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Research Festival

CALLING ALL CELLS: INNATE IMMUNE TALENT

by Aarthi Ashok

Much has been learned in recent years about the receptors of the innate immune system—the most important of which are the toll-like receptors (TLR)—and about dendritic cell function in host defense, observed David Segal, chief of the Immune Targeting Section, NCI, and co-chair of the panel on innate immune recognition. And what much of the new knowledge points to, he said, introducing the talks to follow, is that "most cells of the body can serve some sort of innate immune function."

Aarthi Ashok

David Segal

The Dendritic Cell Bridge

Dendritic cells come into play in the early stages of infection, prior to activation of the adaptive immune system. Two different populations of dendritic cells have been described: the conventional, or myeloid, dendritic cells that secrete primarily interleukin-12 (IL-12) and the plasmacytoid precursor dendritic cells (pDCs) that secrete mostly type 1 interferon (IFN-α or -β).

Aarthi Ashok

Giorgio Trinchieri

Giorgio Trinchieri, a Fogarty Visiting Scholar at NIAID, and his colleagues were interested in determining whether pDCs were the primary source of type 1 IFN during infection in vivo.

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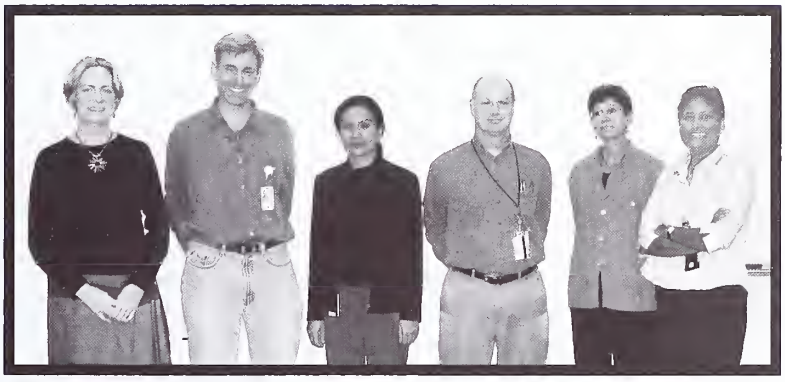
Research Festival

KEEPING UP WITH RNA VIRUSES: EBOLA, DENGUE, WEST NILE, PANDEMIC FLU, ET AL.

by Fran Pollner



Fran Pollner

A Starting Line-Up: *(left to right) Maribeth Eiden, chief, Section on Directed Gene Transfer, Laboratory of Cellular and Molecular Regulation, NIMH; Peter Collins, senior investigator, Laboratory of Infectious Diseases, NIAID; Nidia Oliveira, visiting research fellow, NIMH; Brian Murphy, co-chief, Laboratory of Infectious Diseases, and chief, Respiratory Viruses Section, NIAID; Carolyn Wilson, Center for Biologics Evaluation and Research, FDA; and Kanta Subbarao, senior investigator, Laboratory of Infectious Diseases, NIAID*

Maribeth Eiden itemized some of the factors facilitating the spread of infectious diseases in the 21st century: increased global travel, climate change, poverty, enlarged populations, encroachment of domestic livestock, and inadequate surveillance policies.

She then introduced a panel of investigators from NIAID, FDA/CBER, and her own NIMH lab who are working to control or respond to that spread with sophisticated vaccine development and basic explorations into the mechanisms that enable viruses to jump across species.

NIAID-MedImmune CRADA Aimed at Vanquishing Avian Flu

The global toll of the H5N1 avian flu virus from late 2003 to October 18, 2005, when Kanta Subbarao delivered her talk on vaccines against potential pandemic flu strains, was 117 laboratory-confirmed human cases and 60 deaths—against the backdrop of 150 million infected birds and transcontinental spread.

The cases have all occurred in the context of a poultry outbreak, direct human contact with infected poultry, and person-to-person transmission of

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A Penny Saved Is a Penny Earned

Michael Gottesman

As we get increasingly urgent signals of budget challenges ahead, we must anticipate some belt-tightening. I believe we can get through the lean times the way we got through the parking crunch—by unleashing the power of creative problem-solving that NIH has in abundance.

A first step has been identifying our "cost drivers"—some of the large targets where administrative changes and a bit more care and cooperation could save money without cutting deeply into research.

Leading this activity was the Intramural Research Budget Working Group, co-chaired by NIMH Director Tom Insel and me. We conducted an Analysis of IR Budget Obligations in NIDDK, NIMH, NCI, NIDA, and NHLBI.

Barbara Merchant, NIDDK Executive Officer, recently presented the group's findings to the Board of Scientific Directors and is scheduling a presentation to NIH's administrative and executive officers.

The group found that the intramural program spends the lion's share of its budget on salaries and benefits, "other services"—which includes our contracts—and equipment purchases and maintenance.

The group made several good suggestions for administrative changes and flexibility in government hiring and contracting that could generate significant savings in personnel costs if widely adopted.

They pointed to ways to economize on research animal costs and large equipment purchases, including pooling equipment orders and making individual investigators more responsible for animal use. Equipment maintenance contracts, renovations, and inventory of telephone and data lines are other fertile areas for frugality, the group found.

Multiplied over a large number of interactions, even small savings can yield significant funds. I hope that by modifying some of our administrative approaches and procedures, we can make prudent spending a goal that is in everyone's interest.

Specifically, we want to make it possible for saved money to stay within the intramural program that saves it. Or, put another way, to assure that a penny saved is indeed a penny earned for your IC's intramural program

Specifically, we want to make it possible for saved money to stay within the intramural program that saves it. Or, put another way, to assure that a penny saved is indeed a penny earned for your IC's intramural program.

I'd like NIHers at all levels to help their lab, office, and institute save money and even to look beyond their particular place to NIH as a whole: As you come up with ideas for how to pinch pennies in your program—share the wealth and let others know! I will be asking groups across campus to help us spread good ideas for saving money as we clear administrative paths for pooling purchases and cutting maintenance costs.

So think about this, all of you—individual labs and offices, administrative and executive officers, lab managers, staff scientists, tenure-track scientists, fellows, scientific interest groups, lab chiefs, scientific directors. Our office will be forming a trans-NIH committee to work on user-friendly administrative approaches to propagating best practices for pinching pennies within the Intramural Research Program.

—Michael Gottesman
Deputy Director for Intramural Research

How To Succeed as a PI at NIH: The Making of Leaders

by Joan P. Schwartz

Have you ever wondered why you can't get one of your fellows to do something the way you want? Or how about the time you tried to convince your SD that your latest idea was the key to your Institute's future?

Some of us become lab or branch chiefs—or even scientific directors—with no real training in the new leadership talents those jobs entail.

A logical remedy, agreed to in focus groups with lab and branch chiefs and scientific directors we held earlier this year, is to provide training in such skills.

In December 2003, 18 NIH tenure-track and senior investigators piloted a course on leadership skills. This test course was developed and run by UCSF Professor Ed O'Neil, who had been enlisted by the NIH Committee on Scientific Conduct and Ethics.

O'Neil began the pilot by asking participants whether we thought we would learn something useful. The group was initially dubious. We had struggled to fill out the Myers-Briggs Type Indicator (MBTI) and the 360 Skillscope. The MBTI assesses preferences in making decisions, acquiring information, and engaging others. The Skillscope matches self-perception of leadership skills, attributes, and organizational effectiveness with the perceptions of others who work with you. This entails detailed evaluations from five or six peers. Would the results be worth so much effort? participants wondered.

But by the end of the 36-hour retreat, all were convinced that we understood—or at least had a better perception of—our own leadership styles. In addition, we had developed strategies for improving our leadership skills. We agreed the course could be a useful exercise for all scientists at the NIH, few of whom get such training.

We were also convinced that O'Neil was the right person to teach these skills—he understands how to engage scientists, despite their natural resistance to this training. For example, in one enlightening exercise, O'Neil asked the introverts (as defined by the MBTI) to gather in one corner of the room and the extroverts in the other corner to discuss what it was about the other group that made it so difficult to work with them. It was surprising but reassuring to the introverts to see 20 scientists in their corner and just four in the extroverts' huddle.

A Course for the Tenure Track

The bottom line was that the Scientific Conduct and Ethics committee—12 of whose members participated in the pilot—concluded that the retreat formed the basis of a very useful course for NIH tenure-track investigators. We added a few modules to make a two-day, off-campus retreat with the added benefit of giving attendees an opportunity to start networking with other tenure-track colleagues.

The course has now been held three times, with the fourth session planned for April 2006. Participants complete the MBTI and Skillscope in advance, so that the results can be compiled and shared with them during the course, intensifying its personal relevance.

Retreat Day One features an introduction to leadership in scientific settings, followed by modules on:

- Leadership styles and preferences (MBTI and Skillscope)
- Teams: working through, motivating, and developing others
- Giving developmental feedback
- Practice in giving feedback and a debriefing
- Introduction to goal setting

The second day includes a session in which personal goals are discussed, fleshed out, and made more realistic. Also that day, and in the evening sessions, are modules on criteria for tenure; handling BSC reviews; and dealing with conflict, an interactive session led by NIH ombudsman Howard Gadlin that addresses such issues as conflicts among collaborators about the direction of a project and its publications, authorship, and publicity.

Members of the Scientific Conduct and Ethics Committee lead a session on mentoring, using case studies of problems and mentoring gone awry. For example, what do you do when a new postdoc wants to finish a paper from her graduate work and needs to do a series of experiments unrelated to your lab's goals? Small groups hash out each case and then discuss with the whole group how it could have been handled.

About half of NIH's tenure-track investigators have now attended the course. Ninety percent rated it excellent to outstanding. Specific comments include:

- "I thought it was great. . . ."
- "Ed O'Neil is an incredibly talented group facilitator, who presented information directly applicable to our numerous research responsibilities. Seeing how our Skillscope and MBTI data could be used positively for professional growth was great. The inclusion of senior researchers in the seminars and small groups was appreciated because of their imparted experience in handling challenges."
- "Everything was very good and useful. I wish I had this course early in my tenure-track."

Joan Schwartz

Several people who did take the course early in their tenure track have asked whether they can take it again—they felt they would get more from some sessions with more experience under their belts.

. . . and Now for Senior Investigators

In light of such reactions and the success of the course, the Committee has now decided to offer a version for senior investigators. This course will be offered as individual modules—one or two per day—on the Bethesda campus, starting in January 2006. Ed O'Neil will lead some of the sessions, which will be offered on a first-come, first-served basis to no more than 30 participants.

Dates	Modules
January 26	Leadership Styles and Preferences: MBTI discussion—A.M. Skillscope—P.M.
February 22	Teams: Working through, Motivating, Developing Others—A.M. Managing Up—PM
March/ Early April	Dealing with Conflict, Enhancing Your Mentoring Skills, Negotiating/Hiring Skills

Watch for an e-mail announcement in early December to sign up; this could be your chance to improve your leadership and management of recalcitrant people—even in the NIH bureaucracy.■

Innate Immune Talent

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In 2001, they characterized the mouse counterpart of human pDCs. Using specific antibodies in vivo to deplete this murine pDC population, they were able to establish that mice subjected to microbial challenge could no longer synthesize type 1 IFN.

Moreover, they were able to show that this inhibition of type 1 IFN production was pathogen specific. pDCs express high levels of TLR7 and 9 but lack TLR3 and 4. Pathogens recognized by TLR9 trigger a large type 1 IFN response, which is completely inhibited upon antibody depletion of pDCs. Type 1 IFN production persists, however, in the face of infection with pathogens that signal through TLR3, or a cytoplasmic double-stranded RNA receptor such as RIG-I, despite antibody-mediated depletion of pDCs. Their data, Trinchieri said, point to a role for the classical dendritic cells in type 1 IFN production during later stages of viral infection.

Optimal production of the pro-inflammatory cytokine IL-12 during infection requires type 1 IFN. Trinchieri showed that pathogen stimulation of multiple TLRs, which trigger classical dendritic cells, resulted in optimal IL-12 production.

A Closer Look At TLR3

Aarthi Ashok

Jessica Bell

TLRs are a class of germ-line–encoded pattern recognition receptors that are truly our first line of defense against pathogens, said Jessica Bell, a postdoc in the Laboratory of Molecular Biology, NIDDK.

In humans, 10 members of this family have been described to date (TLR1 to 10). Bell and co-workers probed the molecular structure of TLRs in an attempt to ascertain how so few could recognize so immense a range of pathogens and foreign molecules.

