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NCRR Reporter

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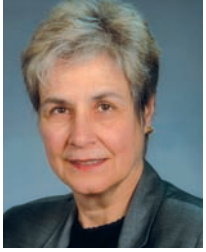
CRITICAL RESOURCES FOR YOUR RESEARCH

U.S. Department of Health and Human Services

Preventing HIV Infection Using Microbicides

At Tulane University's National Primate Research Center, Ronald Veazey is developing a microbicide gel that could block sexual transmission of HIV.

FROM THE DIRECTOR



Slowing the Spread of AIDS Through Translational Research

Because women are particularly vulnerable to HIV infection and the number of women with AIDS continues to rise at a staggering rate, finding effective methods to protect them against the disease is a critical health objective both in this country and around the world. Efforts are underway on many fronts; this issue of the Reporter highlights the work of researchers at NCRR-supported resource centers who are developing affordable and easy-to-use microbicide gels and creams to prevent sexual transmission of HIV.

The article that follows is not only a story of remarkable science, but it also illustrates the process by which discoveries at a cellular level are translated into preclinical studies and then clinical trials. The spectrum of studies described includes work in mice to understand how to prevent cell-to-cell transfer of HIV; the use of monkeys to find ways to block viral access; and large-scale human clinical trials that test different compounds for their ability to prevent widespread HIV infection.

It is also the story of how pre-clinical and clinical resources enable the discoveries made by talented and devoted researchers, which ultimately impact human health. The same kind of support and synergy will be facilitated in the Clinical and Translational Science Awards (CTSAs) announced last year. In fact, the CTSAs are designed to alleviate the roadblocks inherent in translational research and further accelerate the research process. Currently located at 12 academic health centers around the country, the CTSA grantees are developing the resources to train and advance a cadre of well-trained multi- and inter-disciplinary investigators and research teams and giving them access to innovative research tools and information technologies. The CTSA consortium will eventually grow to 60 sites, dedicated to creating transformative, novel, and integrative homes for clinical and translational science.

Our goal with the CTSAs and all resources supported by NCRR is to provide researchers with the tools and connections—whether to other researchers or to patients and communities—to speed the process of discovery and multiply opportunities to improve human health, whether it is protecting women from HIV or the hundreds of other diseases and conditions that affect people around the world. We would like to see the number of women living with HIV, over 17 million today, decrease. The research you will read in the next few pages gives us reason for optimism.

Barbara Alving, M.D.

Barbara Alving, M.D.
Acting Director, NCRR

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CRITICAL RESOURCES

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Research Briefs

NCRR Reporter

This quarterly publication of the National Center for Research Resources fosters communication, collaboration, and resource sharing in areas of current interest to scientists and the public.

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On the Cover: At an NCRR-funded primate center, researcher Ronald Veazey and his colleagues have developed experimental microbicide gels that can protect primates against vaginal infection with simian HIV. His team has also developed oral formulations of a compound in the gels, which have had similarly promising results.
PHOTO BY PHILIP GOULD

QUICK TAKES

New tools and updates from NCRR resources

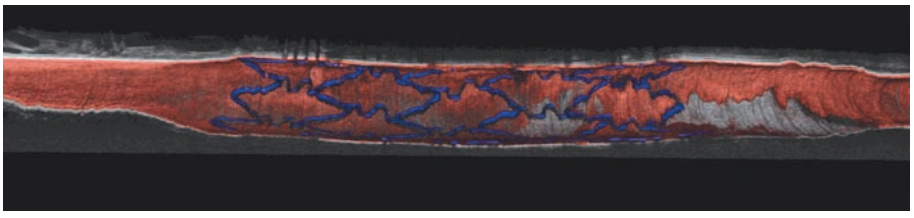
Virtual Biopsies

A new imaging technique provides inner 3-D views of porcine blood vessels in vivo, according to a report in the December 2006 issue of *Nature Medicine*. Researchers used a minimally-invasive catheter to deliver a tiny imaging probe to pigs' arteries, obtaining microscopic images. The same technique could help physicians inspect coronary arteries for high-risk plaques or damaged tissue.

The imaging probe, measuring less than 1 mm in diameter, works by rotating an optical fiber that is shaped to focus and direct infrared light into the vessel wall. The researchers measured the back-scattered light collected by the probe and then used computations to create longitudinal or cross-sectional views of the vessels.

