems. I expect this new approach to include a system that will provide you with frequent, regular progress reports.

I recognize that some of the problems will be remedied only over time as positions at NIH become vacant or new ones become available; however, those that reflect mismanagement or misconduct must be remedied quickly and visibly.

Thank you for your cooperation. Please keep me personally informed of all developments.

Donna E. Shalala

Paving a Career Track

Over the past year, *The NIH Catalyst* has reported steady progress toward the creation of a new career development policy for intramural scientists at NIH. The policy is the product of an extensive series of discussions and input, including the 1988 Institute of Medicine Report, the SDs' Career Development Retreat, the Report of the Women Scientists Task Force and the Task Force on the Intramural Research Program. The SDs' Draft policy was distributed to all intramural scientists in the second issue of *The NIH Catalyst* (April, 1993). Since then, we have received extensive comments through the FAX-BACK and at roundtable discussions with intramural scientists and DDIR staff. A prime feature of the new policy is the tenure track concept. The policy also incorporates family leave and extend-the-tenure-clock provisions. These measures will help provide the flexibility that is essential to make the policy a success for people with family obligations or whose research portfolios include extensive clinical investigations that require more time to yield results.

The extend-the-clock and family leave elements of the new policy required approval by the Office of Personnel Management (OPM)/PHS. We are happy to report that on July 28, James H. Eagen, Director of OPM, sent a memo to the Director, Division of Personnel Management, NIH, stating, "After careful review, we agree in principle with your proposal to change the policy." We are now completing the steps required to implement these policies, including preparation of a formal program brochure and a Tenure Track Agreement which will be signed by all parties concerned — the tenure candidate, the scientific director, personnel officer, and branch or section chief. We are also exploring the possibility of establishing a career counseling service or designating a coordinator knowledgeable about the tenure track program to address the questions and concerns of fellows.

Leadership and Follow-Through

As can be seen by the efforts of the Task Force, the Office of Research on Minority Health, the Office of Equal Opportunity, and the memo reprinted at left, the momentum for improving our NIH workplace comes from the top as well as from individuals within the Intramural Research Program. These efforts deserve the support of the entire NIH community. The efforts to build a better workplace will also benefit from creative, constructive ideas from all quarters. I believe our science can benefit by having a workplace free of real or perceived inequalities which erode morale, cooperation, and collegiality, thereby reducing our productivity and creativity.

Lance A. Liotta, Deputy Director for Intramural Research

LETTERS TO THE EDITOR

Forward with the Faculties

We strongly support the establishment of discipline-based faculties [see*The NIH Catalyst,* April 1993, p. 2] such as those proposed by the Report of the Task Force on the Intramural Research Program ("Klausner Report"). We agree that "the ultimate resource and greatest strength of the Intramural Research Program [IRP] is its scientific staff" and that there is "under-utilization of this staff in those processes that deeply and fundamentally affect the institution." We also concur that this might be significantly ameliorated by the development of trans-Institute faculties. Faculties would give voice to the IRP staff, strengthen the collegial atmosphere of the IRP, enhance communication between scientists, and present an organizational structure to the larger biomedical community that will allow the IRP to more readily attract and retain outstanding scientists.

The faculties should have access to funding to support educational activities — for example, Gordon Conference-style meetings of NIH scientists. Because research affords excellent educational opportunities, most NIH scientists now participate in the mentoring of students and postdoctoral fellows. It seems sensible to provide a general organizational framework for trans-Institute-based training opportunities. There are several diverse educational programs within the IRP, and we understand that there will be enhanced opportunities for mentorship once the M.D.-Ph.D. program — to be sponsored in conjunction with the Uniformed Services University for the Health Sciences — is initiated. Faculties are an appropriate vehicle for overseeing the formal aspects of these educational programs, and may assume other functions as they develop. For example, faculties may provide expert opinion when requested by Scientific Directors regarding recruitment, tenure, and promotion.

WE BELIEVE THAT FACULTIES CAN BE STRUCTURED SUCH THAT THEIR OVERALL IMPACT WILL BE POSITIVE AND EMPOWERING TO THE NIH SCIENTIST.

We believe that faculties can be structured such that their overall impact will be positive and empowering to the NIH scientist. We think they will provide a forum where individual scientists can be heard, and where shared opinions can help shape research directions and the allocation of resources. Inter-Institute faculties will, we believe, serve this purpose considerably more effectively than existing Assemblies of Scientists.

Sanford Markey, Roscoe Brady, Micheal Rogawski, Daniel Gilbert, William Potter, and Husseini Manji
Counselors of the NIMH/NINDS/NIDCD Assembly of Scientists

Questioning the Committment to Clinical Research

Dr. Henry Metzger's letter on clinical research [see *The NIH Catalyst,* June, 1993, p.3] has elicited spirited discussion among many clinical investigators. While Dr. Metzger's expressions of support are appreciated, there are those who perceive the current environment as unsupportive to clinical investigators.

They view several major trends as indications that NIH leadership may in fact be withdrawing from intramural clinical research:

- The hospital in-patient census is chronically low, suggesting unwillingness to invest in patient research, making it difficult to accomplish major research initiatives, and making it difficult to have enough clinical material for training or for maintaining senior staff skills.

- Several Institutes appear to perform little patient-oriented research and have few important studies published from intramural efforts, particularly efforts involving inpatients.

- Training programs in clinical subspecialties are diminishing in number in the Clinical Center and no longer include cardiology, pulmonary, nephrology, or gastroenterology programs. Those programs that exist have substantial difficulties attracting first-rate clinical associates.

- Senior consultants are often outdated in their understanding of subspecialty medical management in areas outside of their narrow foci. This trend, and the trend to hire consultation services on contract, diminishes the quality of care that can be provided to Clinical Center patients.

- Much of the discussion of the future of the Clinical Center focuses on cutting costs rather than on maximizing productivity with whatever resources are available. There is little movement towards cooperative, inter-Institute efforts in cross-cutting areas like gene therapy or bone marrow transplantation.

Many of us continue to be proud to work at NIH, and see this as a unique facility for performing important and innovative clinical research. To produce excellent clinical research, however, there must be a critical mass of patients and investigators to maintain the scope and caliber of services necessary for clinical care. Clinical investigators must also have the resources to perform their work and promotion potential to advance their careers. Steps in this direction would assure them that NIH remains committed to clinical research on the Bethesda campus.

Henry Masur
Chief, Critical Care Medicine Dept., CC

In Future Issues. . .

- National Foundation For Biomedical Research
- Profile of the new NIH Director-Designate Harold Varmus
- Science Education Efforts at NIH

FAX-BACK Feedback

Below is a sample of the FAX-BACK comments we received for each topic raised in the June issue.

On the Task Force on the Status of Women Scientists at NIH

"Appropriate. Pay inequities should be addressed ASAP. Appalling that so few women are invited to named lectures." — *S. Bale, NIAMS.*

"A good beginning, but don't sit back now — follow-through and implementation are important steps." — *C.E. McKinney, NIMH.*

"The recommendations are excellent and should be implemented. NIH reflects the discrimination against women in academia. It should reject the academic system, which definitely favors men." — *Anonymous.*

"They are fine as far as they went. Promotions should have been investigated. Your finding of a pay differential was only the tip of the iceberg." — *Anonymous.*

Editors note: Promotions are being investigated. The Task Force's findings on promotions were omitted in The NIH Catalyst *because they are undergoing further analysis.*

On Problems With the Tech-Transfer Process

"Time required for CRADAs to get through the system." — *D.N. Johnson, NIDA.*

"Material transfer agreements (MTAs) — no strings and complications." — *S.P. Markey, NIMH.*

"The MTAs — the process is too complicated." — *Anonymous.*

'The NIH Office of Technology Transfer (OTT) has publicly stated the following priorities: 1) the success of its staff as evidenced by successful patents and licences; 2) the viability of its contract patent-attorney staff; and 3) the OTT relationship with patent licencees. The actual NIH inventors have no rights or standing in this operational model. When conflict arise, the OTT attorneys always get their way. NIH inventor scientists are told, 'You have no rights and no choice but to do what we say.' I would be happy to share my documentation of this policy with anyone who is interested. Who is going to stand up for NIH inventors in this 'lawyer system run amok?'" — *H. Stevenson Perez, NCI.*

On the Park or the Quad?

"The Park. However, in both designs, the new MLPs [multi-level parking structures] are too close to buildings (31C and proposed support buildings) and obscure sight lines. Don't make NIH look like Tyson's Corner! Look at separation of existing MLP and duplicate. Also, does the disappearance of Building 30 mean that all the tower construction and the removal of at least one magnificient magnolia were for nothing?" — *S. A. Newton, NIEHS.*

"The Park. It appears less regimented."— *D.N. Johnson,.NIDA*

"The Park — retain the campus-like environment." — *C.E. McKinney, NIMH.*

Editors note: It is no longer the Park or the Quad — the Park and the Quad have been combined to form the Quark — a blended design incorporating the most popular features of both earlier schemes. ■

Reunion Task Force Aims to Facilitate NIH-ADAMHA Merger

by Susan Blumenthal and Wendy Baldwin, Task Force Co-Chairs

The NIH Reunion Taskforce, established in 1992 to facilitate the merger of the three former ADAMHA research institutes NIDA, NIAAA, and NIMH into the NIH family, is putting together a series of activities to spark collaboration among NIH biomedical researchers and their behavioral research colleagues.

The task force is considering such activities as an NIH-wide orientation and education program for all new research fellows, trans-program research fellowships in areas that cut across institute interests, and Gordon Conference-style seminars and research workshops. In addition, the task force is organizing a series of seminars to examine such issues as psychoneuroimmunology, neuroscience, and behavior; gender, racial and age differences in pharmakokinetics and toxicology; compliance and adherences; family research; and AIDS risk behavior.

In October 1992, when Bernadine Healy announced the NIH-ADAMHA merger, the research community greeted the news with excitement. But it also had questions about how the merger would affect the status of existing programs and the funding streams and review mechanisms, and where and how the priorities of the new institutes would fit into the framework of NIH's research agenda. To hear and respond to such questions and to facilitate the transition, a task force of 33-members representing each ICD and the OD was formed. The task force's job also included finding ways to heighten the visibility of the behavioral and neurosciences, and to introduce the NIH community to ongoing research on mental and addictive disorders.

Taking a three-pronged approach to our year-long charge, we are focusing on the conduct of science in the intramural and extramural communities, and on the celebratory aspects of the enlarged and renewed NIH community.

Proposed activities involving both the intramural and extramural communities include a series of regional symposia on the relationship of mental and physical illness, a leadership forum involving the NIH community and advocacy groups, and a dinner to bring together legislators, researchers and advocacy organizations.

We welcome your suggestions on other programs and activities. Fax your suggestions to us at 402-8679. ■

NIH faces the future: Summer students attending a Town Meeting at Building 1 on July 8. Lance Liotta, Deputy Director for Intramural Research, described the opportunities for young scientists at NIH. See FAX-BACK question 1, back cover.