The TLRs contain leucine-rich repeat (LRR) domains that, unlike other LRR proteins, contain insertions in specific positions. Bell suspected that these insertions might be involved in antigen recognition.

Using a baculovirus secretion system and affinity chromatography, Bell was able to produce the apo crystals that led to the determination of the first structure of TLR3—a curved solenoid that resembles a Slinky toy with an extended β-sheet structure on its concave surface.

The structure contained 11 glycosylation sites out of a potential 15, as well as two bound sulfate ions. When a 19-base pair RNA molecule is modeled onto the receptor using the sulfate ions as landmarks for the nucleotide's phosphate backbone, Bell noted, the glycosylation sites may direct the target to the binding site.

Generation of co-crystals of TLR3 and double-stranded RNA are underway, and Bell hopes to embark on a mutational analysis of the predicted RNA binding sites on TLR3.

Innate Immunity To Protozoan Parasites

Aarthi Ashok

Felix Yarovinsky

Innate immune recognition of eukaryotic pathogens is little understood. Felix Yarovinsky and his colleagues in the Laboratory of Parasitic Diseases, NIAID, have shown that MyD88-knockout mice, which lack the downstream signaling from TLRs, fail to produce the IL-12 necessary to combat the parasite *Toxoplasma gondii*. This finding was the first that pointed to a role for TLRs in the recognition of and defense against parasitic pathogens.

The team then fractionated parasite extracts to identify a single protein—a 17.5-kDa novel profilin—that was the actual trigger for cytokine production from dendritic cells and hence a potential TLR ligand.

Using cells from various TLR-knockout mice, Yarovinsky demonstrated that the parasitic profilin protein is recognized by TLR11. Interestingly, profilins from several other protozoans, including *Cryptosporidium parvum* and the malarial parasite *Plasmodium falciparum*, are recognized by TLR11. Hence, TLR11 appears to have evolved to recognize so-called apicomplexan parasites, and innate responses are clearly critical in the clearance of these parasites.

TLR11-knockout mice can resist the acute phase of parasitic infection but succumb to the chronic phase. Yarovinsky is now trying to identify the source of the residual IL-12 that helps these mice combat the acute infection.

Innate Immunity To Intestinal Reovirus Infection

Aarthi Ashok

Brian Kelsall

Brian Kelsall, a senior investigator in the Laboratory of Clinical Investigation, NIAID, and his co-workers have been exploring the role of mucosal immunity during infection with the double-stranded RNA-containing reovirus.

Reovirus infects the epithelium overlying the Peyer's patches in the gut and is cleared by a combination of innate and adaptive immunity in about 10–14 days.

Dendritic cells (DC) are prominent in Peyer's patches. Kelsall found that while reovirus does not actively infect DCs, DCs can capture reovirus structural proteins from infected apoptotic epithelial cells and present them to CD4+ T cells in vitro. In addition, they demonstrated that type 1 IFN is produced in Peyer's patches upon reovirus infection and is critical for reovirus clearance.

Reovirus did not induce type 1 IFN production from pDCs in the Peyer's patch; interestingly, Kelsall said, the investigators found that Peyer's patch pDCs did not make type 1 IFN in response to any stimuli, perhaps due to conditioning by factors present in the mucosal microenvironment.

They concluded that type 1 IFN production by a non-pDC source in the Peyer's patch is critical for early immunity. This type 1 IFN may also be involved in driving DC activation, resulting in acquired immune responses to this model intestinal virus infection.

Innate Immune Activation

Aarthi Ashok

Daniela Verthelyi

Studies have shown that innate immune activation using CpG motifs (DNA sequences from bacterial molecules) protects mice against several pathogens, especially hemorrhagic pathogens.

Daniela Verthelyi, a senior staff fellow in the Center for Drug Evaluation and Research, FDA, and her colleagues have evaluated the immunoprotective ef-

Research Festival

Molecular Epidemiology Comes of Age: On the Trail of Non-Hodgkin's Lymphoma

by Fran Pollner

The prognosis for patients with follicular lymphoma has been notoriously unpredictable. But gene expression profiling at the time of diagnosis, NIH scientists are saying, may change that by revealing genes associated with good and poor immune response and corresponding survival.

"Morphologic similarities can mask a surprising degree of molecular heterogeneity that underlies outcome differences," Sandeep Dave observed, citing outcome data related to molecular subtype gathered in Lou Staudt's NCI lab, where he is a clinical fellow.

Subtypes of diffuse large B cell lymphoma (DLBCL) that "look the same under the microscope" nonetheless exhibit different oncogene mechanisms, respond differently to treatment, and carry a different prognostic message.

Five-year survival for the ABC subtype, which expresses NF-κB, is 31 percent; five-year survival for the GCB subtype, which does not express NF-κB, is 60 percent. NF-κB, Dave said, might thus be a therapeutic target in the ABC subtype of DLBCL.

Dave was one of the panelists at a Research Festival symposium that explored the expanding repositories of data—genetic, environmental, and medical—related to the risk of non-Hodgkin's lymphoma (NHL). The need now, they agreed, is to determine which factors and combinations of factors can inform preventive and therapeutic strategies in the clinical setting.

According to data from the SEER case-control study of NHL, Sophia Wang noted, NF-κB is indeed "emerging as a signficant player" in DLBCL risk.

The SEER data, she said, focused on 15 single-nucleotide polymorphisms from seven genes for inflammatory cytokines and uncovered two—TNF-G308A and LTA-252G—that singly and, especially, in combination were significantly associated with DLBCL. A follow-up study is exploring gene-environment interactions, said Wang, an investigator in the Division of Cancer Epidemiology and Genetics, NCI.

Patricia Hartge, deputy director of the DCEG Epidemiology and Biostatistics Program, itemized environmental and host factors that have been implicated in the etiology of NHL—the incidence of which, in contrast to Hodgkin's disease and independent of the advent of AIDS, with which it is associated—has been steadily increasing since 1950.

SEER data, Hartge said, suggest that chlordane insecticides, but not herbicides, are associated with NHL and that foods containing antioxidants and folates may be protective.

Among risky host factors are immune suppression; a family history of NHL, Hodgkin's disease, or multiple myeloma; autoimmune diseases such as Sjögren's syndrome, lupus, and rheumatoid arthritis; and hepatitis C.

Genetics, she said, may elucidate susceptibility and mechanisms. She predicted new large epidemiological studies that would look at survival and cause, a major advance over previous studies.

A newly described genetic disorder of programmed cell death—autoimmune lymphoproliferative syndrome (ALPS)—can presage lymphoma, Michael Lenardo, a senior investigator in the Laboratory of Immunology, NIAID, reported.

Characterized by the loss of a general antineoplastic mechanism associated in 75 percent of cases with an FAS receptor mutation, ALPS patients typically present with lymphadenopathy and splenomegaly by the age of 5—and there is typically a delay of 10 to 40 years between ALPS onset and lymphoma.

In a study of 130 individuals in 39 families with ALPS and FAS mutations, 10 people eventually developed 11 instances of B and T cell lymphomas, Lenardo said.

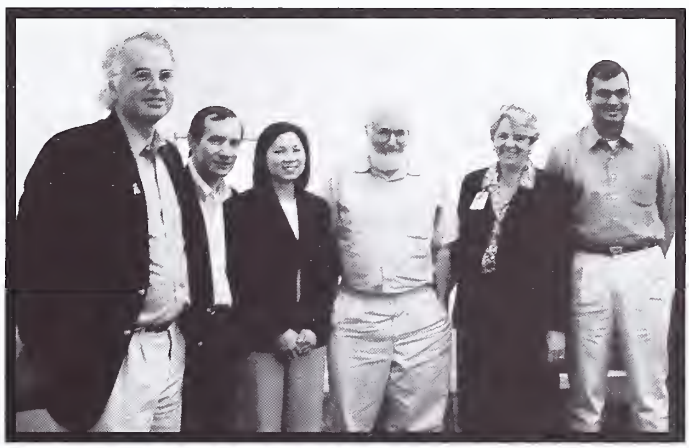
Jumping from the United States to the world stage of molecular epidemiological data, Nathaniel Rothman pointed to the International Lymphoma Consortium (InterLymph), as a growing source of "solid clues that could translate to clinical implications for survival."

Established in the late 1990s, InterLymph has amassed data from about 9,000 cases in North America, Australia, and Europe, said Rothman, a DCEG senior investigator.

The Genetic Polymorphism Working Group has been studying Th1 and Th2 proinflammatory response and has found a consistent association with lymphoma risk for haplotypes with both TNF and LTA variants and, to a less consistent extent, with an IL-10 variant.

With the ability to conduct whole genome scans, it will become possible to distinguish associations that are real from those that are not, Rothman said.

Stephen Chanock, a senior investigator and head of the Genomic Variation Section, NCI, observed that the population groups most extensively studied to date are Caucasian. Asian and African groups, he said, need similar scrutiny, which will elucidate what genetic variants are common across populations and the role of environmental factors. ■



Celia Hooper

***Non-Hodgkin's Lymphoma Panel**. (left to right) Stephen Chanock, Michael Lenardo, Sophia Wang, Nathaniel Rothman, Patricia Hartge, and Sandeep Dave*

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fects of one family of CpG oligonucleotides (D series) in rhesus macaques challenged with the parasite *Leishmania major*. Their data show that the D-series oligos can confer protection, inducing resolution of disease lesions within a few days of treatment. Moreover, these immunoprotective effects are evident in SIV-infected immunocompromised macaques.

There is a catch, however. The tails of the D-series oligos have a tendency to polymerize and aggregate in solution, changing the characteristics of the product. To navigate around this roadblock, the team first had to create D-series oligos with additional protective groups on the poly G tail that allowed them to become activated only upon entry into cells. These novel D-series oligos showed significant protective effects against Leishmania infection in macaques, albeit with some induction of local inflammation. ■

Fran Pollner

The Line-Up: *(left to right) Maribeth Eiden, Peter Collins, Nidia Oliveira, Brian Murphy, Carolyn Wilson, and Kanta Subbarao*

Keeping Up with RNA Viruses

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genetically identical virus within the family of an infected person.

There is no rapid human-to-human spread, Subarrao observed, but there are reasons for concern:

- Poultry outbreaks are increasing.
- There is evidence of genetic drift, with new H5N1 genotypes having arisen since its first appearance in 1997.
- Antiviral resistance is increasing.
- Ducks, tigers, and leopards have died of H5N1 avian flu and ferrets and cats can be infected in the laboratory.
- H5N1 viruses can infect people and cause severe disease, and people have no immunity against it. Were it to become transmissible person-to-person, it could cause a pandemic.

The "H" in H5N1 stands for hemagglutinin, a key viral surface protein, of which there are 16 subtypes that have been isolated from birds. There are currently vaccines to prtect humans against H1 and H3.

Subbarao, a senior investigator in the Laboratory of Infectious Diseases, NIAID, and Brian Murphy, laboratory co-chief, are heading a team of NIAID scientists that will work its way through all the rest—H2 and H4 through 16—under a CRADA agreement with MedImmune, Inc., of Gaithersburg, Md.

Each of these avian flu vaccines will be administered intranasally; the live attenuated bird virus will contain the existing backbone of the influenza A component of MedImmune's FluMist® flu vaccine, a live virus that has undergone attenuating mutations.

The project may take up to 10 years, Subbarao said in an interview with *The NIH Catalyst*. She estimated that it would take about two years to develop vaccines against each subtype and gather initial data from the ensuing phase 1 clinical trial, which will involve healthy adults. The clinical trials will be conducted in an inpatient setting during summers to limit the risk of the expected small amount of shed vaccine virus combining with any circulating wild-type influenza A viruses.

Closing In on Dengue and West Nile

Murphy and his colleagues in the NIAID Laboratory of Infectious Diseases have been working on live attenuated vaccine constructs against two flaviviruses—dengue and West Nile.

Murphy is aiming to create a tetravalent vaccine aganst the four dengue serotypes that are responsible for 50 million infections a year.

Meanwhile, the team has introduced a deletion mutation (delta 30) into each of the four wild-type serotypes to achieve attenuation; thus far, good results have been seen with two (DEN1 and DEN4) of the constructs.

The attenuated recombinant DEN4 construct, for instance, "induced good antibody titers" in tests with 80 human volunteers, Murphy said, adding that mild neutropenia and rash were the notable but not dangerous side effects and systemic symptoms were rare.