The technique was developed at the Wellman Center for Photomedicine at the Massachusetts General Hospital in part through NCRR funding. This type of 3-D microscopy could bridge the gap between low-resolution radiological techniques and excisional biopsies. Unlike other existing high-resolution microscopy techniques, images can be obtained in a matter of seconds.

The technique, named optical frequency-domain imaging, can also be used to examine the gastrointestinal tract or detect early cancerous lesions.



■ A 3-D image of a porcine artery and stent (in blue) is created using a new type of laser microscopy.

■ Scientists have uncovered the purple sea urchin's 23,300 genes.

Sea Urchin Genome

The sea urchin is a favorite animal model among developmental biologists, just like the fruit fly and the worm. But it has one advantage over its fellow invertebrates: its genome is closer to that of humans on the evolutionary scale, according to a study published in the November 10, 2006 issue of *Science*. The study describes, for the first time, the sequence and analysis of the 814 million DNA bases that make up the genome of the purple sea urchin *Strongylocentrotus purpuratus*.

The scientists used a two-pronged strategy to sequence the urchin genome: whole-genome shotgun sequencing and a library of bacterial artificial chromosomes (BAC), clones that carry inside them very large pieces of sea urchin DNA. The NCRR-funded Sea Urchin Genome Resource at the California Institute of Technology provided the BAC library and the DNA for shotgun sequencing.

The sea urchin genome spells out about 23,300 genes. Surprisingly, genes previously thought to be unique to vertebrates also were found in the sea urchin. This realization will allow scientists to perform functional studies in a simple animal model that shared a common ancestor with vertebrates long ago.

Stem Cells Increase Insulin

Researchers at the NCRR-funded Adult Mesenchymal Cell Resource at the Center for Gene Therapy in Tulane University have successfully used adult human stem cells to increase insulin production in a mouse model of diabetes. The work was published in the November 14, 2006 issue of the *Proceedings of the National Academy of Sciences*.

In type 2 diabetes, the pancreas cannot make enough insulin to help process blood glucose into energy. In this study, the researchers injected human multipotent stromal cells (hMSCs)—cells that have the potential to become different types of cells in the body—derived from human bone marrow into the left cardiac ventricles of immunodeficient mice. The human cells did not appear to differentiate into insulin-producing cells. Rather, the hMSCs migrated to the pancreas and seemed to induce the development of endogenous mouse cells that produce insulin. The levels of insulin in the treated mice were found to be twice that of control diabetic mice.

Scientists believe these versatile hMSCs could be used in the future for treating high blood glucose in diabetic human patients. The cells can be obtained from a patient's bone marrow and grown in culture for later transplant back into the patient. ■

PHOTO BY CHARLES HOLLAHAN(TOP), PHOTO COURTESY OF WELLMAN CENTER FOR PHOTOMEDICINE, MASSACHUSETTS GENERAL HOSPITAL (BOTTOM)

Exploring the Potential of HIV Microbicides

As the number of HIV-infected women escalates worldwide, vaginal microbicides may help slow the spread of AIDS.

The face of the AIDS epidemic has changed considerably in the last quarter of a century. Although the disease was first identified in homosexual men, today women comprise half of the world's nearly 40 million HIV-infected individuals. Most of these women became infected through heterosexual contact.

Women are particularly vulnerable to HIV infection because their mucosal exposure to the virus during intercourse is greater than men's. Condoms can help prevent HIV transmission, but their use is not under a woman's control. Because of these and other vulnerabilities, new HIV infections now arise more rapidly among women than among men in many parts of the world.

Public health officials have long called for new HIV prevention methods for women—methods that are inexpensive, easy-to-deliver, and under their control. This need is especially urgent, given that a cure for AIDS and development of a safe and effective HIV vaccine has proven elusive.

Microbicides are substances, typically formulated as gels or creams, that can be applied topically to the vagina to prevent sexual transmission of HIV or other pathogens. The products, now being tested in preclinical and clinical studies, may offer women the protection they need. Mathematical models predict that if only 20 percent of women in the developing world used a microbicide in just half of their sexual encounters, 2.5 million HIV infections could be prevented over a three-year period.

NCRR-funded resource centers are helping scientists to pursue

■ Christopher Miller has found that microbicides, if applied soon after intercourse, may prevent a widespread HIV infection. His research suggests that HIV infection can remain limited to the vagina and cervix for about four days prior to systemic infection.