FEATURE

A Lab In The Limelight

by Celia Hooper

Since the middle of July, the phone in Building 37, Room 4A13, has been ringing off the hook. Photo crews regularly invade the lab, whose principal occupant, Dean Hamer, has been featured on almost a half-dozen network TV shows, from "Good Morning, America" to "The McNeill-Lehrer News Hour." *The Washington Post* splashed the lab's news across the front page—above the fold, where the headline would show prominently in newspaper boxes, but where news about science is rare. And every major news service and daily newspaper in the country covered the lab's discovery when *Science* officially lifted its embargo on the news at 6:00 p.m. on July 15.

The news, of course, was that Hamer and his associates in NCI's Gene Structure and Regulation Section of the Laboratory of Biochemistry had discovered a genetic marker on the X-chromosome that appears to be linked to homosexuality in men. The timing of the publication in *Science* happened to coincide with President Bill Clinton's decision on the controversial "don't ask, don't tell, don't pursue" policy on gays in the military, only swelling the tidal wave of publicity.

Yet, say the new science celebrities, rather than creating a chaotic disaster, the commotion has been tolerable—even helpful in one way. "We've gotten lots of new volunteers for our studies," says Hamer. "We are still collecting data to repeat the study, so that is very useful." Angela Pattatucci, a postdoctoral fellow and co-author on the study, is working her way through a six-page list of potential study subjects while plowing ahead with the lab's other ongoing study: a survey of the genetics and health of lesbians and their relatives.

The studies of gay men and lesbians have dual intellectual goals. Researchers in Hamer's lab are interested in understanding the molecular basis for the complex human behaviors that constitute sexual orientation, but they are also hoping to understand cancer in unique study populations. With gay men, the questions center on Kaposi's sarcoma, which is six times more prevalent in homosexual men with AIDS than in men who have contracted the disease via contaminated blood products or intravenous drug paraphernalia. With lesbians, the questions center primarily on breast cancer. Pattatucci cites meta-analyses suggesting that lesbians have a higher-than-normal incidence of breast cancer. Lesbians also have an unusual profile of risk factors, including reduced use of oral contraceptives—which could lessen the risk of some cancers—and lower fecundity—which could increase the risk of breast cancer.

"This is an extremely exciting study," says Pattatucci, who will head the survey of lesbians. The study will include pairs of lesbian sisters and their families; lesbians who have non-sib lesbian relatives; and lesbians who have no lesbian relatives, but who have heterosexual sisters willing to participate. "It's a multifactorial study," says Pattatucci, who would welcome collaborators with interests in cancer, sexuality, susceptibility to HIV and other sexually transmitted diseases, alcoholism, substance abuse, eating disorders, sexual assault, childhood and other abuse, and other psychological trauma. The genetic part of the study will include at least 40 families, but even with this number of pedigrees, Pattatucci expects that finding a genetic marker associated with female homosexuality will be considerably more difficult than it was in the study of gay men, in which an X-linkage quickly jumped out of the data.

NCI's Dean Hamer and his recently famous team: (from left) Stella Hu, Victoria Magnuson, Dean Hamer, Angela Pattatacci, and Nan Hu.

As the whirlwind of publicity attending Hamer's study of gay men began to subside, members of the research team began to reflect on their debut in the limelight. "The coverage has been amazingly well-balanced and largely accurate," says Hamer. "They have covered the caveats quite well and refrained from grandiose and scientifically incorrect statements like calling this 'The Gay Gene.' I think a lot of the credit for that goes to the Cancer Communications office that helped me to prepare materials for the press, like a glossary and a question-and-answer sheet." Hamer also credits the communications office with helping to keep the focus "on the science and the potential outcome of the science, rather than my personal life or that of my research group." Hamer also briefed NIH and HHS officials on the lab's findings in advance.

Hamer, as first author on the *Science* paper, felt it was his responsibility to handle the bulk of the press inquiries, but his co-authors also received attention from friends and colleagues. Pattatucci, who fielded a handful of interviews, says the effects of the notoriety haven't registered yet. "If you ask me a year from now, I might have a better handle on my feelings about all the press. We're all so much in a whirlwind right now!"

Stella Hu, originally from Shanghai and trained in Canada, has remained enmeshed in her work but says she has never witnessed such extensive publicity for a scientific research project. So far, she says, the feedback she has received from colleagues and friends has been mostly positive. "Everybody realizes this is significant," says Hu. "This is one of the first times people have tried to show a correlation between a complex behavior and a molecular basis ... It's not just homosexuality, but human behavior that we are trying to understand through molecular biology."

Nan Hu, a postdoctoral fellow who also hails from China, says working in the Hamer lab has been quite a learning experience. In previous work in Beijing, and with Janet Rowley at the University of Chicago, her focus was cancer cytogenetics. "I came to Dean Hamer's lab to learn molecular biology," says Nan Hu. "This is very different for me, working on homosexuality." Nan Hu says she has learned a lot from her experience. Most of the things she has learned have been scientific, but she says working on a more politically volatile subject has also shown her some unique facets of American culture.

Victoria Magnuson says publication of the high-profile paper has "been really exciting. Things came together a lot faster than I thought they would. I have only been here since November," says the postdoctoral fellow. "It's nice to have a *Science* paper already." But Magnuson says the hard work has just begun. Now she must dig in with linkage disequilibrium studies to zero in on the actual gene or genes in the Xq28 region that lie at the heart of the study's correlation.

Hamer says his main reaction to the media commotion was to take a long-overdue, four-day vacation, brushing up on his backhand and doing some swimming. Seeing himself on TV, he was surprised by his somber image. "I looked a lot more serious than I usually am." Generally, he says, the whirl of publicity "has been very exciting, and the overall reaction has been positive." But in the long run, he says, "I have no desire to be a media star—what I enjoy is doing science." ■

More Than Meets the Eye:
IL-8 Receptor Reveals Multiple Roles and Personalities and Poses Evolutionary Puzzle

by Seema Kumar

As recently as two years ago, researchers thought that the interleukin-8 (IL-8) receptor found on neutrophils was just another cytokine receptor — a signal-transducing molecule that binds proinflammatory peptides, thereby switching on host defenses, wound healing, and chronic inflammatory disorders. But over the past two years, immunologists have discovered that there is more to IL-8 receptors than meets the eye: "IL-8 receptors and binding sites have turned up in different sizes and forms, in the most unlikely places, and performing the most unusual functions," says Philip Murphy of NIAID, an expert on the receptors. The phenomenon, he says, is a "fascinating piece of molecular magic" that now has scientists wondering about the evolution of the IL-8 signaling system.

Louis Miller is Chief of Laboratory of Malaria Research, NIAID.

Researchers began to appreciate the complexity of the IL-8 receptor in mammalian biology as they discovered four functionally distinct types of IL-8 receptors. The first indication that different forms of IL-8 receptors exist came two years ago when Walter Darbonne and colleagues at Genentech in San Francisco found an IL-8 receptor, oddly enough, on red cells. Odd, because red cells do not perform classic white-cell immune functions and, therefore, had no discernible use for IL-8 binding sites. Then last year, two groups of researchers in Switzerland and at Genentech, and Murphy also found something unusual about the white-cell IL-8 receptor itself: unlike most G-protein-coupled receptors, the IL-8 receptor is promiscuous, or able to bind multiple ligands. Moreover, says Murphy, although the IL-8 receptors on white and red cells are both promiscuous, and while both bind IL-8, they bind, in addition, a different suite of related ligands.

Then, a few months ago, the story took another twist when Murphy and his colleagues found a homolog of IL-8 receptor in another unlikely place — in *Herpesvirus saimiri* (HVS), a cousin of the human Epstein-Barr virus, which infects primates. This discovery was the first demonstration of a signal-transducing viral homolog of a G-protein coupled receptor. But the latest and most intriguing in this series of surprises about the IL-8 receptor is a finding by Louis Miller, Chetan Chitnis, and their colleagues at NIAID and Richard Horuk and his colleagues at Genentech. In the August 27 issue of *Science*, the researchers report that the IL-8 binding site on the red cell is the Duffy blood group antigen — the receptor for invasion of the malaria parasite, *Plasmodium vivax*. Africans and 65% of African Americans who are Duffy negative, or lacking the antigen, are resistant to *P. vivax* malaria. The finding implies that receptor blockade can be used as a therapy for *P. vivax* malaria. "Creating a form of IL-8 that blocks *vivax* invasion but does not activate neutrophils could be a useful pharmacological tool," says Miller.

Researchers are excited about the new finding and its clinical implications, and are intrigued by its implications for the evolution of the IL-8 receptor. Because Duffy-negative individuals show no deleterious physiological effects from lack of the red-cell receptor, researchers are stumped by what function the receptor serves in individuals who possess it.

"We don't know the structure of the red-cell IL-8 binding protein or its amino acid sequence and, therefore, we can't say anything about the evolutionary origin of the IL-8 binding protein of the red cell relative to the other cells," says Murphy. "We do know the structure of the [white-cell] IL-8 receptor and the structure of the viral IL-8 receptor, so we can draw conclusions about the evolutionary origin of the IL-8 receptor of the virus relative to human receptor." Murphy's conclusion is that the virus has stolen the gene from the host and incorporated it into its own genome. He points out that since "viruses don't hold on to junk, the receptor must be there for a reason,"possibly to help the virus to replicate more efficiently in the host."

Recently tenured Philip Murphy of NIAID is an expert on IL-8 receptors.

What is most intriguing to researchers is that these different forms of IL-8 binding sites exist on different cell types and appear to perform the most unusual functions. "We don't know whether the functional triangle defined by IL-8 binding activities in red cells, viruses, and white cells is an outcome of purely divergent evolutionary processes, or convergent processes, or just another quirk of nature," says Murphy. "The lack of sequence and functional information about the red cell IL-8 binding site restrains our ability to speculate about what mother nature is doing with these different forms of IL-8 binding sites."

The Human IL-8 Receptor

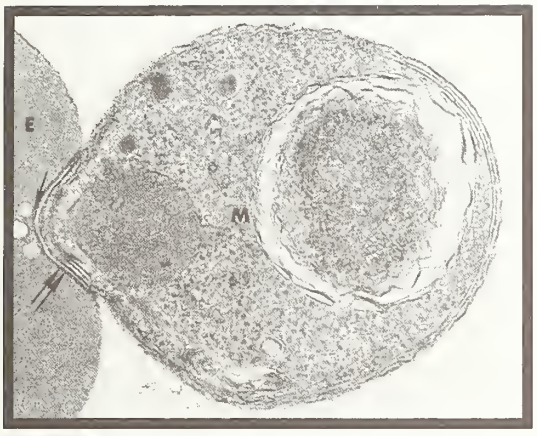
Until 1990, scientists had only known of the IL-8 receptors on neutrophils. IL-8 is a prototype for a family of at least seven neutrophil chemoattractants that flag down circulating quiescent leukocytes and cause them to change and to exert functions in the tissue that they do not perform in the blood. Scientists had known of two IL-8 receptors in neutrophils, IL-8RA and IL-8RB. IL-8RB binds IL-8, but also two other proinflammatory peptides, GRO/melanoma growth stimulatory activity (GRO/MGSA) and neutrophil-activating peptide-2 (NAP-2), that belong to a family of α chemokines. The α chemokines and β chemokines are polypeptides that regulate trafficking and effector functions of phagocytes and lymphocytes and play an important role in host defense, inflammation, and wound healing.