A second approach, which involves making antigenic chimeric viruses between the DEN4 delta30 virus and the DEN2 or DEN3 wild-type virus, has generated good vaccine candidates for these two serotypes. A tetravalent vaccine with these four DEN viruses looks good in monkeys, Murphy said.

Preliminary results in monkeys suggest that a boost at day 30 does not elicit a secondary antibody response; however, a four-month interval, he said, was effective at boosting the antibody response against each of the four serotypes.

Especially significant in low-income countries, Murphy added, is that the equivalent of "one flask could immunize 100,000 inexpensively." He anticipates initiating clinical studies of a tetravalent dengue vaccine in 2006–2007.

The team, led by Alexander Pletnev, is taking a similar approach in developing a vaccine for West Nile virus, which causes "a couple of thousand" infections a year in the United States. Safety studies in mice are encouraging, and one chimeric construct in early human testing was found to raise protective antibodies in seven of nine volunteers in the absence of any symptoms.

Getting the Drop On Respiratory Viruses

Peter Collins, a senior investigator working with Murphy and other NIAID researchers, discussed his work developing an intranasal vaccine vector for highly pathogenic viruses such as SARS, Ebola virus, and avian flu virus.

Initial studies have used live attenuated human parainfluenza virus (PIV3), an important cause of respiratory tract disease in infants and children, as a vector.

PIV3 has a track record: First, the NIAID team used recombinant DNA techniques to develop live attenuated PIV3 strains; a PIV3 intranasal vaccine is currently in clinical trials. The researchers developed the further strategy of using attenuated PIV3 as a vector to express the protective antigens of additional pediatric viral pathogens, such as human respiratory syncytial virus (RSV). This bivalent PIV3/RSV vaccine is also now in clinical trials sponsored by MedImmune.

Collins described how attenuated PIV3 can also be used to express protective antigens of highly pathogenic agents such as SARS and Ebola virus.

The intranasal route, by drops or nasal spray, has the advantage of directly stimulating local respiratory tract immunity, as well as systemic immunity—a clear plus because the respiratory tract frequently is the portal of entry and egress for viruses; and for respiratory pathogens, it is the major site of viral replication and disease.

Using the SARS-S glycoprotein alone, an intranasal PIV3 vector provided protection against the SARS coronavirus in tests with rodents and nonhuman primate. In addition, an intranasal PIV3 vector expressing the Ebola GP glycoprotein protected guinea pigs against an otherwise lethal dose of Ebola virus.

Although these vectored vaccines have promise for use in infants and young children, they probably will not be effective in adults, who have naturally acquired immunity that will restrict the replication of the vector, Collins observed.

Therefore, the investigators are developing nonhuman PIV viruses as vectors, such as low-virulence strains of Newcastle disease virus (NDV). In initial studies, NDV expressing a test antigen proved to be both highly attenuated and highly immunogenic as an intranasal vaccine in nonhuman primates.

Unearthing Ebola's Conserved Domains

The world first became aware of the Ebola virus in 1976; in the past 10 years, there have been 15 Ebola virus outbreaks—notably in Congo (now Zaire), the Sudan, and Côte d'Ivoire.

A natural reservoir has not been identified; incubation is five to seven days;

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symptoms start with generalized ague and progress to disseminated intravascular coagulation, with death in 50–90 percent of cases.

"But survivors develop antibodies against the virus, and this is the basis for a vaccine strategy," said Carolyn Wilson, of the FDA Center for Biologics Research and Evaluation. (She noted that Nancy Sullivan and her colleagues at the Vaccine Research Center have developed a DNA-prime/adenovirus vector–boost Ebola vaccine currently in clinical trials.)

Working with "the Zaire virus," Wilson's lab has been focusing on an Ebola vaccine strategy that targets conserved domains. They've been mining the glycoprotein section—the heavily glycosylated GP1, which mediates receptor binding, and GP2, which mediates fusion. They used site-directed mutagenesis to functionally screen 15 amino acids from the GP domain and found two that are critical for viral entry and "are conserved across all filovirus strains, including Ebola and Marburg," Wilson said.

Further, Wilson showed that a peptide derived from one of these conserved domains was able to block infection by strains of Ebola virus. Now armed with "proof of concept," the team is continuing its investigations with these two conserved amino acids—F88 and F159.

How Much Can a Koala Bear?

Nidia Oliveira, a visiting research fellow at the NIMH Laboratory of Cellular and Molecular Regulation, traced the meanderings and permutations of a retrovirus that jumped from feral mice in southern Asia to Old World primates to a New World pet primate in San Francisco—and more recently has been identified in koalas in Australia, where it has gained its greatest lethality.

About 60 percent of koala bear mortality is attributable to neoplasia. Infected koala bears have a neoplastic disease rate that correlates with viral load. The KoRV retrovirus, Oliveira said, has been isolated in 100 percent of Queensland koalas—"it's in their genomes," she said, noting that the rate is about 30 percent elsewhere in southeastern mainland Australia.

Asian rodents are the reservoir for the GALV-like virus that emerged to infect gibbon apes in Thailand and became WMV in woolly monkeys and KoRV in marsupials.

Sequence comparisons establish the lineage, Oliveira said, noting that GALV, WMV, and KoRV use the same receptor—PIT1—in gaining entry, but that KoRV also uses orthologs that GALV and WMV are unable to use. The KoRV envelope, she said, has an extremely broad host range, and the challenge now is to discern what part of the envelope accounts for that range and for the virus' species-jumping ability. ■

Bush Uses NIH Visit To Call for Emergency Bird Flu Measures

by Celia Hooper

On November 1, President Bush paid his fourth visit to NIH—this time to call on Congress to approve a total of $7.1 billion in emergency funding to arm the nation against pandemic bird flu. Joining Bush were DHHS Secretary Michael Leavitt, other cabinet officers, some members of Congress, and representatives of international bodies involved in responding to avian influenza strain H5N1.

After noting the difference between ordinary seasonal flu and devastating pandemic avian strains that struck the world's immunologically naive populations in 1918, 1957, and 1968, Bush said the country must act preemptively. "There is no pandemic flu in our country or in the world at this time, but if we wait for a pandemic to appear it will be too late to prepare."

World health experts see no signs H5N1 has yet evolved the capacity to be transmitted from person to person—essential for a pandemic—but they are wary of the strain because of its immunological novelty and formidable 50 percent mortality in people infected through direct contact with birds. Citing the substantial 2003 toll of SARS in dollars and lives, Bush said, "A global influenza pandemic that infects millions . . . could be much worse."

To head off such a fate—and simultaneously equip the country against other flu strains and bioterrorist hazards—Bush called on Congress to approve a $7.1 billion legislative package to support:

- Global biosurveillance and disease containment ($251 million)
- Federal purchase and stockpiling of an avian flu vaccine currently in clinical trials ($1.2 billion for 20 million doses)
- Federal purchase and stockpiling of two antiviral medications ($1 billion)
- Grants to cut the time needed for vaccine development and production ($2.8 billion)
- Liability protection for the vaccine makers
- Planning and coordination of local, state, and national efforts for responding to health emergencies ($583 million)

Bush said a cache of first-generation avian flu vaccine, like stockpiles of Tamiflu and Relenza antivirals, would be given to "first responders" and populations especially vulnerable to the flu if a pandemic broke out.

The hope for grants to U.S. pharmaceutical companies is creation of production "surge capacity" and egg-free culture techniques that would enable them to bring on-line a new flu vaccine—sufficient to immunize the nation—within six months of the start of a pandemic.

For the present, Bush urged everyone to get their annual shots against garden-variety seasonal flu. "I had mine," he said. ■

For a complete transcript of Bush's remarks, see
<http://www.whitehouse.gov/news/releases/2005/11/20051101-1.html>.

NIH-Duke TPCR

Applications are being accepted for the 2006–2007 NIH-Duke Training Program in Clinical Research. The deadline for applying is **March 1, 2006**.

Geared to physicians and dentists who want formal clinical research training, the program is geared to part-time study as a complement to concurrent clinical training.

Courses are presented via videoconference at the CC. Academic credit may be applied toward the degree requirement (24 credits of graded course work and a 12-credit research project) for a Master of Health Sciences in Clinical Research from Duke University School of Medicine.

Applications are available in the Office of Clinical Research Training and Medical Education, Building 10, Room B1L403. Additional information is available at the website:

<http://tpcr.mc.duke.edu>.

or by mail questions to:

<mailto:tpcr@mc.duke.edu>.

Enrollment is limited. Individuals seeking funding for participation should check with their IC. Successful applicants will be notified by July 1, 2006. ■

PRAT Fellowships

The NIGMS Pharmacology Research Associate (PRAT) program is now accepting applications for positions to begin October 2006. Applications must be received by **December 16, 2005**.

PRAT is a three-year competitive research fellowship to support training at NIH or FDA laboratories for postdoctoral fellows whose research focuses on the pharmacological sciences and related areas. Fellows with more than one year of research experience at NIH or FDA are not eligible.

Applicants must identify a preceptor — any willing tenured or tenure-track scientist at NIH or FDA—in their application. For more information or application materials, contact the PRAT program assistant at 301-594-3583 or

<mailto:prat@nigms.nih.gov>.

Hair Today, Gone Tomorrow

NIDCR's Hynda Kleinman Takes Off for New Horizons

by Tara Kirby

Hynda Kleinman did not expect to use bikini waxing as a lab technique or to invent the next great remedy for baldness when she came to NIH 30 years ago.

But her long and productive history of basic and translational research, which has landed nine patents—including one for Matrigel basement membrane matrix, among the top 20 NIH royalty generators—has indeed featured some surprising developments.

"I started with angiogenesis," Kleinman said, tracing this particular journey in a parting interview with *The NIH Catalyst*, "and then I wound up in wound healing, and then . . . in hair. It really was a stretch."

Poised to exit NIH at year's end, the NIDCR cell biology chief credits the NIH environment with having enabled her to follow her research down the parallel angiogenic pathways related to cancer, heart disease, wound healing, and hair restoration.

Tracking Thymosin β–4

The protein that sent Kleinman on this hair-raising adventure is thymosin β–4 (Tβ4), a small actin-binding peptide. At the NIH Research Festival in the fall of 2000, she elaborated on its potential role in metastasis (see "Aiming to Control a Double-Edged Sword," *The NIH Catalyst*, March-April 2001); at the Research Festival in the fall of 2005, she elaborated on its wound-healing and hair-growth properties.

Topically applied to the wounded skin of rodents, Kleinman said, Tβ4 speeds wound closure by increasing collagen deposition and blood vessel formation, with help from its anti-inflammatory and antimicrobial activities. It is also showing promise in other types of healing, such as corneal re-epithelialization and cardiac repair. The surprising observation, she said, was hair growth accompanying wound closure—and, beyond that, hair growth even on unwounded skin.

There are currently two patents for Tβ4; it is being tested in wound healing in Phase 2 clinical trials for the elderly, diabetes patients, and epidermolysis bullosa patients, and it has more recently been licensed for hair-growth studies.

Kleinman became interested in Tβ4 in the early 1990's, when her group searched for genes involved in angiogenesis. They found that Tβ4 was upregulated sixfold in tube-forming endothelial cells.

To understand the clinical relevance, they tested it on wounds and saw a dramatic enhancement in healing. Kleinman applied for a patent through NIH, and NIH developed a CRADA with RegeneRx Biopharmaceuticals to develop Tβ4 as a wound-healing drug.

It was in their effort to develop a formulation for clinical use that the group discovered the new potential application of hair growth. Deborah Philp, a fellow in Kleinman's lab, noticed that around the edges of a Tβ4-treated wound, the rats' skin resembled the bottom of an old broom. "It looked like big, thick straw coming out of the edges of the wound, and it was a darker color and much thicker" than normal hair, Kleinman recalled.

As a result, she laughed, "we had many days when we were in the lab, bikini-waxing mice," to test whether this effect could be repeated on unwounded skin. Kleinman and colleagues obtained a separate patent covering Tβ4's effect on hair growth, and it has now been licensed to two companies to be developed as a hair-loss treatment.

And there may be more to come from Tβ4 "We are obviously interested in stem cells," Kleinman said, because Tβ4 "focuses them to migrate and differentiate." Also, the cellular receptor for Tβ4 is unknown. It may function as a transcription factor, since it is found in the nucleus. She is eager to explore what other clinical applications may exist.