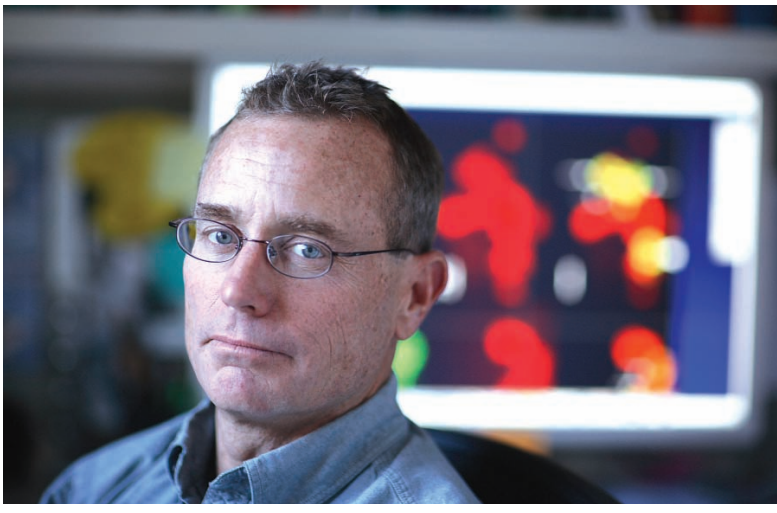


PHOTO BY TIMOTHY ARCHIBALD

■ Women of Galufu, Malawi, a small village in southern Africa, prepare food after the funeral of a female villager who died of AIDS a few hours earlier. Poverty in the village has increased dramatically in the last few years because of drought and AIDS.

novel approaches to microbicides on several fronts. Investigators supported by NCRR's Research Centers in Minority Institutions (RCMI) Program are conducting preclinical studies of an effective yet inexpensive microbicide based on a common product additive. An NCRR-supported General Clinical Research Center (GCRC) is helping efforts to examine both how microbicides react to the human vaginal environment and how the vagina reacts to the compounds. And studies with macaque monkeys at the NCRR's National Primate Research Centers (NPRCs) are uncovering new roles for microbicides and developing approaches that target the molecular interactions between HIV and the immune cells it invades.

In the 1980s, scientists working at NCRR-funded NPRCs laid the groundwork for much of today's HIV research when they discovered the simian immunodeficiency virus (SIV), a close relative of HIV that infects nonhuman primates. SIV infection in macaque monkeys has since become a vital animal model for the study of HIV infection, treatment, and prevention.

Soon after the discovery of SIV, Christopher Miller, a researcher at the California NPRC at the University of California, Davis, and his colleagues showed that they could infect monkeys in a way that mimics sexual transmission of HIV in humans by applying SIV to the genital mucous membranes of macaques. Studies with these monkeys have shown how SIV spreads systemically from genital mucosal sites.

"This is a particularly good model for understanding what happens in human HIV infections, because these nonhuman primates have the same sort of anatomy and physiology as do humans, and they also have 28-day menstrual cycles, just as women do," says Miller. "Especially for studies that hopefully will be predictive of how an intervention affects human patients, we want the animal model to be as close as possible to the real thing."

Miller and his colleagues recently found that new SIV infec-

WOMEN AND AIDS

The number of women with HIV infection and AIDS has increased steadily. By the end of 2005, of the 38.6 million people living with HIV, almost half (17.3 million) were women, according to the World Health Organization. The vast majority (76%) of HIV-infected women live in sub-Saharan Africa. In the United States, the proportion of adult HIV cases among women has more than tripled in the past two decades—from 8% in 1985 to 25% today, according to United Nations and U.S. Centers for Disease Control and Prevention statistics. HIV infection disproportionately affects the nation's African-American and Hispanic women. Together, these two ethnic groups represent less than 25% of all U.S. women, yet they account for 79% of women with HIV nationally.

PHOTO BY PER-ANDERS PETTERSSON/GETTY IMAGES

tions in female macaques remain limited for about four days to a relatively small number of cells, primarily in the vagina and cervix. "There's something of a delay between the time that the virus comes in contact with the genital tract and the time that full-fledged systemic replication of the virus occurs," says Miller. This finding suggests that microbicides might do more than just block sexual transmission of HIV. If a woman has already become infected through sexual contact, a microbicide, if administered soon enough, might prevent or limit a wider systemic HIV infection. "It gives people hope and a rational basis to keep exploring interventions that are aimed at an early timepoint," he says.

At the Tulane NPRC, another NCRR-funded primate center, Ronald Veazey and his colleagues are testing a promising new type of microbicide called a fusion inhibitor. These agents inhibit infection in a specific and targeted way by preventing the binding, or fusion, between glycoprotein molecules on the outer coat of HIV particles and the receptors for those glycoproteins on the surface of immune cells.