Last year, Sunil Ahuja, working with Murphy, cloned the genes for α chemokine receptors IL-8RA and IL-8RB and discovered a related pseudogene named IL-8RP that had recently become extinct. Murphy and his colleagues were impressed by the coevolutionary complexity displayed by the IL-8 system — ligand promiscuity for IL-8RA and IL-8RB, receptor promiscuity for multiple α chemokines, replication of genes encoding both ligands and receptors, and gene extinction for IL-8RP. The three struc-

turally related genes together form a gene cluster on chromosome 2 within a superfamily of G-protein-coupled receptors that tend to be dispersed throughout the human genome.

The Red-Cell Binding Site

Meanwhile, researchers at Genentech had discovered that the red cell IL-8 receptor differed from IL-8RA and IL-8RB and was even more promiscuous than IL-8RB, binding to both α and β chemokines but not all β chemokines. "It is fascinating that this ... binding site on red cells apparently can bind molecules that even the leucocyte receptors that have been designed for these molecules cannot cross-bind," says Murphy. But because red cells are incapable of performing classic white cell functions, researchers hypothesized that "maybe the IL-8 receptor on red cells is not a signaling receptor, but just a binding protein for IL-8 and other chemokines," says Miller. "Because the number and concentration of red cells in the blood is much greater than that of the white cells, the total binding sites available [for IL-8] on the red cells would be much greater than in the white cells. So Darbonne at Genentech suggested that the red cells could be acting as a ligand sink for IL-8, thereby preventing inflammatory responses that could be deleterious to the host if they occurred intravascularly," says Murphy.



Red-cell invasion by malaria parasite: Arrows show the merozoite (M) forming a junction on the erythrocyte (E) surface.

At Genentech, Horuk and his colleagues continued studying this unusual receptor and its binding activity with different chemokines. During these studies, they observed that whenever they used red blood cells from African Americans, the chemokines did not bind to the receptors. "At first, they thought something was wrong with their assay," says Chitnis. But when they repeated the experiment, it confirmed the observation. Horuk then remembered something he had learned in his college microbiology courses — that certain malaria parasites cannot invade red cells of individuals in whom the invasion site, called the Duffy blood group antigen, is missing. Miller had made the discovery 20 years ago. Horuk tracked Miller down at NIAID.

"When Horuk contacted us, we were excited and sent him Duffy-typed blood from both Caucasians and African Americans, 5 to 20% of whom are positive," says Chitnis in Miller's lab. Red blood cells from the 11 Caucasians and a few African Americans who were Duffy positive bound to radiolabeled IL-8; red blood cells of Duffy-negative African Americans did not, suggesting that the Duffy blood group antigen and the red-cell IL-8 receptor were one and the same. In subsequent studies, researchers confirmed this equivalence when they found that monoclonal antibodies to the Duffy antigen could block binding of the chemokines. Horuk and his team also found that the sizes and biochemical properties of the IL-8 receptor and the Duffy blood group antigen were similar. With little doubt left that the two must be the same, Chitnis in Miller's lab then showed they could block binding and invasion of red cells by *P. knowlesi* using IL-8 and other chemokines as competitive inhibitors.

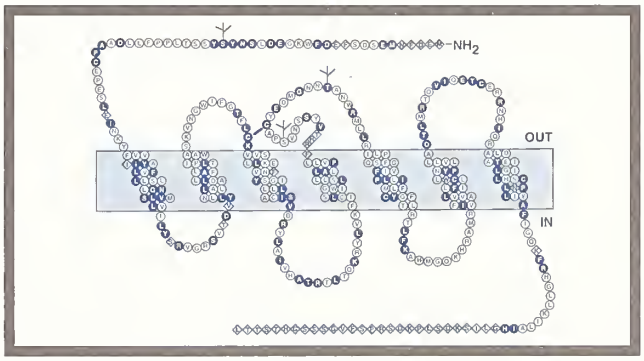
In IL-8, Chitnis sees "a molecule that can block parasite invasion, and so potentially, one can develop a drug based on these molecules [that] blocks invasion but does not bind neutrophils," says Chitnis.

Researchers do not yet have an explanation for the observation that African American individuals have lower neutrophil counts; whether or not Duffy-negativity has anything to do with it remains to be seen. Scientists speculate that it may be related to the relative proportions of cells residing in the blood vs. tissues. Lacking vast pools of IL-8 receptors on red cells, Duffy-negative individuals are likely to have higher concentrations of soluble cytokine, and concomitantly, greater activation of neutrophils and thus fewer cells in the blood stream, suggests Chitnis. Although such a theory might explain the lower neutrophil count in Duffy-negative people, it does not answer the central mystery in this work: What is the evolutionary and physiological function of the IL-8 receptor on red blood cells?

The IL-8 Receptor's Viral Homolog

Whereas humans living in regions where malaria is endemic may have obtained a survival advantage by *losing* the IL-8 receptor from red cells, a herpesvirus may have gained its evolutionary success by *acquiring* an IL-8-receptor gene. Murphy and Ahuja stumbled onto this knowledge when they found that the gene encoding IL-8RB is homologous to a gene called ECRF3 in the *Herpesvirus saimiri* (HVS) — a herpesvirus that infects squirrel monkeys and is evolutionarily related to the human Epstein-Barr virus (EBV). The similarity between key regions of ECRF3 and IL-8RB is 33% — enough to suggest that the two are evolutionarily related.

"ECRF3 is a signal-transducing receptor for the same chemokines that IL-8RB is a signal transducing receptor for, but interestingly, even more sensitive to certain a chemokines than is its mammalian counterpart," says Murphy. "We believe that *continued on page 8.*



Proposed transmembrane topography of IL-8 receptor B. Blue circles show positions in the sequence that are identical to corresponding positions in the viral homolog; white circles show positions where the sequence of IL-8RB and the viral homolog differ; and diamonds represent positions in the IL-8RB that are absent in the viral homolog sequence.

IL-8 Receptor

continued from page 7.

HVS has stolen the gene for IL-8RB from primates ... The virus incorporated this into its genome and changed it considerably, so that it is only 33% identical to the parent, but because [the gene] is still there ... its got to be there for a reason."

Murphy can only speculate what this reason could be. "What we know right now is that the kind of signal ECRF3 can deliver to the inside of the cell is the same type of signal that the IL-8 receptor delivers to the inside of the cell," says Murphy. The signal elicits transient elevations of calcium in cells, an indication that ECRF3 may provoke activation responses similar to those mediated by the IL-8 receptor. If this is the case, Murphy says, "HVS could be deliberately overexpressing a version of the IL-8 receptor in infected target cells in order to make those cells ... more sensitive to environmental concentrations of α chemokines." Murphy speculates that the greater sensitivity to cytokines may contribute to the efficiency of replication of HVS.

Murphy's lab is pursuing several details and interesting chapters in the viral IL-8 receptor story. The team has not yet demonstrated that the ECRF3 gene is expressed in infected cells. And the team is also looking for a human herpesvirus homolog of ECRF3. It might exist, "but we just don't know where it is yet," says Murphy. The EBV does not have an ECRF3 homolog.

HVS can also be oncogenic. For example, human T cells infected with HVS become transformed in vitro. And non-natural primate hosts infected with HVS develop fatal leukemias. "We don' t know to what extent that transforming activity is due to ECRF3," says Murphy. "But the reason why that is worth considering is that there are other genes, encoding G-protein- coupled receptors (mas oncogene, serotonin receptors), that are already known to have transforming activity." Researchers also know that the IL-8RB is activated by GRO/MGSA — originally identified by its ability to promote the growth of melanoma cells in vitro. "It is possible that ECRF3 is mediating a growth signal for target cells that could bleed off into a growth signal," says Murphy. Murphy plans to follow up on these leads.

Future Directions

Murphy is also interested in the more global questions of the evolution of IL-8 receptors. Miller, who has been interested in malaria as a selective force for certain genes, is also interested in this area. "When you have a disease from a single organism [like malaria] that kills 25% of the population each generation, you realize that any change in the genetics of the population that will lessen mortality would be selected for over a period of time," says Miller. "We know that the evolution of some genes — sickle cell anemia, hemoglobin C, and thalassemia, and the newly discovered association of HLA 53 to malaria resistance — are all related to increased resistance to malaria." Miller is now giving broad consideration to the advantages of not having a sink for chemokines in the presence of malaria. The Duffy-IL-8-receptor findings led Miller to wonder whether a hypertension gene involved in sodium transport of a particular type may have conveyed an advantageous resistance to malaria. The gene, more prevalent in Africans and African Americans, becomes deleterious with a typical Western, high-salt diet.

Perhaps the most important next step will come with the sequencing of the red-cell IL-8 receptor. Knowledge of the structure will allow researchers to make genetic, structural, and evolutionary comparisons with the IL-8 receptors on white cells and go on to perhaps answer the central mystery of the function of the red-cell IL-8 receptor.

"The fact that [the IL-8 receptor on red cells] binds ligands that the [other] known mammalian IL-8 receptors do not bind suggests very strongly that it is going to turn out to be quite different from those encoding the white-cell receptors and the viral [ECRF3] receptor," says Murphy.

The gene sequence will tell the researchers whether the IL-8 receptor on red and white cells have achieved their corresponding abilities to bind IL-8 through convergent evolution, divergent evolution, or coincidence. This knowledge, in turn, may then point to answers to the central mystery: What are the physiological and evolutionary advantages of the presence and absence of IL-8 receptors on red cells and viruses? ■

The Parasite's Perspective:

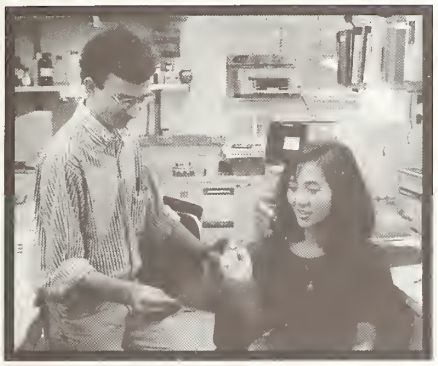
Malaria parasites enter red blood cells by binding to specific proteins on the red cells — *Plasmodium vivax* and *Plasmodium knowlesi* parasites do so by binding to the Duffy blood group antigen. Most strains of *P. falciparum*, the leading and the most deadly cause of malaria in people, enter by binding to sialic acid on glycophorin A. To do this, each parasite has specific proteins called erythrocyte-binding antigens, or EBAs, designed to lock onto Duffy or sialic acid depending on the parasite species.

Although the EBAs of *P. vivax*, *P. knowlesi*, and *P. falciparum* are different from each other, Kim Lee Sim and Chetan Chitnis in Louis Miller's laboratory at NIAID have found that an important similarity in the structure of the EBA genes — a conserved, cysteine-rich domain — accounts for the parasite's ability to bind different epitopes on red blood cells. Chitnis and Sim believe that the new cysteine motif has excellent potential as a candidate for a malaria vaccine.

"We think the cysteines give the protein the ability to properly fold into the ... shape needed to bind different epitopes," says Chitnis. "The cysteines provide the scaffold, and the differences in the rest of the amino acid sequence [allow] it to bind to different proteins."