Tracking Gender Bias at NIH

The intensity of her research program not withstanding, Kleinman also found time to take the lead in bringing to light and brightening the status of women scientists at NIH. As chair of the Task Force on the Status of NIH Intramural Women Scientists, she guided the development of a 1992 report on gender inequities at NIH and recommendations to eliminate them; several of these recommendations have been implemented (for an overview, see "Ten Years and Counting: Have NIH Women Scientists Advanced Since the Task Force Report?" *The NIH Catalyst*, September-October 2001).

Kleinman is now part of the Second Task Force on the Status of Intramural Women Scientists, which is examining the progress women have made since the first report. A report is currently in preparation and should be available within the next few months.

Tara Kirby

When NIDCR's Hynda Kleinman turns out the lights of her lab for the last time in December, she will leave behind a host of fans—including scientists and students who have profited both from her mentoring and her pioneering policy making. She will also leave a legacy of royalties from her scientific discoveries during her 30-year sojourn at NIH.

Chief of the Cell Biology Section in the dental institute, Kleinman's basic research has focused on extracellular matrix components and cellular receptors involved in tumor growth and metastasis, angiogenesis, and nerve regeneration. Along with the intellectual fruits of this research, it has also yielded Kleinman authorship on nine patents, including one for Matrigel—one of the 20 top royalty-generating patents at NIH.

Her service to current and future generations of scientists at NIH is reflected in her work as chair of the Task Force on the Status of NIH Intramural Women Scientists and as a recipient in 1999 of the Association for Women in Science, Bethesda chapter, Mentoring Award. Says Joan Schwartz, assistant director, OIR, "She has set a real example for the women scientists on this campus in terms of fighting for our rights [and] keeping our consciousness raised at all times."

The biggest change, in Kleinman's view, is that women have more visibility and recognition as scientists. Pay equity and resources have also been tackled, she said, and there is more awareness in general of women's issues—all important advances.

However, she noted, the percentage of tenured women (approximately 20

percent) has remained lower than the percentage of women who are postdoctoral fellows (at least 40 percent). "That's been the painful reality, that women haven't advanced up," Kleinman said. Moreover, there are "still subtle, back-door issues that remain to be resolved" and will be addressed in the second task force report.

The report will also address mentoring, particularly for tenure-track women at NIH. "I would say that many women at NIH feel very isolated," Kleinman said. Mentoring, she said, is something that NIH, as an institution, should work on. She suggested the need for a structured mentoring program.

Mentoring has been one of Kleinman's favorite activities at NIH and one for which she has been honored. Listening is a major part of mentoring, she advised—"sometimes people just need to let off steam." There's also promoting productivity and creativity, getting a sense for what someone's best potential is and keeping her or him on track, and generally keeping up morale—these are the key elements of good mentoring, Kleinman said. And it pays off: "If you mentor people well, they'll be more productive, they'll work harder, and they'll want to contribute more to their lab."

Tracking a New Path

Kleinman is leaving NIH for a position at Washington, D.C.'s George Washington University— professor in the Department of Biochemistry and Molecular Biology; she also plans to work with industry on new model systems for Tβ4.

Despite the translational research component of the NIH Roadmap, Kleinman said, "I don't feel it's valued," in that achievement is still measured by the number of *Cell*, *Science,* and *Nature* papers produced; still, she predicts "that's going to change with time."

The bottom line about NIH in Kleinman's life, though, she said, is that it "has been good to me—there's no question that this has been a creative and fast-moving environment" for research. Not having to be "constrained by what my grant says I have to do," she noted, made her more productive.

Before she starts her new job, Kleinman will be riding off into the sunset—literally. She's planning a bike trip across the United States with her husband. ■

Ed.Note: The NIH Catalyst would like to bid Hynda Kleinman, who has served us so well on our Board of Editorial Advisors, a very fond adieu and the very best of luck.

Osteoporosis Attracts Royal Attention at NIH

In an unprecedented week of high-powered visits to NIH, the United Kingdom's Prince Charles and his wife, Camilla, the Duchess of Cornwall, dropped by on November 3 for a chat about osteoporosis.

The British royals' visit came just two days after President George Bush used NIH as a backdrop in calling on Congress to pass legislation preparing the country for pandemic flu (see p. 7).

Branson

Hands Across the Ocean: *Camilla, Duchess of Cornwall, meets NIAMS Director Stephen Katz outside the Clinical Research Center, as NIH Director Elias Zerhouni approaches Prince Charles (back to camera)*

After the Prince and Duchess greeted a small crowd of NIH patients and staff gathered in the lobby of the new Clinical Center, NIH Director Elias Zerhouni escorted them to the Medical Board Room and led a meeting with scientific experts, patients, advocacy groups, HHS officials, and NIAMS director Stephen Katz.

The Duchess, who is President of the U.K.'s National Osteoporosis Society, had both a grandmother and her mother with the disease, which affects one in three of women over the age of 50 in the U.K. and one in five women of that age in the United States.

Katz says he told the royal couple that one of NIH's greatest challenges is "disseminating information about medical breakthroughs, not only to patients but also to the medical professionals who interact with patients." Particularly critical is information about—and then actual changes in—lifestyle in one's younger years that can prevent bone loss later. "Translating knowledge into behavior changes is a very critical challenge for all of us," Katz told the Duchess.

—Celia Hooper

Branson

Command Performance: *NIAMS Director Stephen Katz (seated at table, far right) delivers a specially requested talk on osteoporosis to Camilla, Duchess of Cornwall, during her visit to NIH November 3. To Katz's right is NIH Director Elias Zerhouni; to Camilla's left is her husband, Prince Charles.*

Youth vs. Age

Medical Student Disrupts Progeria Mechanisms While Learning Research Ropes in Collins Lab

by Karen Ross

When NYU medical student Brian Capell first decided to apply for the HHMI (Howard Hughes Medical Institute)/NIH Research Scholars Program, he had no idea that 18 months later he would have participated in a major scientific breakthrough that may offer hope to children afflicted with progeria, a rare but devastating disease that causes premature aging.

Capell first heard about the Research Scholars Program, which gives medical students the opportunity to do one year of research at NIH, from an alumnus of the program, a fellow student at NYU. He applied during his third year of medical school.

Capell had some experience doing clinical research, and he was intrigued by the idea of immersing himself in a year of basic research. "I didn't even think I'd be accepted at first," he recalls. But accepted he was. He arrived at NIH in July 2004, along with 40 other Research Scholars from medical schools across the United States.

The Scholars normally spend their first two weeks visiting labs in order to decide where they would like to do their research. Capell interviewed with several labs, but he already had an idea of what he wanted to do. A month earlier, he had met NHGRI Director Francis Collins at a scientific meeting and was fascinated by the work in his lab (Molecular Genetics Section, Genome Technology Branch).

After discussing possible projects with Collins, Capell says, his decision was "pretty easy." He joined the Collins lab and started working on progeria.

Children with progeria age extremely rapidly, so that by the age of three or four, they have the body of an 80- or 90-year old. They lose their hair, their skin wrinkles, and, most importantly, they develop severe cardiovascular disease. Most die by the age of 12 or 13 from heart attacks or strokes.

In 2003, Collins' lab discovered that progeria is caused by a defect in a protein called lamin A. Lamin A is part of the nuclear matrix, a network of proteins that gives structure and organization to the cell's nucleus.

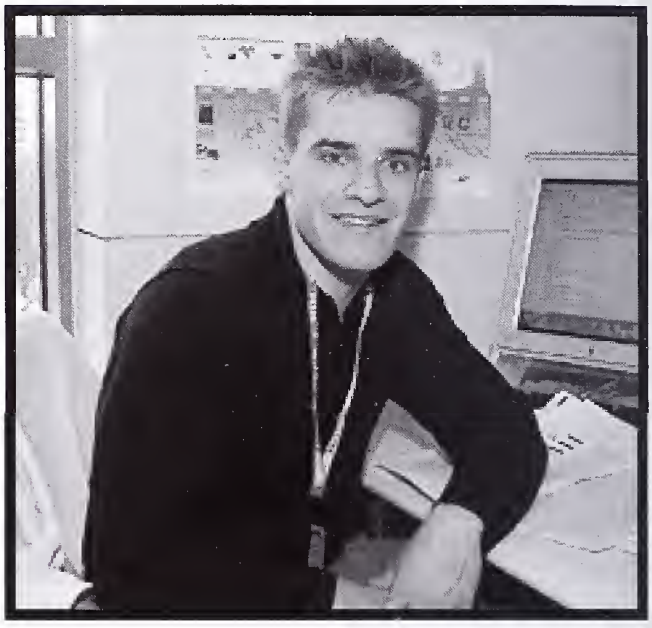
In progeria patients, lamin A lacks 50 amino acids that are critical for targeting it to the nuclear matrix. The mutant lamin A gets stuck in the nuclear membrane, causing the nucleus to assume an irregular lobulated, or "blebbed," morphology.

No one knows how, or even whether, the blebbed nuclei contribute to the symptoms of progeria. Some researchers speculate that progeria cells are very fragile and break down in high-stress areas of the body such as the large arteries, causing cardiovascular disease. Others think the distorted nuclear shape may disrupt gene expression.

The culprit behind the mislocalization of lamin A in progeria patients is a 15-carbon lipid chain referred to as a farnesyl group. Normally, a farnesyl group is added to lamin A shortly after it is synthesized in the cytoplasm, which directs the protein to the nuclear membrane. Then the farnesyl group is cleaved off, and lamin A moves from the membrane and into the nuclear lamina. In progeria patients, the farnesyl group is properly attached to lamin A; however, because of the missing 50 amino acids, the farnesyl group's cleavage site is deleted and it remains in place, thus trapping lamin A at its intermediate destination, the nuclear membrane.

Capell and Collins reasoned that if the attachment of the farnesyl group was blocked in the first place, the defective lamin A might remain harmlessly in the cytoplasm.

Together with NHGRI staff scientist Michael Erdos, NHGRI postdoctoral fellow Renee Varga, and colleagues from the University of North Carolina, Chapel



Maggie Bartlett

A gentleman and an HHMI/NIH scholar: *Brian Capell at home in his Building 50 office, a few steps from the bench where he conducted basic research with dramatic implications for a devastating genetic disease*

Program Particulars

The HHMI/NIH Research Scholars Program, established in 1985, brings U.S. medical and dental students to NIH to do a year, or occasionally two years, of research. The Howard Hughes Medical Institute funds the program; NIH provides advisors and mentors and lab space and equipment.

Scholars arrive in the summer and spend the first few weeks visiting labs to find one that fits their research interests. In addition to their lab work, the students attend weekly lectures given by NIH or HHMI scientists.

The Scholars live together in the Cloister (Building 60) in dormitory-style rooms or small apartments, a living situation that encourages bonding with and learning from their peers.

HHMI funds cover living expenses, books, and travel to a scientific meeting as well as health and life insurance. Scholars are also eligible to take classes at FAES.

Highly competitive, the Research Scholars Program accepts only 42 students each year out of 150–200 applicants.

"The HHMI program at the NIH is a real win-win," says NHGRI Director Francis Collins. "[It's] an opportunity for motivated medical students to make contributions to cutting-edge research and an opportunity for NIH to have talented and energetic young scientists on our campus."

Application deadline for the cycle beginning in the summer of 2006 is January 10, 2006. For more information on the program, visit

<http://www.hhmi.org/cloister/program.html>.

Hill, the University of Michigan, Ann Arbor,and the Progeria Research Foundation, Capell used two strategies to prevent the attachment of the farnesyl group to lamin A.

He mutated the amino acid in lamin A that normally forms the link to the farnesyl chain, and he used drugs called farnesyl transferase inhibitors (FTIs) that interfere with the enzyme that adds the farnesyl group to lamin A. In both cases, lamin A no longer got stuck in the nuclear membrane, and the blebbing of the nuclei in progeria cells was dramatically reduced. Capell was first author in the study reporting these findings in the September 6, 2005, issue of *Proceedings of the National Academy of Sciences*.

FTIs are already in Phase III clinical trials in the treatment of myeloid leukemia. The drugs were originally developed to inhibit cancer-causing proteins that require farnesylation to function.

If the drugs work as well in an animal model of progeria as they did on progeria cells, says Collins, "we might be able to leapfrog over a decade or more of basic research and move to a clinical trial within a year or two."