Veazey studies fusion inhibitors that target a type of cellular receptor called CCR5. As one of the main receptors that HIV uses to infect cells, CCR5 appears to play a major role in HIV transmission across mucosal surfaces, like those in the vagina. When these fusion inhibitors bind to a cell's CCR5 receptors, they block viral access to the receptors and in some cases also trigger cellular changes that reduce the number of receptors on the cell's surface. These mechanisms greatly limit viral entry points into the cell.

Veazey and his colleagues have found that both vaginal and oral administration of CCR5-based fusion inhibitors protects macaques against infection. "We've shown tremendous proof of concepts in blocking this receptor," says Veazey. "Blocking CCR5 seems to be all that is necessary to prevent transmission of the AIDS virus, at least in the monkey model."

In one study, the researchers administered three experimental microbicide gels, alone and in combination, to female macaques and found that all three were protective against vaginal infection with simian HIV. In addition, significant protection was achieved when two of the agents—known as Compound 167 and BMS-378806—were administered in combination. The combination gel was protective even when applied up to six hours before viral exposure. In a separate study, Veazey and colleagues found that orally administered formulations of Compound 167 can prevent vaginal infections of simian HIV in macaques.

Scientists are now working to develop more cost-effective molecular preparations of fusion inhibitors. "Clearly, the CCR5 point of attack is extremely effective. Our major obstacle now is to develop a fusion inhibitor that can be produced economically," says Veazey. Clinical trials of the fusion inhibitor gels are now being planned.

Another novel approach to microbicide development is being pursued in preclinical studies at the NCRR-funded RCMI at Meharry Medical College in Nashville. James Hildreth, director of Meharry's Center for AIDS Health Disparities Research, is investigating an agent called beta-cyclodextrin. Cyclodextrins are simple polymer sugars that are already widely used in a variety of products, including mouthwash, topical creams, food flavorings, and some intravenous medications.

"Cyclodextrin is easy to synthesize, very inexpensive to pro-

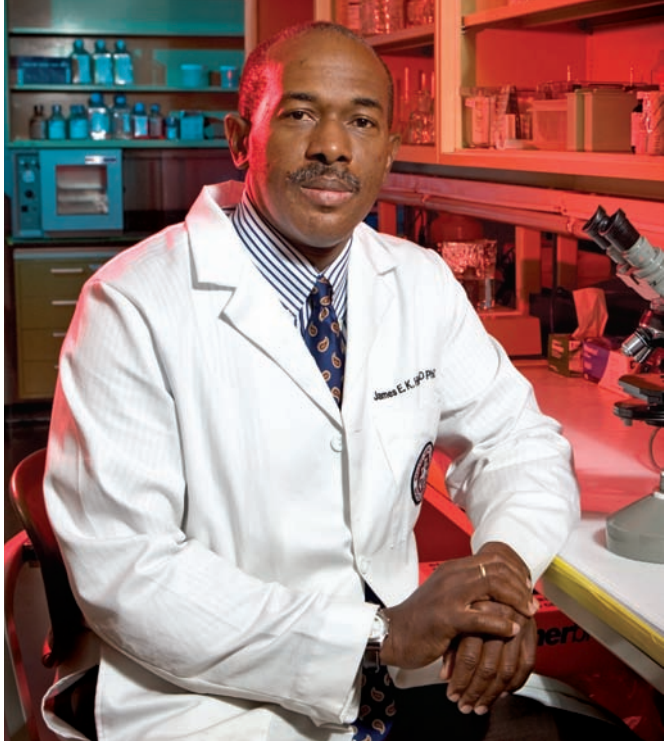
NIH-FUNDED MICROBICIDE CLINICAL TRIALS

With funding from several NIH institutes, two multisite clinical trials are now under way to evaluate the safety and efficacy of the potential vaginal HIV microbicides described below. Although called "microbicides," most of these agents do not actually kill the virus, but instead simply impede its ability to infect host cells.

Tenofovir/PMPA A Phase II clinical study, expected to involve 200 women, will be evaluating the safety and acceptability of a microbicide gel formulation of tenofovir (PMPA). Tenofovir works by inhibiting enzymes that are needed for HIV replication. An orally administered tablet form of tenofovir was approved several years ago by the U.S. Food and Drug Administration for the treatment of HIV infection in combination with other anti-HIV medicines.