During malaria-parasite invasion, the merozoite attaches randomly onto the surface of the red blood cells, reorients itself so that its apical end points toward the red blood cell, and then prepares to form a junction, or a port of entry, into the cell. Scientists had known that to form a junction, the parasite needs to bind red cells proteins. They have been trying to clone the genes responsible for the parasites' ability to bind red cells in

HOPE FOR A MALARIA VACCINE



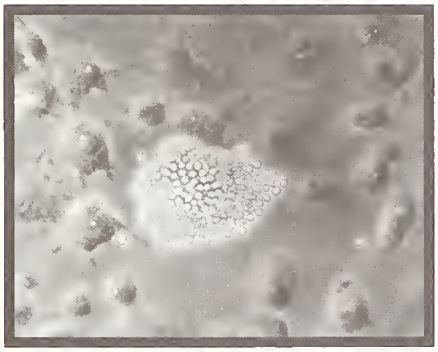
Chetan Chitnis and Kim Lee Sim of NIAID have found that a new cysteine motif allows malaria parasites to bind different epitopes on red blood cells.

the hope that they could devise ways to block invasion.

Three years ago, John Adams at Louis Miller's lab at NIAID cloned the gene encoding the P. ***knowlesi*** EBA. At about the same time, Sim, then at Walter Reed Army Institute of Research at Washington, D.C., and her colleagues identified the gene encoding the EBA of *P. falciparum* and a peptide that elicits antibodies that blocks malaria invasion in mice. Sim and colleagues also found that the parasite's binding protein has a receptor-ligand interaction with red cells and that the binding correlated with how readily the red cell can be invaded. "We also found that red cells from guinea pigs and rabbits, which are not invaded by malaria, do not bind this protein," says Sim. Then last year, Adams, Sim and Miller cloned and sequenced three genes encoding P. ***knowlesi*** 's EBAs and compared their sequences with those encoding P. ***vivax*** 's and *P. falciparum* 's EBAs. They found that all these EBA-encoding genes had a similar structure and noted that the homology among them lay in two cysteine-rich regions. They speculated that these cysteine-rich regions were important for binding to red cells.

Recently, Chitnis tested this speculation by expressing different regions of the vivax protein on the surface of mammalian COS (monkey kidney) cells and comparing their ability to bind erythrocytes. Region II of the vivax protein bound to Duffy positive but not Duffy negative human red blood cells; other regions of the vivax protein did not bind to either Duffy positive or Duffy negative red cells — proof that the red-cell binding domains of vivax and knowlesi proteins do lie in this cysteine rich region. The data, say Chitnis and Miller, provides the first direct evidence that the cloned genes do encode the *P. vivax* and *P. knowlesi* EBAs. Sim, who conducted a similar experiment with the *P. falciparum* EBA gene, came up with a similar proof that Region II of *P. falciparum* is the binding ligand for *P. falciparum*. What was amazing, says Sim, is that the ability of the EBA to bind red cells seems to be wholly a function of the cysteine domain.

Chitnis says the evolutionary conservation and importance of the cysteine-rich domain in *Plasmodium*'s binding make the motif an excellent candidate for a vaccine. "If you can stop [the EBA-red cell] interaction from happening by using antibodies to the cysteine-rich binding domain, then you can block invasion," says Chitnis. "So vaccine [design] can focus on this domain and the hope is that if you can immunize people with just this [region], they will make antibodies that will block invasion," says Chitnis. Moreover, says Chitnis, although parasites often evolve to evade host defenses, minimal variation should occur in the binding domain, again making that region a good vaccine candidate.



Red cell binding by the cysteine rich region of P. vivax *protein expressed on monkey kidney cells.*

Chitnis and Sim are now expressing the *P. falciparum* EBA gene in yeast and making antibodies to it. They are also preparing an in vitro assay to study the ability of parasites to invade red cells in the presence of antibodies.

Meanwhile, an exciting suggestion of a broader importance of the cysteine-rich motif came to Sim and Chitnis from the lab next door. Evidence from David Peterson and Xinzhuan Su in Tom Wellem's lab at NIAID shows that many genes of the malaria parasite contain this motif. One of the genes is very similar to the *P. falciparum* EBA gene and may also mediate erythrocyte binding. Several more genes share many subtle changes in the cysteine-rich domain. These may be involved in other aspects of host parasite interaction. The family of genes identified by Peterson and Su have the same distribution of cysteine residues as the EBAs of *P. falciparum* and vivax, suggesting that the parasite uses this domain in multiple ways. — S.K.■

Recently Tenured

Anthony Basile *came to NIH in 1984 as a staff fellow in the Laboratory of Neuroscience, NIDDK. He is currently a Senior Investigator.*

My laboratory is investigating the neurochemistry of hepatic encephalopathy (HE), a recurrent, neuropsychiatric disorder that accompanies acute or chronic liver failure. HE is most commonly observed in patients suffering from cirrhosis caused by alcohol or liver parasite infestations and constitutes a significant world health problem. The syndrome is characterized by personality disorders, confusion, and motor disturbances, leading to a coma that may last several days. An inexpensive therapeutic intervention that could rapidly reverse the coma and stabilize the mental status of patients while their livers recover would be highly desirable.

One of the neurochemical characteristics of HE is a significant increase in the activity of the primary inhibitory neurotransmitter, γ-aminobutyric acid (GABA). The increased GABA activity is believed to account for global suppression of central nervous system function observed in patients with HE. Agents acting at the benodiazepine (BZ) receptor, which is part of the GABA-receptor complex, are known to enhance the ability of GABA to depress neuronal activity. Substances that bind to the BZ receptor include such widely used drugs as Valium and Xanax. The precise mechanism by which the increase in GABAergic neurotransmission occurs in HE has been a subject of some controversy.

In recent studies, I have measured the changes in the electrical activity of single cerebellar neurons from a rabbit model of HE and found that these cells are more sensitive to depression by GABA than are neurons from normal rabbits. This enhanced sensitivity to depression by GABA could be blocked by administering BZ-receptor antagonists. The BZ-receptor antagonists have no effect on the electrical activity of neurons from normal rabbits. These results suggested that increased concentrations of BZ-receptor agonists could contribute to the pathogenesis of HE in this model. I subsequently found increased concentrations of BZ-receptor agonists in the brain and plasma of several animal models of HE, and in humans who died from acute liver failure with severe HE. Concentrations of BZ-receptor agonists are particularly high in the cerebellum and cerebral cortex, a localization consistant with the psychiatric and motor abnormalities characteristic of HE. Although some of those agents were identified as 1,4-benzodiazepines, subsequent investigations have indicated that six to 12 other substances with BZ-agonist properties are also elevated in HE. Recently, I found that the behavior manifestations of HE could be reversed in animals by administering BZ receptor antagonists. Concurrently, several uncontrolled clinical trials in Europe have indicated that BZ-receptor antagonists may be useful in improving the status of a population of patients suffering from HE. — *A.B.* ■

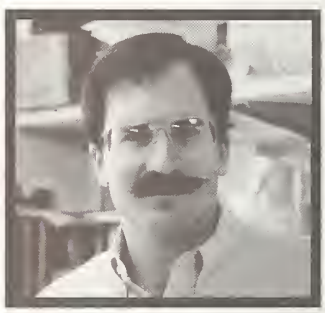
Michael Hamilton, *recently granted clinical tenure as Chief of the Clinical Investigation Section in the NCI-Navy Medical Oncology Branch, came to NIH in 1985.*

Our section of the NCI-Navy Medical Oncology program is responsible for overseeing patient care in inpatient and outpatient wards and clinics, ensuring the progress of clinical trials that grow out of laboratory and clinical interests of the Branch. But we also conduct clinical trials that spring from the discoveries and interests of collaborators in other parts of NCI. We currently enroll 200 to 250 patients per year in our trials, but also care for approximately 1,100 newly diagnosed patients per year who come to the Bethesda Naval Hospital, primarily for treatment of solid tumors. These patients represent an important potential source of clinical materials and subjects for collaborative intramural investigations in cancer prevention, etiology, and pathology, as well as for cancer-treatment protocols.

Our most recent clinical trials will test cancer immunotherapy. In early June, in collaboration with Jeff Schlom, we initiated a phase I trial of a therapeutic cancer vaccine based on *Vaccinia* that has been genetically altered to express the human carcinoembryonic antigen (CEA), a protein expressed in some breast, lung, and gastrointestinal tract tumors. In collaboration with Jay Berzofsky, we are now planning an immunotherapeutic trial targeting mutated *p53* in lung cancer patients. Within the next 18 months, we also hope to launch trials targeting *ras* mutations.

I developed my experience in the design and management of multi-institution clinical trials during the seven years I spent in the Cancer Therapy Evaluation program in NCI's Division of Cancer Treatment. My work there included supervising the large National Adjuvant Trials that examined the value of adjuvant chemotherapy (such as 5-FU and levamisole) in postsurgical treatment of colon cancer patients. — M.H. ■

Frederic Kaye *began Fellowship training in Medical Oncology at the Clinical Oncology Program at NCI in 1984. He has been on the Senior Staff of the NCI-Navy Medical Oncology Branch since 1988.*



My laboratory group studies the role of tumor-suppressor genes in the etiology of human malignancy. We have focused our efforts on the retinoblastoma gene (*RB*), which we identified as a target for mutational inactivation in the majority of small-cell lung cancer samples. In addition, we have isolated and characterized a series of stable mutant RB products from these human tumor samples, and from this have delineated important "tumor-suppressor" domains within the *RB* protein. We have also demonstrated that the tumorigenicity of *RB*(-) lung cancer cells can be suppressed by the stable reintroduction of the wild-type *RB* gene.

We are currently interested in studying mechanisms of RB-mediated tumor suppression. One approach we have taken is to identify and define

the functional properties of a new family of cellular *RB*-binding peptides. We believe that the study of these binding proteins will provide important clues to pathways that control cell growth and differentiation in *RB*-meditated tumor suppression. — F.K. ■

Paul Klotman *first came to NIH in 1989, on sabbatical from Duke University. He decided to stay and is now chief of the Viral Pathogenesis Section of NIDR's Laboratory of Oral Medicine.*

My laboratory's research has focused on the role of extracellular matrix molecules and their receptors in HIV-related syndromes, such as HIV-associated nephropathy. We have generated transgenic mouse models to express HIV's regulatory and envelope genes and have found that these mice develop a syndrome that resembles HIV-associated nephropathy in man. My laboratory demonstrated that HIV-1 infection induces the expression of specific integrins on the T-cell surface that allow the cells to adhere to components of extracellular-matrix-proteins in kidney tissue. Once adherent, HIV-infected T cells and macrophages become more invasive and express elevated levels of proteolytic enzymes that degrade normal tissue architecture. I believe that this constant remodeling of kidney tissue with simultaneous damage and regeneration contributes to kidney destruction because the organ is dependent upon normal structure to achieve normal function. What remains unclear is whether kidney tissues can sustain viral replication. In addition to the findings in the kidney, we have observed that transgenic mice manifest psoriasiform lesions and a syndrome of growth retardation that has many similarities to that observed in pediatric AIDS patients. My laboratory is currently focusing on ways to use these mice to explore potential molecular-based therapies for AIDS and HIV-associated nephropathy. — P. K. ■

Andrew Larner, *a staff scientist at the FDA's Center for Biologics Evaluation and Research, came to NCI's Laboratory for Pathology in 1985. He joined CBER in 1988.*

My research has focused on understanding the mechanisms by which interferons (IFNs) activate the transcription of early-response genes that govern a cell's initial response to cytokine signals.