Capell got permission to extend his stay at NIH by one year, so that he can test FTIs in a mouse model of progeria. "I wanted to see the results through here, to see what happens," he says.

Capell is equally enthusiastic about life outside the lab. The HHMI/NIH Scholars live together in Building 60 on the NIH campus where, Capell says, "It's amazing how much [we] really talk about science." Monday nights, an NIH or HHMI scientist presents his or her research over dinner, and each Thursday night one of the Scholars speaks.

In addition to a stipend that covers their living expenses, the Scholars receive funds for books, classes, and travel to a scientific meeting. Capell used his travel money to present a poster on preliminary progeria findings at the Gordon Research Conference on Human Genetics and Genomics in Newport, R.I.

Capell will return to NYU in May or June 2006, but isn't sure yet of the exact path his career will take beyond medical school. Based on his experiences at NIH, he knows he wants to combine research and clinical activities.

Collins thinks Capell is well suited to pursue that course. He says Capell "has shown all of the attributes of a future successful physician-scientist—intellectual curiosity, experimental adeptness, fearlessness about trying new approaches, a willingness to work hard, and a heartfelt desire to help those who are struggling with terrible diseases." ■

2006 Demystifying Medicine for Ph.D.s

The 17-week Demystifying Medicine course begins **January 10, 2005**, and will be held from 4:00 to 6:00 p.m. in the Building 50 ground-floor auditorium (Rooms 1227 & 1233). Classes will be available through Breeze.

For academic credit, register with FAES at **<http://www.faes.org>**; otherwise, registration is at the Listserv: **<http://list.nih.gov/archives/demystifyingmed/html>**.

The schedule appears below and also at **<http://www1.od.nih.gov/oir/demystifyingMed/>**.

For more info, contact Win Arias at **<arias1@mail.nih.gov>**.

Date	Speakers	Subject
	January	
10	Harvey Alter (CC) Barbara Rehermann (NIDDK)	Hepatitis C: A Silent Global Disease
17	Ruth Kirschstein (OD) Ellie Ehrenfeld (NIAID)	Polio: Past and Present
24	Steven Holland (CC) John Robbins (NICHD)	Tuberculosis: The Ever-present "White" Plague
31	David Henderson (CC) Kanta Subbarao (NIAID)	Avian Influenza: Another Pandemic?
	February	
7	Ron McKAY (NINDS) Cynthia Dunbar (NHLBI)	Stem Cells: Frontiers and Applications
14	Gary Nabel (NIAID) John Robbins (NICHD)	Diseases of Potential Terrorism: Ebola and Anthrax
21	Henry Masur (CC) John Coffin (NCI)	HIV: Advances and Retreats
28	Tom Wellems (NIAID) Rick Fairhurst (NIAID)	Malaria: The Number-One Killer
	March	
7	Elias Zerhouni (OD); Jennifer Lippincott-Schwartz (NICHD) **This session held in Wilson Hall, 3rd floor, Building 1**	Imaging: A New Frontier for Organs and Cells
14	John Hardy (NIA) Katrina Gwinn-Hardy (NINDS)	Parkinson's Disease: The Shaking palsy
21	Baruch Blumberg (Fox Chase) Jay Shapiro (Hopkins/NASA)	Astrobiology: Clinical and Basic Research
28	George Kunos (NIAAA) Barry Hoffer (NIDA)	Addiction: Cannabinoids and Other Drugs
	April	
4	Philip Gordon (NIDDK) Jack Yanowski (NICHD)	Obesity: A National Epidemic
11	Elizabeth Nabel (NHLBI) Francis Collins (NHGRI)	Genetics, Aging, and Heart Disease: New Insights
18	Richard Anderson (NCI) Snorri Thorgeirsson (NCI) Win Arias (NICHD)	Hepatocellular Cancer: An Increasing Global Problem
25	Alan Schechter (NIDDK) Jeffrey Miller (NIDDK)	Sickle Cell Anemia: Treating a Molecular Disease
	May	
3	Michael Gottesman (OD/NCI) Win Arias (OD/NICHD) and others	Finale: Symposium on Career Opportunities for PhD Postdocs

Summer Poster Day Epilogue

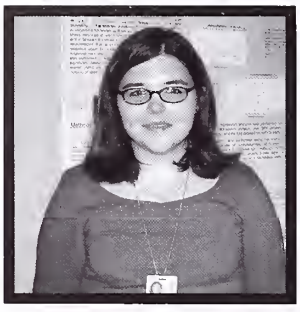
Effects of Early Rearing Experience on Stress and Dominance in Rhesus Macaques

Brittany Copp, Tulane University, New Orleans, Louisiana
Preceptor: James Higley, Laboratory of Clinical and Translational Studies, NIAAA

The stress response to a perceived threat and the coping mechanism used to return to normal behavior is thought to be heavily influenced by early experiences such as the quality of maternal care.

Copp looked at stress levels (measured by cortisol levels) in rhesus macaques that were either reared normally by protective mothers (MR) or raised by surrogates with minimal social interactions (SPR). She found that as stereotypies, or repetitive motor movements, increased, the SPR animals showed increased cortisol, while MR animals that showed low levels of these stereotypic movements exhibited high cortisol. This was indicative of an abnormal response to stress in the SPR group.

Interestingly, while MR animals used social contact as a coping mechanism during stressful conditions, the SPR animals avoided social contact. Copp believes that this may be due to SPR monkeys' finding it difficult to initiate social behavior and also because they may not find such contact comforting.



Aarthi Ashok

Brittany Copp

The physiological response to stress has also been shown to differ based on the dominance rank of the monkeys. All female rhesus monkeys inherit their rank from their mothers, and Copp was therefore interested in understanding ranking in the SPR group, whose members have no clear inheritance. In a short study during a previous summer, she was able to demonstrate that the SPR animals consistently ranked lowest in the group of MR and SPR animals. She used this information in her current study to correlate stress levels with rank or perception of rank in the case of SPR animals.

By measuring stress using MHPG (monoamine metabolite of norepinephrine) levels in these monkeys, Copp found that in the MR group, increasing MHPG levels correlated with increasing rank, while in the SPR group, increasing MHPG levels correlated with decreasing rank. This suggested that the lower-ranking animals of the SPR group perceived normal activities as more stressful than the MR animals. This response may provide the reason for their lower ranking within the group. However, larger studies are needed to confirm these findings, as the small sample size of Copp's study meant that these correlations did not reach significance.

"I will miss the monkeys," Copp said, talking of her plans for graduate school while pointing to a picture of Stella, a mother macaque, "but I have had two excellent summers here."

—Aarthi Ashok

Immunofluorescent Analysis of Chemokine Trafficking

Megan McCain, Washington University, St. Louis, Missouri
Preceptor: Fred Indig, Confocal Imaging Facility, Research Resources Branch, NIA

Chemokines are a class of cytokines that are secreted by cells that play an important role in human immune response against foreign antigens. Chemokines that are endocytosed by cells such as macrophages are degraded and presented as antigens on their cell surface. These chemokine antigens can be recognized by either CD8+ or CD4+ T cells, which then become activated to participate in the immune reaction. Recognition by the CD8+ or CD4+ T cells requires that the antigen be presented on the cell surface by MHC class I and class II molecules, respectively.

Antigens presented by MHC class I molecules are degraded by cytoplasmic proteasomes, whereas MHC class II antigens are processed in the lysosomes. Which pathway is used in the presentation of chemokine antigens on macrophages?

Aarthi Ashok

Megan McCain

With the assistance of Purevdorj Olkhanud and Arya Biragyn of the Laboratory of Immunology, NIA, McCain addressed this question in her study of the intracellular trafficking of a fluorescently tagged chemokine, MIP3α, in mouse macrophage cells. She followed the fluorescent chemokine in these cells by first allowing them to bind MIP3α at 4° C and then inducing endocytosis of the chemokine by shifting the cells to 37° C. McCain fixed the cells at various time points after the shift to 37º C and stained them with antibodies against clathrin, proteasomes, and lysosomes.

Her confocal microscopy images show that MIP3α co-localized with clathrin within two minutes of the 37 ºC shift, demonstrating that the chemokine is internalized by clathrin-dependent endocytosis. Ten minutes after internalization, the chemokine co-localized with both lysosomes and proteasomes but no longer with clathrin.

This result led McCain to the unique finding that by gaining access to proteasomes and lysosomes, the chemokine could be presented by both the MHC class I and the class II pathways. By 60 minutes, almost all chemokine staining was lost, suggesting that the cells are able to completely degrade this chemokine within an hour.

"We could specifically target cancer cells by tagging a toxic molecule onto a specific chemokine whose receptors are highly expressed on tumor cells," McCain suggested, noting the implications of the research for cancer immunotherapy.

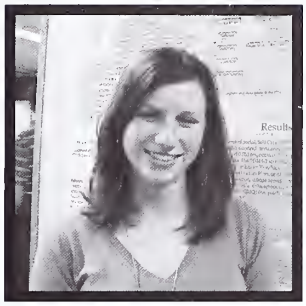
—Aarthi Ashok

Gender and Lung Function

The Women's Health Special Interest Group is hosting a talk on "Gender Differences in Lung Function and Response to Environmental Agents," presented by Darryl Zeldin, senior investigator, NIEHS. The talk and ensuing discussion will take place **Friday, December 16**, from 11:30 a.m. to 12:30 p.m. in Wilson Hall, Building 1, 3rd floor. For sign language interpretation, contact Vicki Malick five days before the seminar at **<malickv@od.nih.gov>**.

The Functional Performance of Children with Smith-Magenis Syndrome

Amy Zolko, University of Maryland, College Park
Preceptor: Michaele Smith, Rehabilitation Medicine Department, CC



Annie Nguyen

Amy Zolko

Smith-Magenis Syndrome (SMS) arises from a partial or full deletion of band p11.2 on chromosome 17 and is characterized by a distinct pattern of physical, behavioral, cognitive, and functional abnormalities.

Zolko's project aimed to provide physical therapists and other involved health practitioners with detailed documentation of the level of functional motor skills attained by children with SMS compared with age-matched peers. Reasonable expectations regarding both the rate of skill development and the achievable functional level could be translated into additional interventional strategies.

The research team used two assessment methods—the Pediatric Evaluation of Disabilities Inventory (PEDI) and the Peabody Developmental Motor Scales (PDMS-2)—to assess functional motor skills in SMS patients. The PEDI is a parental questionnaire designed to monitor individual progress in daily functional activities such as brushing teeth, dressing, walking, and peer interaction. Used by clinicians, the PDMS-2 estimates a child's motor competency on subtests that measure reflexes, locomotion, ability to sustain body control, and object manipulation. Twenty-eight children, ages five months to seven years, with confirmed SMS diagnosis were enrolled in the study and monitored annually for several years.

Both the PEDI and the PDMS-2 showed that children with SMS scored below the mean for their age group for all subtests. Nonetheless, they eventually did achieve the desired milestones, such as running, skipping, and stair climbing, only at a slower rate than normal children. However, it is not certain that children with SMS will accomplish all the motor skills gained by unaffected children or at what age they can be expected to achieve them. Typically, children with SMS are not as coordinated as their unaffected peers.

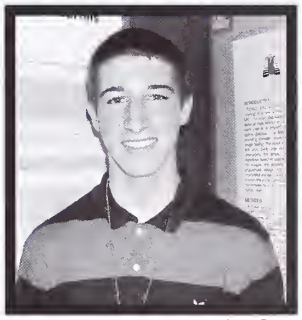
The study suggested that children with SMS should be monitored annually to detect any motor delay as they grow older and that prolonging physical therapy may be effective in helping them obtain higher-level functional skills. The investigators plan more studies to determine the effects of prolonged therapy.

Zolko is in her last year at the University of Maryland at College Park and plans to pursue graduate study in physical therapy.

—Annie Nguyen

The Prion Protein Protects Against Harmful Aggregation of Huntingtin

David Rogawski, Williams College, Williamstown, Massachusetts
Preceptors: Lois Greene, Kyung Jin Lee, Evan Eisenberg, Laboratory of Cell Biology, NHLBI



Karen Ross

David Rogawski

Rogawski worked on an intriguing connection between Huntington's disease and the prion protein PrP^C, which, in its misfolded, "infectious" form is responsible for a family of fatal neurological illnesses, including mad cow disease.

Despite its potential to cause devastating disease, PrP^C is found in the brains of most mammals and may play an important role in neuronal functioning.

Rogawski investigated the normal function of PrP^C and discovered that it helps protect neurons from the symptoms of Huntington's disease. Huntington's is a neurodegenerative disease caused by a mutation in the *huntingtin* gene.

The defective huntingtin protein aggregates inside brain cells, eventually killing them.

Working with cultured cells that carried the mutant huntingtin protein, Rogawski found that depleting PrP^C accelerated the formation of huntingtin aggregates.

He observed that PrP^C affected the qualitative appearance of the aggregates. Cells with a normal complement of PrP^C tended to have one large huntingtin aggregate, whereas those that lacked PrP^C had numerous, smaller aggregates.

He then showed that introduction of a mouse version of PrP^C into PrP^C-depleted cells reduced the rate of aggregate formation.

The group's findings raise the possibility that PrP^C may have a general neuroprotective function, preventing unwanted protein aggregates from forming in otherwise-healthy neurons.

—Karen Ross

Understanding the Evolution of Vaccine-Derived Polioviruses

Simone Berkower, Yale University, New Haven, Connecticut
Preceptor: Elena Cherkasova, Laboratory of Method Development, CBER, FDA

Annie Nguyen

Simone Berkower

The live attenuated virus upon which the oral polio vaccine (OPV) developed by Albert Sabin is based has been observed to revert to wild-type virus after inoculation into the human host.

These strains, known as vaccine-derived polioviruses (VDPVs), have regained virulence and occasionally result in paralysis and polio outbreaks. This increased risk is especially significant in developing countries, where the oral construct is used almost exclusively due to its low cost and easy administration.

Berkower and her colleagues set out to elucidate the underpinnings of these changes. The team analyzed vaccine-derived strains isolated from healthy children after OPV administration. The children were given three doses of vaccine, and stool samples were collected approximately once a week after each dose. Viral RNA isolated from stool samples was synthesized into its complementary DNA via reverse transcription.

Subsequent DNA sequencing revealed amino acid mutations in capsid proteins in or near antigenic regions matching those of its wild-type predecessor or homotypic wild polioviruses.

The researchers point to errors made by the RNA polymerase during replication of the viral genome as one cause of the live attenuated Sabin 1 virus mutation.

They speculate that common antigenic alterations in evolving OPV strains largely reflect attempts to eliminate fitness-decreasing mutations acquired either during the original selection of the vaccine or already present in the parental strains.

Studying the patterns of mutation of VDPVs may provide new insights into viral evolution, the researchers note, as well as better approaches to crafting future vaccine constructs and vaccination policies.

Berkower, now a student at Yale University, spent the two NIH Summer Research program summers after her junior and senior years at the Sidwell Friends High School in Washington, D.C. She plans to continue her research endeavors at Yale.

—Annie Nguyen

The 'Embedded Librarian': NIH Informationists Become Team Players

by Cindy Clark

Many a research team at NIH could probably use another knowledgeable person—perhaps someone to perform complex searches in targeted databases or to help with projects like protocol development. Informationists from the NIH Library are filling that role, working as adjuncts on NIH research teams.

An informationist* is a professional librarian with extensive training in a specific subject area—such as chemistry, immunology, or technology transfer. Through the NIH Library, informationists provide personalized information services that break the usual bounds of librarian services—they become active members of the teams they are assisting. Informationists fulfill their assignments not only in person but also via e-mail, BlackBerry® or Palm® hand held, fax, or telephone, as needed, to groups located beyond the NIH campus.

As NIH Library Director Suzanne Grefsheim explained at a recent gathering at the Library of Congress, "Informationists need to know more than just how to search PubMed®. They need to know anatomy, physiology, and the specific specialty of the groups with whom they work. Ideally, they need to be both an information scientist and a subject specialist." The 14 informationists now on staff at the NIH Library are involved in 28 different groups at NIH. Following are three representative profiles.

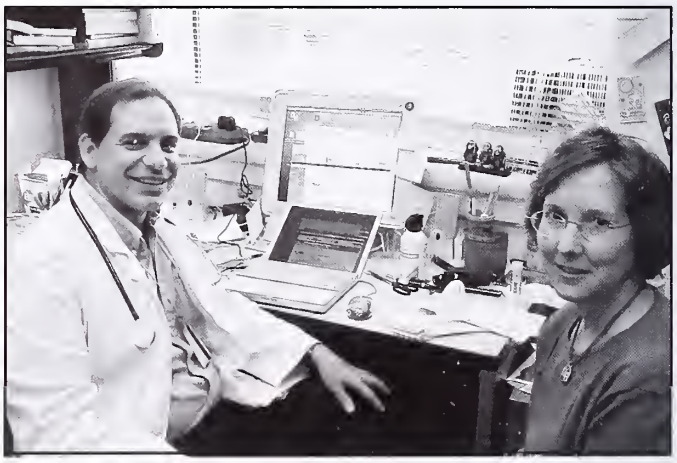
*The term "informationist" was proposed in "The Informationist: A New Health Profession?" in *Annals of Internal Medicine* (F. Davidoff, V. Florance, **132**: 996, 2000). Two years later, the Medical Library Association convened at NLM to discuss the role of the informationist or "librarian-in-context." And recently, the NIH Library's informationists participated in a presentation at the Library of Congress on the "embedded librarian." Today, these three terms are often used interchangeably.

Work in Clinical Settings

Diane Cooper received her Master of Science in Library Science (MSLS) and is credentialed by the Academy for Health Information Professionals. Her experience is in the field of health services. In addition to taking classes required of informationists—Principles and Practices of Clinical Research and Ethics of Human Subject Research—Cooper has also taken classes in immunology and endocrinology.

She has been working with components of the Interinstitute Endocrinology Program (including NICHD's Developmental Endocrinology Branch, Pediatric Endocrinology Branch, and Gynecology Consult Service and NIDDK's Clinical Endocrinology Branch) for almost two years.

A grateful Alejandro Ayala, an NICHD endocrinologist, especially values the time Cooper spends researching and critically appraising the ever-increasing volumes of online biomedical information. Her work, he said, "complements and scrutinizes our work. It is a very important safety mechanism in times when physicians and scientists are faced with a remarkable amount of information and pressured by time constraints."



Michhael Walden

Informationist Diane Cooper meets frequently with mentor Alejandro "Alex" Ayala, an endocrine clinician with NICHD, in his office, at the whiteboard, or via e-mail. Cooper also provides informationist services to several endocrinology groups at NIH and to clinicians in the Indian Health Service located throughout the United States.

Cooper notes that questions demanding additional research often come up during the usual course of making rounds. She cites the following as an example: What is the normal response of aldosterone to the ACTH infusion test? (ACTH is a pituitary hormone that stimulates the adrenal cortex to produce cortisol and aldosterone. It is mostly used as a diagnostic test for adrenal insufficiency, but there are fewer data on the normative values of aldosterone response to ACTH.) Are there any studies comparing normal subjects with patients with adrenal insufficiency?

She has learned that what appear on the surface to be easy questions usually involve time-consuming searching and synthesis of the literature. In the above example, she was able to identify three studies that answered the clinicians' questions.

Cooper is also responsible for creating a nationwide virtual informationist service for the Indian Health Service (IHS). Her IHS clients are located at more than 300 sites in 35 states.

To identify who needed informationist services and what their priorities were, Cooper conducted or collaborated on three surveys within a six-month period. She determined that the primary users of the service are the clinicians in American Indian and Alaska Native hospitals and health-care facilities. She also learned that their greatest needs are for clinical protocol information and patient health-care education materials at the point of service.

Cooper provides the needed services to the IHS clinical staff by answering questions via e-mail and at websites and through articles published in *The IHS Primary Care Provider* and the *IHS OB/GYN Newsletter*. She also attends conferences to meet clients face-to-face. ■

Work at Home and Abroad

Pam Sieving joined the staff at the NIH Library as its first informationist. She holds masters degrees in linguistics and in library science and is the former director of library services in the department of ophthalmology and visual sciences at the University of Michigan. Sieving's clients include NEI and the CC Tracheotomy Consult Service. Her two main clients in NEI are the retina and genetics groups.

Sieving attends NEI cinical conferences and consult meetings. She reports on such subjects as the social implications for children with certain diseases, economic impacts, literature reviews, animal models, portfolio reviews, and reports to Congress. She also assists with the creation of search strategies and alert services. Much of her work involves searching for evidence-based medicine, and she is currently working with others on a systematic review of complementary and alternative therapies for ocular surface disease.

According to Emily Chew, deputy director of the Division of Epidemiology and Clinical Research, Sieving "has conducted incredibly comprehensive searches for both protocol development and manuscript preparation." She's also helped train NEI fellows on how best to avail themselves of library services.

Hine Phonthachack

Pam Sieving, the NIH Library's first-hired informationist, works with NEI staff, the CC Tracheotomy Consult Service, and the editor of the journal ORL-Head and Neck Nursing.

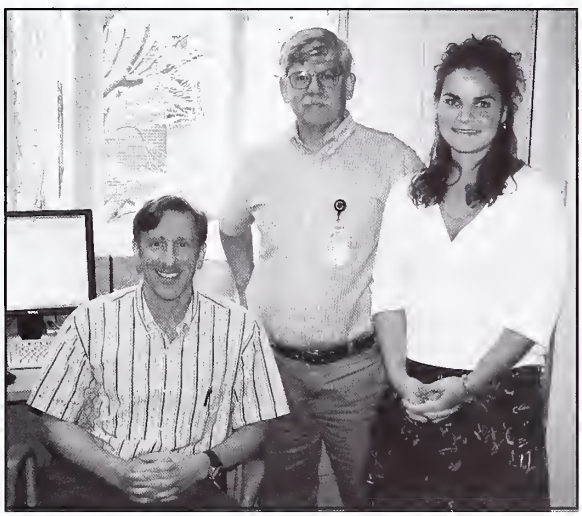
Sieving was also a part of a U.S. contingent, including some NEI staff, that traveled to three cities in India earlier this year to explore opportunities for collaborative vision research. Her expertise revolved around facilitating communications, enhancing access to information resources, and enhancing the use of evidence-based medicine.

In the CC Tracheotomy Consult Service, Sieving helped create evidence-based guidelines for tracheotomy patient care. She attends rounds with a physician, a speech therapist, a respiratory therapist, a social worker, and a wound-management nurse.

Recently, Sieving began working with Susan Rudy, an NIDCD research nurse practitioner and editor of *ORL-Head and Neck Nursing*. Sieving provides editorial support including searching for quotations for editorials, editing references, and analyzing articles as part of the peer-review process. ■

Work in Bioinformatics

Doug Joubert has a Master of Library Information Science degree and is currently pursuing a Master of Science in Biotechnology from the University of Maryland University College, headquartered in Adelphi, Md. Joubert's interest in technology made him a good fit for working with a group from the CIT Mathematical and Statistical Computing Laboratory (MSCL), led by lab chief Peter Munson. Joubert was tapped to assist with bioinformatics on a trial basis. The trial was extended, and Joubert has been the group's informationist for almost a year, spending about 50 percent of his work time on data analysis for MSCL.



Hine Phonthachack

Doug Joubert (left) plumbs databases with CIT MSCL lab chief Peter Munson and systems analyst Jennifer Barb

Joubert's primary project, Munson said in an interview, is to organize and further explore the potential relevance of the genes that emerge in microarray studies. Investigators can "wind up with tens of hundreds of new leads for genes," Munson said; the informationist can then follow through, using automated library tools to ferret out the relevant literature on any given collection of genes and evaluate the quality of evidence supporting the investigator's study.

"If it's true in yeast," Munson mused, "it might be true in cancer."

To be most effective in his projects with MSCL, Joubert said that he learned the basics of Linux, UNIX®, and PERL. Tools that he frequently uses include NCBI databases such as Entrez Gene, Entrez Protein, AmiGo, Expression Analysis Systematic Explorer (EASE), MedMiner, MatchMiner, MILANO, and Ingenuity Pathways Analysis®. For keeping up with innovations and trends in biotechnology, Joubert says he can turn to "over 60 special interest groups in biology, medicine, genes and genetics, and proteins and proteomics at NIH." He also participates in journal and book clubs of the Association for Computing Machinery and the Medical Library Association. ■

To contact NIH informationists, visit the NIH website at **<http://nihlibrary.nih.gov/LibraryServices/Informationists.htm>**.