Members of my laboratory have designed a cell-free system that permits IFNs to activate the assembly of several transcription complexes. Using these systems, we have been able to demonstrate that both a membrane-associated tyrosine kinase and phosphatase are required to activate the formation of these complexes. We now want to determine the molecular relationships between the IFNα or IFNγ receptors, the tyrosine phosphatase(s), and the tyrosine kinase(s), and the transcription factors, as well as to investigate whether there are any other molecular interactions needed for signaling by these cytokines.

Phorbol esters and the expression of the oncogenic viral proteins, adenovirus E1A, and the human papilloma virus (HPV) E6/7 gene products inhibit IFN-induced gene expression. Several cell lines derived from human cancers that are IFN-insensitive are also defective in interferon signaling. Inhibition of IFNα-induced formation of the transcription complex ISGF3 in these cell lines involves the expression of a cellular "competitor," which disrupts the formation of the ISGF3 transcription complex. I would like to understand the mechanism by which this competitor(s) functions, both because our studies indicate that this may be a novel means to regulate assembly of the ISGF3 transcription complex, and because such a repressor may be an important factor in cell transformation. Using an HPV⁻ cervical carcinoma, we are purifying a protein that disrupts the formation of ISGF3.

In certain types of cells, such as human fibroblasts, IFN-induced gene transcription is greatly diminished after cells are treated with IFN for 6 hours. We are now completing a series of experiments that indicate that a nuclear protein tyrosine phosphatase is partially responsible for decreasing the transcription of these early-response genes with extended exposure to IFN. These studies provide the first evidence that tyrosine phosphatases located in the nucleus play an important role in the regulation of gene expression. — A.L. ■

Peter Nara, *Acting Head of NCI's Virus Biology Section of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, came to NIH in 1984 as a medical staff fellow.*

The natural course and subsequent dynamics of lentiviral transmission and disease, following infection of nonhuman and human primates and other animals, provide a basis for our understanding of the human immunodeficiency virus - type 1 (HIV-1). My laboratory's effort over the past 5 years has been to characterize the mechanisms of host responses and viral genetic variation and escape from humoral immunity, and to develop physiologically relevant, in vitro tissue-culture systems for HIV-1, with the goal of applying this information to the development of an effective AIDS vaccine. The work has provided information on the molecular mechanisms and processes of immunoglobulin-mediated viral inactivation (neutralization) and identification of epitopes on the viral envelope (gp120/41), such as the hypervariable region (V3), that are subject to neutralization.

Studies of viral variation and host immune response in HIV-1-infected chimpanzees have provided insights into the dynamics of early viral-replication events. These studies demonstrated that clonal expansion of a virus subpopulation occurs initially. Subsequently, humoral selection and viral variation lead to

continued on page 12.

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continued from page 11.

sequential replacements of closely related virus populations. The most important observations from the study were the recognition that viral escape from humoral immunity is mediated by a mechanism called "distant-site variation" with evidence of "original antigenic sin" (OAS), a phenomenon described earlier for the influenza virus. OAS, in essence, serves to limit the host's polyclonal humoral immune response for a given multi-epitopic antigen. In the case of HIV-1, it appears that an effective neutralizing antibody response is made to the V3 loop of the initial strain of HIV-1 infecting a host; however, spontaneously occurring neutralization escape mutants (which showed no evidence of amino acid substitutions in V3) are poorly neutralized by the animal's serum. The mechanism for this phenomenon appears to be the continued expansion of B-cell clones (stimulated initially following infection) by the antigenically closely related viral escape mutants.

These data, as well as other studies, have led us to the notion that the HIV-1 envelope may have evolved epitopes (decotopes) that are capable of decoying and disregulating the normal polyclonal humoral and cell-mediated immune responses to the viral populations that establish themselves after infection. We are currently pursuing novel strategies for refocusing the immune responses away from decotopes to the more conserved epitopes, which are responsible for viral infectivity and pathogenesis. We are also collaborating on clinical studies of people undergoing the acute phase of HIV-1 infection and attempting to identify the in vivo sites of viral replication; of the quantitative, biophysical, and genetic properties of the virus; and of the innate and specific immune responses that serve to limit, control, or prolong viral replication in the body. — P.N. ■

Michael Norcross *joined NIH as a postdoctorial fellow in NIAID's Laboratory of Immunology in 1982. He is now a Staff Scientist in the*

Food and Drug Administration's (FDA's) Center for Biologics Evaluation and Research.

Our research is directed at the early molecular events of HIV-1 binding and entry into human cells. We are also studying how these events modify the immune function of receptive cells. Our laboratory is characterizing the host-cell surface molecules that mediate virus entry, and we have found that one component of this pathway is a surface proteoglycan related to heparin. These heparan sulfate proteoglycans function in concert with the CD4 receptor and other surface proteins during viral binding and fusion events.

We find that, following virus binding, multiprotein complexes are formed of host and viral proteins. These complexes allow penetration of the virus while generating functional signals that can be detected in vitro. Depending on the extent of T-cell-receptor engagement, these co-stimulatory signals lead to either negative or positive functional T-cell activity, which is consistent with the clinical picture of both T-cell dysfunction and hyperactivity in AIDS.

Currently, a major focus of our research is the identification and cloning of the other molecules in these virus - host-cell complexes that are responsible for the various cell tropisms of different strains of HIV-1, including types that infect normal monocytes and primary T cells. Our goal is to clarify molecular mechanisms of virus-cell interactions to provide a foundation for the development of AIDS vaccines and therapies. — M.N. ■

John O'Shea, *Acting Head of the Leukocyte Cell Biology Section in the Laboratory of Experimental Immunology (LEI), Biological Response Modifiers Program, Frederick Cancer Research and Development Center, NCI.*

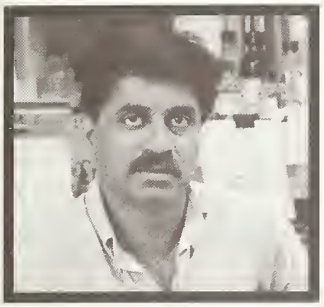
My interest since coming to NIH in 1981 has been the structure and function of receptors on cells of the immune system. Initially, I worked with Michael Frank, John Gallin, and Eric Brown in NIAID on complement receptors on phagocytic cells. I then joined Rich Klausner and Larry Samelson to study signal transduction mediated by the T-cell-antigen receptor.

In 1989, I started my own group in LEI in the Biologic Response Modifiers Program, focusing on the role of protein tyrosine kinases in lymphocyte activation. Because of the interest in large granular lymphocytes, or natural killer (NK) cells, in the LEI, I also became interested in receptor-mediated signal transduction in NK cells. We and others showed that the Fc receptor on NK cells was structurally and functionally very similar to the T-cell receptor (TCR). We showed that it also uses a signal-transducing subunit (TCR-zeta) that was thought to be associated only with the TCR. Also, through its interaction with TCR-zeta the receptor is uniquely associated with and coupled to a non receptor protein tyrosine kinase. We showed that protein tyrosine kinase activity is essential for receptor-mediated signaling.

Our continued comparative study of receptor-mediated signaling in T cells and NK cells has led us to the cloning of two novel protein tyrosine kinases with extremely limited tissue expression. These kinases are expressed in inactivated NK cells, but not in resting T cells. They are expressed in T cells and monocytes upon activation. One kinase is a member of the Janus family of protein tyrosine kinases, which have duplicated catalytic domains and appear to be critical to the relay of signals from cytokine receptors. The other kinase is homologous to Csk, or c-src kinase, a kinase-kinase. Csk negatively regulates the function of src-family tyrosine kinases by phosphorylating the conserved carboxy-terminal tyrosine. We are now elucidating the function of the two NK-cell tyrosine kinases.

We also study the expression of T-cell-signaling molecules in lymphocytes derived from animal and human cancer. We made the rather surprising observation that the zeta chain of the TCR and the T-cell-specific tyrosine kinase, Lck, are downregulated in T cells isolated from animals and humans with tumors. We documented this downregulation in two mouse models, and our results have been reproduced in other models around the country. Our latest data show that more than 90% of patients with renal cancer have abnormalities in their tumor-infiltrating lymphocytes. We are currently trying to dissect the cell biology behind these defects. — J.O. ■

Arun K. Seth *joined NCI in 1982 as a visiting fellow in the Laboratory of Molecular Oncology (LMO), NCI. He currently heads a group of seven researchers.*



My research in the Cellular Biology and Biophysics Section at NCI's Frederick Cancer Research and Development Center focuses on two primary areas: the functional role of proto-oncogenes, particularly the ets family of genes, as transcription factors and the genetic events involved in the progression of breast cancer.

While at the Basic Research Program in the Molecular Mechanisms of Carcinogenesis Laboratory in Frederick from 1983 to 1987, I developed methods for production, purification, and molecular characterization of the proto-oncogene protein mos. This work made it possible to demonstrate for the first time that the protein sequences of v-mos and c-mos are identical and that the *mos* product binds ATP and possesses ATPase activity.

Since 1987, my work at LMO has focused on understanding the functions of the ets family of genes in cells and animal systems. My colleagues and I showed that ets genes could affect the growth properties of mouse and human cells, and using gene-transfection studies, we demonstrated that the *ets*-1 and *ets*-2 genes could transform certain mouse and rat fibroblasts and cause tumors in nude mice. We went on to show that the *ets* proteins could activate gene transcription by binding to specific purine-rich DNA sequences that have been characterized as ets binding sequences (EBS). These are now known to be present in a variety of promoters and enhancers of many eukaryotic genes, ranging from *Drosophila* to humans. Our work has shown that the *ets* -1 gene is regulated by its own product and that the *ets*-1 protein can transactivate transcription from various cellular and viral promoters and enhancers, including p53 and HIV-1. We are also using mice bearing *ets*-1 and *ets*-2 transgenes under the control of heterologous promoters to study the genes' role and expression during murine embryogenesis.