Excerpts of informationists' interactions with clinical and basic research teams are presented in a 13-minute documentary, "Expert Searching at the NIH," produced by the NIH Library and MAPB for the 2003 Medical Library Association conference. Copies of the CD for staff use or presentations are available on request.

For more information, contact Susan Whitmore (301) 496-1157 or **<susan_whitmore@nih.gov>** or Suzanne Grefsheim at (301) 496-2448 or **<grefshes@mail.nih.gov>**.

Recently Tenured

Glinda Cooper *received her Ph.D. in epidemiology from the University of North Carolina at Chapel Hill in 1993. She then joined NIEHS as a staff fellow, became a tenure-track investigator in 1998, and is currently a senior investigator in the Epidemiology Branch.*

Glinda Cooper

Autoimmune diseases affect 5 to 8 percent of the United States population and are a leading cause of death among women younger than 65 years old.

Despite the impact of these diseases, they have been relatively neglected with respect to epidemiologic research—the study of the distribution of disease within a population—an approach that can provide insights into disease mechanisms, prevention, and prognosis.

My research has focused on the prototypical disease, systemic lupus erythematosus, or lupus.

Lupus is a severe, disabling disease that can lead to significant morbidity and mortality, particularly from renal and cardiovascular disease. The vast majority (more than 85 percent) of lupus patients are female. Although it has long been known that genetic susceptibility plays an important role in lupus, there is growing interest in environmental influences on the development and progression of this disease.

A central piece of my research is the Carolina Lupus Study, the first population-based case-control study of hormonal and occupational risk factors for lupus conducted in the United States.

Quite surprising, given the female predominance in lupus in humans and the role of estrogen in disease progression seen in lupus mouse models, none of the markers of estrogen exposure we examined were associated with risk of lupus.

These markers included earlier age at menarche and later age at menopause, use of hormone replacement therapy or hormonal contraceptives, and number of pregnancies and live births. Many of these exposures are associated with the risk of breast cancer, suggesting that lupus does not fit that model of an "estrogen-related" disease.

Several occupational cohort studies have examined the association between silica exposure and the risk of various systemic autoimmune diseases, but ours was the first group to include an assessment of silica exposure in a population-based study of lupus. Our findings were striking. We observed a silica dust dose-response across levels of exposure, with an approximate twofold increased risk in the medium-exposure category and a fourfold increased risk in the high-exposure category. As expected, the prevalence of high exposure was lower among women than men, but the associations were seen in both groups.

Our success in carrying out a population-based study of lupus that contains a strong environmental component has encouraged others to consider the contribution that environment can make to lupus and to other autoimmune diseases. Several new studies are being developed, intramurally and extramurally, by other investigators incorporating aspects of our data-collection instruments and hypotheses.

I also continue to pursue an interest in ovarian function, an area that includes menstrual cycle patterns and the timing of ovarian failure or menopause. The effect of potential environmental endocrine disruptors on ovarian function is an issue that is particularly relevant to the mission of NIEHS.

We were the first to report an association between serum levels of the persistent organochlorine DDE (a breakdown product of the pesticide DDT), and earlier age at menopause, with effects of similar magnitude to that seen with smoking.

In the Agricultural Health Study, a joint NCI-NIEHS prospective cohort study of more than 50,000 licensed pesticide applicators and their spouses, we found an association between pesticide use and an increased risk of long cycles, missed periods, and intermenstrual bleeding among premenopausal women.

Menstrual cycle characteristics and timing of natural menopause may be sensitive markers of biological effects of environmental exposures, and I am continuing to examine these outcomes in studies of environmental pollutants.

Patricia Gearhart *received her Ph.D. in immunology in 1974 from the University of Pennsylvania in Philadelphia. She did postdoctoral work at Johns Hopkins University, Baltimore, on a Helen Hay Whitney fellowship. She was a staff associate at the Carnegie Institution of Washington in Baltimore and an associate professor in the Bloomberg School of Public Health at Johns Hopkins University before joining the Laboratory of Molecular Gerontology, NIA, in 1995. She became a tenure-track inves gator in 2001 and is currently a senior investigator in the Unit on Antibody Diversity.*

Patricia Gearhart

I like to start out my seminars with a slide from the "War of the Worlds" movie that came out in the summer of 2005. In the movie, humans are being pulverized by invading Martians, and extinction is imminent. Then, suddenly, the Martians drop dead! They died because they didn't have the activation-induced deaminase (AID) molecule, which would have allowed them to become immune to earth's microorganisms.

The idea of having an unlimited repertoire of proteins able to tackle any foreign antigen, even ones the human race has never seen, is mind-boggling. Immunity is partly based on a random slew of mutations that occur in antibody genes, followed by selection of those B lymphocytes that can express the mutated proteins with the highest affinity.

Thus, when you're injected with a vaccine, some of your B cells can bind to the antigen and produce low-affinity antibodies of the IgM type. Soon after, the B cells start a process called somatic hypermutation—somatic because these mutations are not present in your germ-line genes and hypermutation because the genes undergo mutation at a frequency that is a *million* times as high as any other gene in your body.

I became interested in the generation of antibody diversity as a graduate student at the University of Pennsylvania. This was in the pre–molecular biology era, so we studied different proteins with anti-antibodies. The hybridoma era came next, and it was possible to sequence antibodies at the protein level to identify different molecules.

In 1980, I showed that B cells initially make IgM antibodies using a few germline genes and then switch to making IgG antibodies with many mutations in their genes.

As a staff associate at the Carnegie Institution of Washington, I had the opportunity to learn gene cloning and DNA sequencing. I was able to show in 1983 that the range of mutations extended over a two-kilobase region around the antibody variable gene. In other words, hypermutation was targeted to a small region of the chromosome.

While on the faculty at Johns Hopkins University, I realized the mechanism producing hypermutation would only be solved by studying DNA repair. I had the good fortune to come to NIA in Baltimore in 1995 and work in a repair lab headed by Vilhelm Bohr.

In 1998, we published the first paper to show that a mismatch repair protein, MSH2, played a powerful role in the mutation of certain nucleotides. Then in 2001, we were the first to identify the involvement of the error-prone DNA polymerase η in the hypermutation process. But we still didn't know the mechanism.

The whole story came together with the separate contributions of researchers from Japan, England, and elsewhere.

First, in 2000, the Japanese team discovered that the AID protein (which the Martians didn't have) initiates hypermutation and heavy chain class recombination. AID is expressed only in B cells; people who don't have AID have recurring infections because they can't mutate their variable genes or make IgG.

Then, in 2002, the mechanism was solved when researchers in England reported that AID works by deaminating cytosine in DNA to form uracil. Uracil is a foreign nucleotide and will cause mutations. MSH2 and its partner, MSH6, enter the pathway by binding to the uracil mismatches and then recruiting DNA polymerase to synthesize errors.

The most satisfying moment in my career came this year when we published a paper that tied these proteins together using biochemistry, genetics, and biology. (T.M Wilson, A. Vaisman, S.A. Martomo, et al. "MSH2-MSH6 stimulates DNA polymerase η, suggesting a role for A:T mutations in antibody genes," *J. Exp. Med.* **201**:637, 2005).

The final frontier is to determine how AID is targeted to immunoglobulin genes. We are trying a variety of approaches to solve this enigmatic puzzle. Inappropriate targeting by overexpression of AID can produce mutations in other genes and DNA strand breaks. B cells therefore walk a fine line between generating antibody diversity and tumors.

Kim Y. Green *received her Ph.D. in 1986 from the University of Tennessee at the Center for Health Sciences in Memphis. She joined the Laboratory of Infections Diseases (LID), NIAID, that year as a postdoctoral fellow in the Epidemiology Section. In 1993, she became a tenure-track investigator in the LID and is currently a senior investigator. She is also an adjunct associate professor at the University of Maryland, College Park.*

Fran Pollner

Kim Green

When I joined the Epidemiology Section, the rotavirus vaccine candidate developed in the laboratory under the guidance of my mentor, Albert Kapikian, was already in clinical trials. It was an exciting time, because data from the Phase III trials were emerging to show that the vaccine was effective in preventing severe diarrhea in infants and young children.

My initial research in the laboratory as a postdoctoral fellow addressed the molecular mechanisms for serotypic diversity among the predominant rotaviruses associated with diarrheal illness. I developed assays that enabled the dissection of the serotype specificity of the antibody response to the vaccine in young vaccinees. These studies showed the importance of serotype-specific antibodies and supported the rationale for the use of a multivalent rotavirus vaccine.

In 1993, I was asked to initiate a research program in the Epidemiology Section to address the role of the Norwalk virus and related viruses (now called noroviruses) in human diarrheal illness. Norwalk virus had been discovered by Kapikian in 1972 but had taken a back seat to the vigorous rotavirus vaccine development effort in the section. My research program began with nothing but frozen stool samples and sera collected from volunteers who had participated in challenge studies at NIH in the early 1970s, along with the newly available sequence of the Norwalk virus genome published by scientists at Baylor University in Waco, Texas.

We initially thought that most of the norovirus strains associated with epidemic gastroenteritis (known colloquially as "stomach flu") would be closely related to the original Norwalk virus strain. However, our work soon revealed that considerable genetic diversity existed among circulating noroviruses and that infection with these viruses was more common than expected, even among younger individuals. Noroviruses have now been established as the major cause of the acute nonbacterial gastroenteritis that often occurs in sharp outbreaks that capture headlines, such as those that have arisen on cruise ships. My laboratory played a key role in some of the early large-scale epidemiologic studies that established the importance of noroviruses in nursing homes and military settings.

We are now convinced that these viruses are worthy of concerted efforts to develop vaccine and antiviral control strategies. My future research will address some of the technical hurdles that must be overcome to pursue this line of research—especially the inability to grow the human norovirus pathogens in cell culture and the absence of an animal model for diarrheal disease. Currently, the only way to study the efficacy of a vaccine candidate, such as recombinant capsid protein, is to immunize adult volunteers and then challenge the volunteers with noroviruses present in stool material.

One approach pioneered by my laboratory was the use of related viruses within the virus family (Caliciviridae) as models to study general features of calicivirus growth, replication, and maturation in vitro. We reported the development of the first infectious RNA system ("reverse genetics") for the family Caliciviridae.

This system, modeled on the cultivatable feline calicivirus (FCV), was based on the transfection of infectious in vitro–transcribed capped RNA molecules derived from a full-length cDNA clone of the feline calicivirus genome into permissive cells and recovery of viable viruses. This system allowed us to introduce mutations into the cDNA clone and

Recently Tenured

study their effect on the growth and replication of the recovered virus.

We also found that we could engineer chimeric viruses with altered antigenic specificities. We are now working on the development of a reverse genetics system for the noroviruses.

In the meantime, we have made progress in dissecting the functions of various norovirus proteins in replication and are exploring potential antiviral inhibitors through the study of recombinant enzymes.

The noroviruses are interesting in that they have a global distribution and are ubiquitous in the environment. They seem to arise out of nowhere to cause acute gastroenteritis outbreaks. We do not yet understand how immunity is developed, and we are just beginning to identify the role of host factors in susceptibility to infection. The field of norovirus research has exciting opportunities for addressing the role of a common and sometimes severe pathogen and in gaining a better understanding of the virus and host interactions that might lead to the development of effective control strategies

Andrew Griffith *received his M.D. degree and Ph.D. in molecular biophysics and biochemistry from Yale University in New Haven, Conn., in 1992. He completed a residency in otolaryngology–head and neck surgery and a human genetics research fellowship at the University of Michigan, Ann Arbor, before joining NIH in 1998 as a research fellow in both the Laboratory of Molecular Genetics and the Neuro-Otology Branch, NIDCD. He is currently acting chief of the Section on Gene Structure and Function and of the Hearing Section, NIDCD.*

Kiyoto Kurima

Andrew Griffith

There are hundreds of genes in which mutations cause hearing loss either alone or in combination with other abnormalities as part of a syndrome. Because most hearing loss phenotypes are nonspecific, the clinical challenge is to determine which gene to test in a patient whose hearing loss may have a genetic etiology.

Inner ear malformations seen on CT or MRI scans are an example of clinically useful markers to guide molecular genetic diagnosis. The most commonly detected malformation, enlargement of the vestibular aqueduct (EVA), is clinically important because affected children often pass newborn hearing screening but experience significant postnatal hearing loss.

Mutations of *SLC26A4* (*PDS*), which encodes the anion transporter pendrin, have been identified in patients with nonsyndromic EVA (NSEVA) as well as in a syndromic form of EVA called Pendred syndrome. The molecular and physiologic details of the pathogenesis of hearing loss caused by *SLC26A4* mutations remain unclear.

In a study of approximately 90 EVA patients at the NIH Clinical Center, we observed that one-third of cases are associated with a thyroid iodination defect as part of Pendred syndrome and mutations of both alleles of *SLC26A4*. One-third of cases are nonsyndromic and associated with only one detectable mutant allele of *SLC26A4*, while another one-third are also nonsyndromic but have no detectable mutations. Identifying the etiology of nonsyndromic EVA in these latter two genotypic groups is a focus of our current research.

Another major goal of my laboratory is identification of the molecular function(s) of the proteins encoded by *TMC* genes. We used positional cloning to determine that dominant and recessive mutations of the transmembrane channel–like gene 1 (*TMC1*) cause hearing loss in humans and mice.

There are eight mammalian *TMC* genes, and their deduced amino acid sequences have no obvious similarities to other domains or proteins of known function. All of the *TMC* genes are predicted to encode proteins with 6 to 10 membrane-spanning domains; we are now completing a study indicating that TMC1 has 6 transmembrane domains and cytoplasmically oriented N- and C-termini. This topologic structure is shared with the large superfamily of multimeric cation channels that includes transient receptor potential (TRP) channels.

We are currently using knockout mouse models of *Tmc1* and other *Tmc* genes to understand the pathogenesis of hearing loss caused by *TMC1* mutations, to characterize the molecular and cellular function of TMC1 protein, and, we hope, to identify other tissues and *Tmc* mutant model systems to study the function(s) of this gene family.

The histopathologic hallmark of *Tmc1* mutant mice is early and rapid degeneration of cochlear neurosensory hair cells, where *Tmc1* mRNA is expressed. In mice that are heterozygous for the dominant *Tmc1* mutation, the degeneration of cochlear hair cells depends upon the mouse strain background.

We have mapped four genetic loci with strain-specific alleles that differentially affect degeneration of hair cells. Because hair cell loss is a final common pathogenetic pathway in many mouse and human hearing loss disorders, such as age-related hearing loss and noise-induced hearing loss, identification of these modifier genes may provide critical insights into these more common but etiologically complex phenotypes.

Vittorio Sartorelli

Indeed, previous work by others on a mouse model of polygenic hearing loss enabled us to identify a hypofunctional variant of plasma membrane calcium pump PMCA2 (encoded by *ATP2B2*) that modifies human age-related hearing loss caused by a mutation of the cadherin 23 (*CDH23*) gene. We plan to assess the contributions of *ATP2B2*—and other modifier genes that might be identified—to age-related hearing loss and noise-induced hearing loss in large case-control cohorts of human subjects.

Vittorio Sartorelli *received his M.D. degree from the University of Brescia, Italy, in 1984; he completed an oncology residency at the University of Milan and postdoctoral work at Stanford University in Stanford, Calif., and the University of Southern California in Los Angeles. He was then an assistant professor of biochemistry and molecular biology at USC before joining NIAMS in 1999 to head the Muscle Gene Expression Group in the Laboratory of Muscle Biology.*

The formation and development of an organism relies on the specification of discrete cell lineages. Because every cell of an organism contains—packed in its DNA—the same genetic information, it is of interest to understand the strategy a cell uses to become, for instance, a contractile muscle cell rather than a firing neuron.

My group in the Laboratory of Muscle Biology addresses the cellular and molecular mechanisms underlying specification, differentiation, and regeneration of skeletal muscle cells. Pivotal in skeletal muscle biology are the myogenic basic helix–loop–helix (bHLH) transcription factors MyoD, Myf5, myogenin, and Mrf4.

After interaction of these proteins with the ubiquitously expressed bHLH E proteins, the resulting myogenic bHLH–E heterodimers bind to and regulate expression from the E-box, a specific DNA motif present at muscle gene enhancers and/or promoters.

The interactions of the myogenic bHLH transcription factors with enzymes that modify the structure of the nucleosome—the basic unit of chromatin—enable tem-

porally regulated formation and recruitment of specific protein complexes at the chromatin of discrete muscle gene loci.

By influencing the structure of the chromatin, such protein complexes ultimately dictate whether or not a given gene will be transcribed. Therefore, a detailed biochemical definition of the protein complexes and the mechanisms that regulate their chromatin engagement is essential to the comprehension of how muscle gene expression and cell differentiation are orchestrated.

Over the past years, we have identified and characterized proteins that are recruited on the chromatin and that either promote or inhibit muscle gene expression. A common strategy used by these proteins to regulate transcription is the enzymatically mediated addition or removal of chemical groups (acetyl, methyl, phospho groups) at defined amino acid residues of histones.

The availability of cell-permeable small molecules that interfere with the enzymatic activities of some of these proteins (histone deacetylases) has afforded us the opportunity to manipulate muscle gene expression both in cell cultures and in animal models. Using histone deacetylase inhibitors, we have been able to increase formation of mature skeletal muscle cells in cell culture and to promote the expression of markers of muscle regeneration in animals in which muscle degeneration was experimentally induced. The next challenge will be to evaluate the therapeutic efficacy of histone deacetylase inhibitors in animal models of Duchenne and limb girdle muscular dystrophies.

Another line of research pursued in my group relates to the mechanisms regulating the expression of muscle-specific ubiquitin ligases involved in mediating skeletal muscle atrophy. Skeletal muscle atrophy is the loss of muscle mass and function that is often associated with cancer, AIDS, diabetes, glucocorticoid treatment, chronic uremia, sepsis, and other diseases. Muscle atrophy also follows denervation, immobilization, and starvation.

While the pathophysiology of all these conditions is often unrelated, reduced protein synthesis and increased protein degradation invariably accompany muscle atrophy, regardless of the triggering cause. Two genes have been identified that are rapidly upregulated during the course of skeletal muscle atrophy and are causally linked to its pathogenesis. These genes, *Atrogin-1/MAFbx* (*muscle atrophy F box*) and *MurF1* (*muscle RING finger 1*), encode for two E3 ubiquitin ligases selectively expressed in skeletal muscle and heart and involved in modifying proteins fated to be degraded by the proteasome.

We are interested in understanding how transcription of these two genes is regulated during muscle atrophy. Our objective is to be able to modulate their expression to spare muscle mass in diseased conditions.

Lino Tessarollo *received his Ph.D. in Biological Sciences from the University of Padua, Italy, in 1987. After postdoctoral training at the Institute of Oncology, Padua, and at NCI-Frederick, he was recruited in 1994 as an investigator at the ABL-Basic Research Program, NCI-Frederick, to develop targeted gene-manipulation technology in the mouse. In 1999, he joined the Mouse Cancer Genetics Program at the NCI Center for Cancer Research, where he directs the Neural Development Section and the Gene Targeting core facility.*

My research focuses on the dissection of signals that control cell proliferation and survival in the mammalian system. Using mouse models and in vitro approaches, we aim to identify specific pathways that can be activated to promote survival of cell populations affected in neurodegeneration—or that can be inhibited to avoid uncontrolled cell proliferation leading to cancer.

Lino Tessarollo

We study the neurotrophin family of peptide growth factors (NGF, BDNF, NT-3, and NT-4/5) and their high-affinity Trk tyrosine kinase receptors (TrkA, TrkB, and TrkC) because they are critical players in the development and maintenance of the mammalian central and peripheral nervous system. These genes have attracted great interest as potential therapeutic targets for the management of neurodegenerative disorders such as Parkinson's and Alzheimer's disease.

Neurotrophin receptors are also frequently overexpressed in human cancer, including pancreatic and prostate carcinoma, Wilm's tumor, and neuroblastoma, particularly those with aggressive behavior and poor prognosis. Thus, while suppression of Trk-activated pathways may contribute to tumor management, strategies aimed at improving neurotrophin-mediated activities may also help stem neurodegenerative diseases.

Over the past decade, many laboratories have contributed to our understanding of neurotrophin receptor signaling. However, most of this information has been generated in vitro. The situation is far more complex in vivo because cellular context and environment may influence how cells respond to signals. To dissect the cell type– and environment-specific effects of the neurotrophin-activated pathways in vivo, we use a mouse model to introduce specific targeted mutations.

For the development of our in vivo systems, we focus on the following molecular properties: a) docking sites on the intracellular portion of the neurotrophin receptors to evaluate whether altering specific pathways can affect cell type–specific responsiveness to neurotrophins; b) truncated Trk isoforms that lack the kinase domain and may have positive or negative modulatory roles on the kinase active receptors; and c) tissue-specific neurotrophin or receptor deletion to dissect both neuronal and non-neuronal functions.

These studies have led us to the identification of a specific docking site in the Trk receptor juxtamembrane region, which in vivo has the ability to potentiate neurotrophin signaling without affecting cell proliferation. Similar results were obtained by the targeting of physiological truncated Trk receptors.

In vivo reduction of these truncated receptors can increase ligand activation of the kinase active receptors and avert neural cell death in a mouse model of neurodegeneration. We are now planning to assess whether these Trk mutations, which potentiate neurotrophin signaling in vivo, may affect tumor development and/or metastatic properties in established tumor mouse models.

These approaches have validated our strategies and helped us to shed more light on the role of Trk isoforms and Trk signaling in vivo. They will also be essential in assessing the risks and benefits associated with augmenting or inhibiting neurotrophin signaling in the clinic.

Moreover, our results suggest new strategies to affect Trk receptor activation in vivo by small-molecule–based approaches—which could circumvent the challenge of delivery to the CNS and specific pharmacokinetic problems associated with the classic neurotrophin peptide–based approach. Clearly, there is still insufficient knowledge on the physiological roles of these genes and their underlying molecular mechanisms of action.

We anticipate that our model systems will help amass more detailed information on how Trk-activated pathways control the maintenance and function of the mature organism.

This expanded knowledge base may help identify potential therapeutic targets within the neurotrophin signaling pathways for the treatment of neurodegenerative diseases and cancer ■

Catalytic Reactions?

If you have a photo or other graphic that reflects an aspect of life at NIH (including laboratory life) or a quotation that scientists might appreciate that would be fit to print in the space to the right, why not **send it to us via e-mail: catalyst@nih.gov>; fax:402-4303; or mail: Building 2, Room 2E26.**

Also, we welcome letters to the editor for publication and your reactions to anything on the *Catalyst* pages.

In Future Issues...

- Obesity Research
- IRP Roundup
- Another Look At Open Access

Kids' Catalyst: Zapped! Static Electricity

Under the right conditions, you'll throw sparks! It could be a little jolt or a very big one, but when it happens, you will be experiencing the effects of static electricity firsthand.

You've seen it or felt it before . . . the socks that magically stick together, that shock from touching a doorknob in winter or just touching someone else. It's all static electricity, and we're going to make a little bit of it today.

Try this when it's cool and dry: Put some shoes on and scuff your feet on the carpet a few times. Now if you touch a doorknob, or another person, you'll be shocked. Ouch! (The more you scuff, the more it will hurt, so be careful.)

But you don't have to wait for the perfect conditions, and you don't have to feel the pain in order to prove something is there. Let a balloon do the sticking for you!

What you'll need for this experiment:

1. At least two balloons, blown up and knotted—the more the better
2. A wool sweater, rug, or cooperative fur-bearing animal (Cooperative is very important! If not, stick with the sweater.)
3. A wall
4. Talcum powder or flour
5. Hand lotion or hair conditioner

Now take the balloon and try to stick it to the wall (without tape, please). You'll see nothing but a falling balloon. Rub the balloon on the sweater for a few seconds and try again. It sticks! But for how long? Do you think the amount of time it sticks will increase the longer you rub the balloon?

Now if this isn't enough, take a look at fields. Instead of sticking the balloon to the wall, hold it over (but not touching) some flour or talcum powder. The powder will fly up and stick to the surface of the balloon. Twist the balloon around, picking up flour, and see if one side of the balloon picks up more than the other. Does it make a pattern?

Take another balloon and create the static charge again, but this time take a dab of hand lotion and put it on the balloon. Now hold it next to the powder. What do you think will happen?

Try sticking balloons to different materials: cloth, leather, metal, yourself. Can you predict which will stick longer?

What you've done by scuffing your feet or rubbing a balloon is to create a charge. Because there is no current (like plugs in the wall), it is called static. So the next time you're walking around and unexpected sparks fly, you'll know why!

—Jennifer White

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