Recently, I have used the methods of subtractive-hybridization and differential display, using cDNA from normal and malignant human breast cancer cells and tissues, to identify and clone genes that are specifically expressed at different stages of malignant disease. To date, our group has cloned and sequenced more than 20 genes, most of them unique, that are differentially expressed in normal and malignant tissues. We also have shown that p53 gene mutations are less involved in male breast cancers than in female malignancies and have described several novel p53 mutations in sporadic breast tumors. — A.K.S. ■

Unnur Thorgeirsson *came to NIH in 1979 as a visiting scientist in the Laboratory of Pathology, NCI. Since 1987, she has led a research group under the Office of the Director, Division of Cancer Etiology, NCI.*

For the past 12 years, I have been studying different aspects of tumor invasion and metastasis. While in the Laboratory of Pathology at NCI, I used an amnion-invasion assay to demonstrate that pro-

tein synthesis, not DNA synthesis, is needed for tumor-cell invasion, and that tissue-inhibitor-of-metalloproteinases (TIMP-1) blocks invasion. I discovered that the invasive and metastatic phenotype, as well as increased type-IV collegenolytic activity, could be conferred on NIH/3T3 mouse fibroblasts through transfection with activated H-*ras* oncogenes. After joining the Division of Cancer Etiology, I continued my work on *ras*-mediated stimulation of type-IV collagenolytic matrix metalloproteinases (MMP). I discovered that a 92-kDa gelatinolytic-type IV collagenolytic MMP was induced simultaneously with the metastatic phenotype in the ras-transfected NIH-3T3 cells. In a rat mammary-carcinoma model carrying a mutated H-ras, I found an association between the 92-kDa MMP expression, increased type-IV collegenolytic activity, and tumorigenicity, but not metastatic capability.

My work has recently started to focus on the multifaceted role of endothelial cells in tumor progression. My coworkers and I have established an immunomagnetic separation technique to obtain pure populations of endothelial cells from a mixed cell population for in vitro studies. We have new data suggesting that interleukin-1-activated endothelial cells contribute significantly to tumor-cell escape from the vasculature through polarized secretion of proteinases toward the underlying basement membrane. Our projects are now centered on human breast carcinoma, and specifically, on angiogenic factors and on the differences between tumor and normal microvascular endothelium. We are also evaluating the role of TIMP-1 as an antiangiogenic factor in breast cancer. Our hope is that further knowledge of tumor angiogenesis and of the special features of endothelial cells lining the tumor microvessels will eventually lead to new therapeutic strategies directed at the vasculature in breast cancer. — U.T. ■

Robert Walker *came to NIH as a staff fellow in 1986. He returned in 1990, joining the Clinical and Molecular Retrovirology Section of NIAID's Laboratory of Immunoregulation, where he is now a Senior Investigator.*

My work centers on the design and execution of clinical investigations related to HIV infection and associated opportunistic diseases. Reflecting the research interests of NIAID's Intramural AIDS programs, this work focuses on immune-based therapeutic strategies and on newer antiretroviral drugs.

Because HIV infection results in progressive CD4-lymphocyte depletion and severe immunodeficiency, candidate immunotherapies for AIDS have been directed at restoring CD4 numbers and function. We have been studying adoptive immunotherapy in the syngenic subjects — identical twins — for several years. These projects have

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Commentary

NICHD's Conjugate-Vaccine Revolution

by Birgit Au der Lan, Laboratory of Developmental and Molecular Immunity, NICHD

Two years ago, the U.S. Public Health Service and the American Academy of Pediatrics recommended that pediatricians routinely administer a vaccine that protects infants and young children against meningitis and other serious diseases caused by the *Haemophilus influenzae* type b (Hib) bacterium. This vaccine has already had a profound effect on the health of the nation's children, as reported in three articles in the *Journal of the American Medical Association* this past January. Before the vaccine was introduced, Hib was the leading cause of bacterial meningitis in the United States. Even when the meningeal infection is successfully treated with antibiotics, children are often left mentally retarded. Indeed, the incidence of hospitalization due to Hib infections has dropped dramatically—to 10% of its previous level, and it is still declining. Adams et al. (1) estimate that the vaccine has prevented between 10,000 and 16,000 cases of Hib infection and between 500 and 800 deaths in the United States. In countries such as Finland and Greenland, where nearly all children are immunized, Hib-related diseases have been virtually wiped out.

"This remarkable achievement exquisitely reflects the bench-to-bedside payoff inherent in the NIH's unique intramural research milieu," says Arthur Levine, scientific director of NICHD. Two NICHD scientists, John B. Robbins and Rachel Schneerson, were the first to make a Hib vaccine that protects infants. But the conjugation technique they developed for this purpose has enabled them to cast a much wider net, developing vaccines against many other life-threatening bacterial diseases. Their collaboration began 25 years ago at the Albert Einstein College of Medicine in New York, when Robbins was an associate professor of pediatrics and Schneerson was an instructor in the same department. Two years later, they came to NIH and have been on campus ever since.

Many serious bacterial diseases, such as typhoid fever and cholera, are caused by pathogens that bear surface polysaccharides, either in the form of an antiphagocytic capsule or as lipopolysaccharides. Although most of these polysaccharides produce antibodies when injected into adults, they are either relatively poor immunogens or have undesirable pharmacological properties, say Robbins and Schneerson. Robbins directs the NICHD's laboratory of Developmental and Molecular Immunity, and Schneerson heads the Section on Bacterial Disease Pathogenesis and Immunity within that Laboratory. Polysaccharides are so-called T-cell-independent antigens; they do not elicit a booster response, and they are not immunogenic in children under 2 years old, the population most at risk for such diseases. These scientists have spent the past 25 years perfecting techniques to overcome these problems.

(From left) Shou Sun Szu, Rachel Schneerson and John Robbins of NICHD.

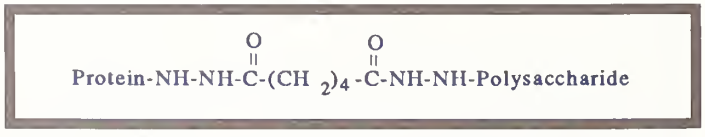
Old Idea Leads to New Vaccines

Robbins and Schneerson used a principle proposed in the late 1920s, first by Landsteiner and then by Avery and Goebel (2, 3), by which small nonimmunogenic haptens are made immunogenic by linking them to proteins. For the Hib vaccine, the scientists link the relatively unreactive, poorly immunogenic polysaccharide to a bifunctional, nucleophilic spacer molecule. This adipic dihydrazide derivative of the polysaccharide is then attached to a suitable protein, such as tetanus toxoid, by carbodiimide condensation. These reactions are mild enough to retain the immunogenic properties of both the protein and the polysaccharide; conjugation with a protein confers T-cell dependence to the polysaccharide, thus making the antigen immunogenic in infants.

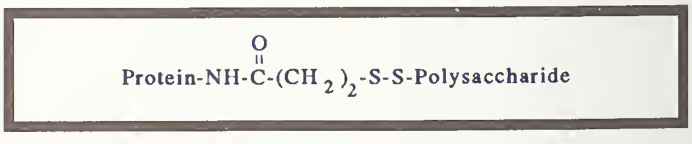
Now manufactured commercially, the Hib vaccine is the first conjugate vaccine to have come into wide use and thus to have made an impact on public health in this country. Robbins and Schneerson have many other vaccines in various stages of development; several have already been shown to be immunogenic in animal studies, and several have advanced to the stage of clinical testing.

When making vaccines with polysaccharide antigens, conjugation is only half the battle—first the polysaccharide must be isolated intact and without loss of its immunogenic properties. Once this has been achieved, the conjugation technique devised for the Hib capsular polysaccharide can be applied to many other polysaccharides. Using the adipic acid dihydrazide spacer, the group has prepared synthetic vaccines against several pathogens, including group B streptococcus (GBS), several pneumococcus types, and most recently, *Cryptococcus neoformans,* the first vaccine ever produced against a fungus.

continued on page 18.



Protein linked to polysaccharide with the N-succinimdyl 3 - (2-pyridyldithio) proionate heterobifuntional reagent. Szu used this type of linker to attach the Vi capsular polysaccharide to recombinant P. aeruginosa *exotixin A. protein.*



Protein linked to polysaccharide with the adipic dihydrazide homobifunctional reagent. Robbins and Schneerson used this type of linker to attach the Hib capsular polysaccharide to tetanus toxoid protein.

Commentary

Autoimmune Diseases: The Role of MHC Class I

Dinah S. Singer, NCI
Edna Mozes, NCI
Leonard D. Kohn, NIDDK

Autoimmune diseases are the result of a breakdown of the immune system, in which excessive B-cell and T-cell reactivity against self molecules results in tissue damage and, ultimately, destruction. A wide array of diseases are autoimmune, including systemic lupus erythematosus (SLE); Grave's disease and Hashimoto's disease of the thyroid; rheumatoid arthritis; myasthenia gravis; and insulin-dependent diabetes. Although environmental factors play a role in the generation of autoimmune diseases, genetic studies have implicated the major histocompatibility complex (MHC), termed HLA in humans, as an important factor in predisposition to disease (1). The proteins encoded by MHC genes fall into four major classes: the class I molecules that trigger cellular immune responses to intracellular pathogens; the class II molecules that activate helper T cells (CD4+) to generate both humoral immune responses to extracellular pathogens and cellular responses to intracellular pathogens; components of the complement pathway; and the recently described components of the peptide transporter system. Because class II molecules function to regulate immune responses, it has been presumed that they play a pivotal role in the induction of autoimmune diseases. But our recent studies have now implicated the class I molecules as having a role in the induction of at least one autoimmune disease, namely SLE (2).

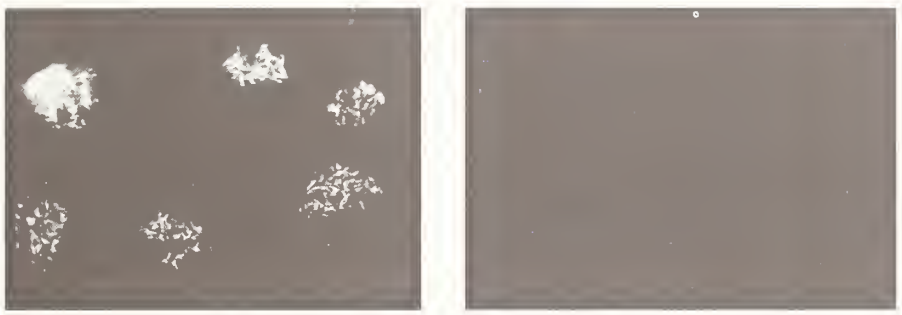


Figure 1. Class I⁻ mice do not develop immune-complex deposits in their kidneys following immunization with 16/6Id. Kidneys from control class I+ mice (left) and class I - deficient mice that had been treated with 16/6Id were tested for the presence of immune-complex deposits. Kidney sections were stained with fluorescein-labeled goat antibodies to mouse immunoglobulin, which will detect any immune complexes. (Reproduced from E. Mozes, L. Kohn, and D. Singer, Science **261**, *91)*

In most autoimmune diseases, affected individuals make antibodies against normal cellular components (3). In some cases, these autoantibodies are largely restricted to a specific organ; in Graves' disease or Hashimoto's thyroiditis, patients have circulating antibodies against thyroglobulin, thyroid peroxidase and/or the thyroid stimulating hormone (TSH) receptor. In other cases, the autoantibodies are targeted at components common to many cells. For example, in one of the best-studied autoimmune diseases, namely SLE, autoantibodies are produced against DNA and nuclear proteins (4).

Although autoantibodies may be ultimately responsible for the pathogenesis of disease, both antibodies and helper T cells are capabl e of eliciting disease, as deduced from studies of experimental SLE in mice (5). In the mouse model, an SLE-like syndrome is induced following immunization of mice with a human monoclonal anti-DNA antibody, called I6/6Id, that was derived from an SLE patient. The sequelae observed include generation of mouse antibodies to 16/6Id, production of anti-DNA antibodies, and antibodies to nuclear proteins, leukopenia, proteinuria, and immune-complex deposits in the kidney (5). Helper T-cell (CD4+) clones specific for the 16/6Id can also induce disease when injected into histocompatible, naive recipient mice (6). Because both antibody and helper T cells are activated through peptides bound by class II receptor molecules, it had largely been presumed that susceptibility to SLE is linked to class II - mediated immune responses.

But we considered another possibility — namely, that abnormal MHC class I expression might trigger certain types of autoimmune disease. Unlike MHC class II molecules, which are only expressed on B cells and antigen-presenting cells (APC6), class I molecules are expressed on nearly all somatic cells, with the exception of brain and germ-line cells (7). However, the level of class I expression differs markedly (but distinctively) among the tissues, such that expression is highest in the lymphoid tissues and, at varying lower degrees, in other tissues. Levels of class I expression in the thyroid, kidney, and liver are among the lowest. This tissue-specific expression is regulated through transcription by the interaction of a silencer factor with a negative regulatory element (8). Although tissue-specific regulation establishes a homeostatic level of class I transcription, additional regulatory mechanisms dynamically modulate that level of transcription.

An example of dynamic regulation of class I transcription by hormones is found in thyrocytes. Thyrocytes cultured in the absence of TSH do not synthesize or secrete thyroid hormone and express moderate levels of class I molecules (9). Treatment of these thyrocytes with TSH triggers a cascade of events associated with normal thyroid function, culminating in the secretion of thyroid hormone (10). We found that concomitant with these TSH-mediated events, transcription of class I genes was repressed (9). Furthermore, secretion of the endproduct, thyroid hormone, induced class I transcription (C. Giuliani, M. Sji, D. Singer, and L. Kohn, unpublished observations). Thus, hormones that regulate tissue-specific functions dynamically regulated class I transcription in the thyroid, and could, potentially, do so in all endocrine tissues.

If class I molecules simply provide immune surveillance for intracellular pathogens such as viruses, why should their expression be so tightly regulated both homeostatically and dynamically? Although class I molecules serve as receptors for viral peptides during a viral infection, under normal conditions, the class I molecule binds cellular peptides and presents them on the cell surface. Tolerance to a basal level of common self-peptides is acquired during thymic "education" of T cells. We speculated that to avoid breaking tolerance and inducing autoimmune responses, class I expression is actively repressed at a time when self-peptide is being synthesized abundantly, such as during hormonal stimulation of an endocrine tissue.

A corollary to this hypothesis is that reduction of class I levels might be protective against autoimmune diseases. Indeed, we found that a drug normally used to treat Graves' disease, methimazole, suppresses class I transcription (11). To test this theory directly, we examined the role of class I molecules in the induc-

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Bone Marrow Unit Chief
continued from page 1.

leukemia (CML) and regenerating the patient's blood cells with primitive marrow cells donated by a relative. A few weeks later, in what Barrett hopes will be a promising new twist, he will perform a second transplant, this time giving the patient mature T cells from the donor. Barrett hopes the T cells will destroy residual leukemic cells lingering in the patient.

The procedure is at once "a safer approach, not highly experimental, but novel," says Barrett, who arrived at NIH in early May. Since May, Barrett has been assembling the extensive team of NIH experts—from diagnostic specialists and radiotherapists to anesthesiologists, nurses, pharmacists, and physical therapists—that must be in place for the first transplant.

John Barrett heads NHLBI's new Bone Marrow Transplant Unit.

Tracing the history leading up to the new protocol, Barrett says that allogenic transplantation at NIH in the early 1970s was not as successful as people had hoped. Since then, researchers have struggled to refine radiation techniques, immunosuppressive drugs, treatment of post-transplant infections, and transplantation procedures. Although some of these measures brought significant improvement, others produced "a series of false starts—each a great leap sideways," Barrett quips.

For example, scientists thought they had discovered the key to successful allogenic transplants when they found that removing mature T cells from the donor marrow greatly reduced graft-versus-host disease, a serious immune complication in which donated cells attack host tissues. "I joined in the enthusiasm for T-cell depletion in the mid 1980s. Our initial impression was that the technique was great. Patients were up and out of the hospital in a month," says Barrett. Researchers suspect that immature donor cells, unlike fully differentiated T cells, can somehow be "re-educated" to recognize the recipient's tissues as "self," thus preventing graft vs. host reactions.

Elation gave way to disappointment, however, as the patients receiving the T-cell-depleted marrow showed a much higher rate of leukemic relapse than did patients who received transplants not depleted in T cells. In studies conducted in his previous position at the Hammersmith Hospital in London, Barrett found an effective treatment for the relapses: a transplant of the donor's circulating T cells. In an apparent graft-vs.-leukemia effect, the donor's blood lymphocytes preferentially seek out and destroy the recipient's leukemic cells.

By building into his scheme both types of transplants—a month apart—Barrett hopes that his new NIH protocol will give patients the best of both worlds—the graft acceptance and good recovery engendered by the T-cell-depleted transplant, and the graft-vs.-leukemia effects and specific beneficial immune reactions of the mature T-cell transplant.

In addition to performing the transplants, a key part of Barrett's work will be analyzing the cellular and molecular interactions associated with the two transplantation procedures. "It's sobering, really," says Barrett. "We've been doing bone marrow transplantation for 20 years, yet we still have 20% mortality for [transplants between] sib pairs ... We don't know nearly enough about tolerance, minor antigen matching, or the genetic basis of the whole system." Barrett says that answers to these and other questions could improve immune therapy as well as bone marrow transplantation.

Scientists don't even have a clue about answers to such basic questions as how the adult body, lacking thymus tissue, is able to re-educate donor marrow cells, says Barrett. And, he adds, "Why there is sometimes a graft-vs.-leukemia response but not a graft-vs.-host response is not clear." An understanding of the graft-vs.-leukemia response, combined with gene-modification techniques, could allow scientists to make improvements to T cells that would boost their longevity and anti-cancer powers. Barrett says that scientists are just beginning to understand the operation of minor histocompatibility antigens that differ from tissue to tissue and that may to be a key to graft rejection, graft-vs.-host disease, graft-vs.-leukemia effects, and post-transplant infections.

As Barrett and his team build up their requisite experience with the first 40 pairs of donor-recipient siblings, he is thinking ahead to other projects. He hopes to branch out to transplants between unrelated individuals, and hopes to collaborate on gene-therapy protocols, including treatment of Fanconi's anemia, that would be based on genetic correction of primitive bone marrow cells. Barrett will also assist ongoing intramural protocols involving autologous marrow grafts, in which a cancer patient's marrow is temporarily removed and then given back. This operation is used, for example, to protect the marrow from powerful chemotherapy that would otherwise kill sensitive marrow cells at the concentrations needed to destroy malignant cells.

Barrett is excited about the projects that are possible at NIH, thanks to a diverse array of collaborators. "There are a lot of things we are in a fantastic position to do at NIH," he says. "The facilities are breathtaking, and there is an amazing field of collaborative potential with many keen, highly intelligent people who are very interested in this research." ■

We are looking for a few good copies of our first [February] issue. Over the past few months, our office has received a number of requests for back issues of *The NIH Catalyst*. We are always happy to oblige, but we have run out of copies of the first issue. Because the cost of reprinting is prohibitive, we are asking for your help in rounding up extra copies. If you happen to have extra copies of the February issue, please send them to us by mail. [For address see back panel.] ■

Clinicsl Center Management Fund
continued from page 1.

recommended this basal bed cost as a fair way to minimize the fluctuations in the budget throughout the year. We also suggested that a system be established to enable Institutes to rent beds or outpatient clinics to provide a mechanism for meeting transient changes in programmatic requirements. After lengthy discussion, the 1989 recommendations were not accepted.

The Clinical Center budget problems did not go away. In 1990, a retreat was held in Easton, Md., for NIH Institute Directors, Scientific Directors, Clinical Directors, and other senior officials to review several issues related to the Clinical Center. Three changes in the budget-assessment process were adopted: adjustments to the budget would be made annually rather than quarterly, thereby eliminating midyear taps on Institutes; budgets would be determined retrospectively, based on the previous year's spending; and budgets would be determined largely by bed allocation. A Clinical Center Oversight Committee to advise the Clinical Center Director on broad issues was also recommended but not implemented.

The changes made after the Easton Retreat brought about significant improvement in the system, most notably, the elimination of midyear taps on Institute budgets. However, certain difficulties remained. The inflationary costs of maintaining Clinical Center vital services increased faster than congressional appropriations for intramural research, adding to the strain on NIH intramural budgets. In addition, the old assessment process, based solely on bed allocation, remained inequitable. Variation in programmatic requirements of different institutes led to differences in the number of beds per square foot of space designated for patient care; thus, some Institutes got much more space per dollar than did other institutes.

Other problems persisted. Programmatic changes by one Institute affected the budget of others. There were no cost incentives to encourage clinical research. Space utilization within the Clinical Center was not reviewed to ensure that it was being used for clinical research. There was no review mechanism for the use of the Clinical Center reserve fund, which had been created to cover unexpected emergencies, and there was little, if any, prospective budget planning at the Clinical Center and the various Institutes.

John Gallin heads the Clinical Center Cost Management Committee.

As a consequence, in November 1992, the new NIH Deputy Director for Intramural Research, Lance Liotta, asked me to convene a committee once again, to evaluate Clinical Center management-fund-assessments. The Committee had representatives from all segments of the NIH community and included Drs. Duane Alexander, James E. Balow, Gregory Curt, Cherie Fisk, and Mark Hallet and Mr. Steven Berkowitz, Ms. Yvonne du Buy, Mr. Stephen Ficca, Ms. Anabel Holliday, Ms. Francine Little, Mr. Gerald Macks, Ms. Kathryn McKeon, Mr. Gerald Osborne, Mr. C. John Slovikosky, and Mr. Hillel Soclof. The Committee completed its report April 1, 1993, and recommended major changes in the mechanism for Clinical Center assessments. We suggested that the Clinical Center budget be divided into four parts: a fixed-cost component based on space and service; variable costs including prospective and retrospective components; and a reserve component for unexpected events. Features of the new system include the following:

- The fixed-cost component, based on space assignments, establishes a fair and clearly defined basis for assigning overhead costs. This component includes a basal access cost to Clinical Center Service Departments.
- The cost of patient-care space will be intentionally set at a much more expensive rate than other Clinical Center space. This high cost for patient care space will make it difficult to use space set aside for patient-care for anything but clinical research.
- A mechanism for Institutes to return, rent, or "moth-ball" space was established. Relinquishing space should save Institutes money.
- Prospective planning has been emphasized. Annually, through an interactive process with Clinical Center management, each Institute will plan its Clinical Center budget. Institutes will be bound to their budget commitments and the Clinical Center will guarantee delivery of promised services at the agreed price.
- An accounting system to track the use of services was introduced to define cost elements.
- Programmatic decisions by one Institute should not affect other Institutes over the short or long term.
- Institute Directors and the NIH Deputy Director for Intramural Research will periodically review space utilization.
- The Medical Board and Scientific Directors will review the use of the Clinical Center reserve fund.
- The new Management Fund Assessment policy is an "experiment" and will be reviewed after three years.

The recommendations were reviewed and approved by the NIH Medical Board, the NIH Board of Scientific Directors, the NIH Institute Directors, and, on June 16, 1993, by Bernadine Healy before she stepped down as Director of NIH.

To assist in the implementation of the new Clinical Center Management Fund Assessment Policy, a new Clinical Center Advisory Board has been established. This Advisory Board will be charged with meeting the objectives of a similar Board proposed at the Easton Retreat of 1990. The Clinical Center Advisory Board will advise the Clinical Center Director and the NIH Deputy Director for Intramural Research regarding issues of Clinical Center space, staffing, and budget. We expect that the new Management-Fund-Assessment policy, together with the Clinical Center Advisory Board and all the other groups already interacting with the Clinical Center, will provide a fair, flexible structure that will support intramural clinical research initiatives into the next century. ■

RECENTLY TENURED
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involved bone marrow and peripheral blood lymphocyte transfers from healthy, HIV-seronegative individuals to their HIV-infected twins. In a current protocol, peripheral blood lymphocytes from the uninfected twin undergo ex vivo expansion and activation with IL-2 and anti-CD3 antibodies and are then transferred to the HIV-infected recipient twin. Preliminary results indicate that such transfers are safe and generally well-tolerated, but are associated with only short-term immunologic benefit. In collaboration with Micheal Blaese's group at NCI and with Harvey Klein's group in the Clinical Center's Department of Transfusion Medicine, we are now initiating a clinical trial to study survival of gene-modified CD4 and CD8 cells in the peripheral blood of HIV-infected patients after the cells are transferred from the identical twin. Syngenic subjects also provide an ideal system for testing candidate gene therapies for HIV infection, and much of our current work with cell transfers will lay the groundwork for further trials with gene-modified cells.

We are also studying the adenine analogue 9-(2-phosphonylmethoxyethyl)adenine (PMEA), in a phase I-II clinical trial. PMEA is thought to act as an inhibitor of the viral enzyme reverse transcriptase. Nucleotide analogues such as PMEA have theoretical advantages over zidovudine and other nucleoside analogues, including longer persistence within immune cells and the ability to penetrate monocyte/macrophages that may serve as a reservoir of HIV. Preliminary results from the human study show evidence of antiretroviral activity at tolerable doses. Future studies will investigate the potential of agents that block tumor necrosis factor-α in the treatment of HIV infection. — R.W. ■

The SDs have invited the following to give lectures in 1993-94.

Mider Lecture	•Anita Roberts and •Michael Sporn, NCI
Dyer Lecture	•Flossie Wong Staal, NCI
NIH Lecture	•Mary-Claire King, Univ. of Calif., Berkeley •Christiana Nusslein-Vollhard, Max Planck, Tubingen, Germany •Kathryn Zoon, SD, CBER/FDA •Patricia Donohue, Mass. General Hospital, Boston
NIH Cultural Lecture	•Jane Alexander, Actress and Director-Designee, Nat. Endow. for the Arts ■

VACCINE
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Neonatal Sepsis and Meningitis

GBS is the leading cause of neonatal meningitis in the United States. It is a natural inhabitant of humans—25% of all women carry the bacterium in their genital tract, and most transfer enough antibodies to their infants to protect them, if the infants are born at term. But for reasons not yet understood, some mothers do not make enough antibodies against this organism. Their infants and all premature infants are at risk for neonatal sepsis caused by GBS. According to Schneerson, the development of a GBS vaccine that induces protective numbers of IgG antibodies in serum-negative women would provide a useful mechanism for preventing of neonatal GBS disease. Clinical trials of a possible GBS vaccine, GBS capsular polysaccharide conjugated to tetanus toxoid, are scheduled to begin soon.

Antibiotic-Resistant Pneumococcal Strains

A looming public-health problem in this and other countries is the ever-increasing antibiotic resistance of many pneumococcal strains, particularly those causing pneumonia and otitis media—diseases that are prevalent in small children, who cannot be protected with existing polysaccharide vaccines. (More children visit their doctor for otitis media than for any other health problem.) Robbins and Schneerson and their colleague Shousun Szu have prepared conjugates of type 6 pneumococcus-polysaccharide tetanus toxoid, type 14 pertussis toxoid, and type 12 diphtheria toxoid, with the goal of providing a new infant vaccine, using the bacterial toxoids as carriers for Hib and critical pneumococcal types. "Such a formulation would expand the vaccine coverage of childhood infections with the fewest number of components," says Levine. The type 6 conjugate is now being tested in Detroit and in Iceland, where the pneumococcal strain that causes otitis media in infants and pneumonia in adults is mostly antibiotic resistant.

Opportunistic Pathogens of AIDS and Other Immunocompromised Patients

"*Staphylococcus aureus* and *Cryptococcus neoformans* are opportunistic organisms, causing systemic infections in immunocompromised individuals," says Schneerson. "For both, capsular polysaccharides serve as virulence factors—allowing the pathogen to escape natural immune defenses—and as protective antigens, serum antibodies facilitate phagocytosis." *S. aureus* is a major cause of hospital-acquired bacteremia, and *C. neoformans*, although a rare cause of disease in the general population, causes meningitis in about 5% of AIDS patients. After isolating the capsular polysaccharides from these organisms, Schneerson conjugated the *S. aureus* polysaccharide to a genetically engineered deletion mutant of *Pseudomonas aeruginosa* exotoxin A and the *C. neoformans* polysaccharide, to tetanus toxoid. Initial trials of these experimental vaccines in adult volunteers, which are being conducted in collaboration with John Bennett of NIAID, have shown both vaccines to be immunogenic.

Taming Typhoid, Shigella, cholera, and E. coli 0157

A major thrust of the group's work is to provide immunization against enteric diseases, for which no effective vaccines have been developed. One such enteric disease that is a threat to populations that are forced to rely on contaminated water supplies is typhoid fever. According to Robbins, "Typhoid fever remains the most common and severe cause of enteric fevers in most of the world." The currently available whole-cell vaccines against *Salmonella typhi* afford protection for only a short time and cause severe local and systemic reactions. A purified capsular polysaccharide, commonly known as the Vi (virulence) antigen, prepared in Robbins' laboratory by Szu, is currently replacing the whole-cell vaccine, but it, too, has shortcomings—only about two-thirds to three-quarters of those receiving the vaccine are protected. And because it is a polysaccharide, it does not protect infants. The NICHD lab is collaborating with the Walter Reed Army Institute of Research on initial clinical trials of a conjugate vaccine incorporating the Vi polysaccharide linked to recombinant, inactivated *P. aeruginosa* exotoxin A or to the heat-labile toxin of *Escherichia coli*. These trials have demonstrated that the conjugate vaccine is significantly more immunogenic than the polysaccharide alone; clinical trials of this improved Vi vaccine are now under way. The conjugation technique for this antigen was also developed by Szu, and uses another bifunctional ligating agent, N-succinimidyl-3-(2-pyridylthio)propionate, which attaches a thiol group previously introduced onto the Vi directly to the amino groups of the carrier protein.

The NICHD scientists are also applying their conjugation techniques to tackle shigellosis, a form of dysentery that is a major world-health problem in infants and children in developing countries, and in overcrowded living conditions. According to Robbins, despite decades of study, efforts

to produce vaccines against Shigellae have proven fruitless, partly because there are no animal models for the disease and partly because the protective immune mechanism in humans is unknown. Robbins and Schneerson have proposed that protection against *Shigella dysenteriae* infections may be afforded by serum antibodies against pyrogenic surface lipopolysaccharide. Recently, they isolated a polysaccharide from the lipopolysaccharide and conjugated it to medically useful proteins, such as tetanus toxoid and recombinant *P. aeruginosa* exotoxin A; trials of this experimental vaccine are now in progress in Israeli Army recruits. Preliminary results suggest induced antibody levels are likely to be protective.

Szu is carrying out similar work to make a conjugate of the detoxified lipopolysaccharide of *Vibrio cholerae*, the agent causing cholera, against which there are no effective vaccines. Cholera is on the upswing in Central and South America, and there are reports of new, exceptionally virulent strains emerging on the Indian subcontinent, which lends considerable urgency to this work. In 90% of the adult volunteers studied so far, Szu's conjugate vaccine elicited vibriocidal antibodies and had no side effects. Szu has also purified and conjugated the lipopolysaccharide from *E. coli* O157, the antibiotic-resistant pathogen that caused the recent outbreak of hemolytic-uremic syndrome in diners eating hamburgers at a fast-food chain on the West Coast. Preliminary laboratory-animal tests suggest that the conjugation approach will also prove effective in preventing this disease. Robbins and Schneerson say the polysaccharide-protein conjugation technology offers considerable promise of eradicating many of these diseases. ■

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AUTOIMMUNE DISEASE

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tion of experimental SLE in class I - deficient mice. Knockout mice that lack cell-surface expression of class I molecules have recently been generated through inactivation of the gene for ß-microglobulin, which is required for assembly and transport of the class I molecules (12). These mice not only fail to express class I molecules, but also fail to develop the CD4-CD8+ T-cell subset. Despite these deficiencies, the mice are generally healthy, mount brisk antibody responses to various immunogens, and readily survive most viral infections.

To determine whether class I molecules are involved in the induction or propagation of experimental SLE, we tested class I^- mice for their susceptibility to SLE following 16/6Id immunization (2). Surprisingly, but consistant with our hypothesis, we found that class I^- mice were resistant to SLE by all of the parameters that could be tested. Although they generated high antibody titres to the 16/6Id, they failed to produce antibodies to either DNA or nuclear proteins. Furthermore, they developed none of the clinical manifestations of SLE. They had neither leukopenia nor proteinuria. Most dramatically, there was no evidence of immune-complex deposits in their kidneys (fig. 1). In contrast, normal mice were highly susceptible to SLE, and manifested all of the symptoms. Our results demonstrated that MHC class I molecules play a pivotal role in the induction of autoimmune SLE.

What is the significance of this finding? First of all, it may provide new approaches and insights into the causes of human autoimmune disease, and of SLE in particular. Researchers have placed a great deal of emphasis on elucidating the role of class II moleculesin SLE. We suggest that a new emphasis be placed on examining the role of class I molecules in autoimmune disease. Second, we may be able to categorize various autoimmune diseases according to their dependence on class I or class II molecules. For example, whereas experimental SLE depends on the expression of class I molecules, recent data suggest that another experimental model of autoimmune disease — mutliple sclerosis — does not depend on class I molecules (D. Singer, L. Quigley, M. Racke, and H. McFarland, unpublished observations). Finally, a better understanding of the disease mechanism may also lead to new approaches to therapy. For example, our studies lead to the prediction that reduction of class I concentrations in normal animals should mitigate experimental SLE . Indeed, in ongoing experiments, we have recently found that agents that decrease class I expression in vivo protect against experimental SLE (D. Singer, L. Kohn, and E. Mozes, unpublished observations). We hope that further studies of the role of class I molecules in autoimmune diseases will lead to a better understanding of the underlying defect in some autoimmune diseases, and perhaps to novel therapeutic approaches. ■

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