BufferGel and PRO 2000/5 A Phase II/IIb clinical trial, with an anticipated enrollment of 3,220 women, will be evaluating the safety and effectiveness of two vaginal microbicides, each administered alone to different volunteer participants. BufferGel is expected to help maintain the normal acidity of the vagina even in the presence of ejaculate, which has a higher pH; studies have shown that HIV may be inactivated at an acidic pH of less than 4.5. The other microbicide, PRO 2000/5, inhibits viral entry into susceptible cells. Both microbicides are being evaluated for their potential in blocking HIV and also other sexually transmitted agents. Study results are expected in 2009.

Primary funding for the clinical trials described above comes from the National Institute of Allergy and Infectious Diseases. Additional NIH sponsors and collaborators include the National Institute of Child Health and Human Development, the National Institute on Drug Abuse, and the National Institute of Mental Health.



■ Physician James Hildreth is studying beta-cyclodextrin, an agent widely used in mouthwash, topical creams, and food flavorings. The compound can prevent cell-to-cell transfer of HIV, an important route of infection for sexually transmitted viruses.

duce, and if it works it will cost about 7 cents per application," says Hildreth. "For developing countries, where the annual income is often only a few hundred dollars per year, we have to produce something that is very inexpensive."

In 2001, when Hildreth was an associate professor at Johns Hopkins University School of Medicine, he and his colleagues discovered that cholesterol plays a critical role in HIV fusion and entry into cells. In later studies, the scientists showed that HIV buds from infected cells at cholesterol-rich regions known as lipid rafts, small fatty globules scattered throughout cellular membranes. "HIV is a thief. It steals proteins and other molecules from the host cell when it buds from that cell," says Hildreth. "In 2003 we showed that HIV particles themselves also appear to have lipid rafts, probably picked up from cellular membranes as the virus exits the cell."

Further studies revealed that beta-cyclodextrin drains cholesterol from both HIV and the host cell membrane. "As you remove cholesterol from HIV particles, you can unplug that lipid raft, and the virus will lose its essential components and become noninfectious," Hildreth says. The compound also enables healthy cells to resist HIV and makes infected cells less able to spread the virus.

By studying mice that contain transplanted human cells, Hildreth and his colleagues demonstrated that beta-cyclodextrin can prevent cell-to-cell transfer of HIV, an important route of infection for sexually transmitted virus. In 24 out of 27 mice, vaginal administration of beta-cyclodextrin blocked the passage of HIV from infected cells in the vagina to uninfected cells in the body.

Encouraged by his mouse research, Hildreth is planning a small clinical trial at Meharry's RCMI Clinical Research Center to assess the safety of a beta-cyclodextrin microbicide in women. Beta-cyclodextrin has already been found safe for human use in toxicity studies related to its various product applications.

Some microbicide candidates have already advanced to clinical trials, although these generally have a less specific and targeted mechanism of action than the CCR5 fusion inhibitors and other agents now being investigated in preclinical studies involving animals. Among the compounds currently in clinical trials, substances called sulfated polyanions, which bind proteins HIV uses to enter human cells, appear particularly promising. The National Institute of Allergy and Infectious Diseases is currently funding a multicenter clinical trial—involving more than 3,000 women in the United States and Africa—examining both a sulfated polyanion called PRO 2000/5 and a gel designed to lower the pH of the vagina (BufferGel) to evaluate their safety and ability to prevent HIV infection in at-risk women. Other NIH-funded microbicide clinical trials are also under way. (See box on page 6.)

Along with their colleagues, Marla Keller and Betsy Herold at the NCRR-supported GCRC at Mount Sinai School of Medicine in New York recently showed that the environment of the human vagina does not lessen the potency of PRO 2000/5. In fact, they demonstrated for the first time that a microbicide can remain highly effective after contact with the human vagina.

The researchers placed PRO 2000/5 into the vaginas of 10 women and then collected cervicovaginal fluid samples. The samples were mixed independently with HIV and herpes simplex virus type 2 (HSV-2)—the pathogen responsible for genital herpes, which is known to increase a person's risk for HIV. When human cells were inoculated with the cervicovaginal samples, the PRO 2000/5, still present in the samples, inhibited both HIV and HSV infection at least 1,000-fold. In a follow-up study, involving 24 healthy women, Herold and Keller determined that daily applications of PRO 2000/5 does not trigger an inflammatory response in cervicovaginal secretions, suggesting that repeated use of this microbicide is safe.

By supporting this type of clinical research, as well as preclinical animal-based studies, NCRR is helping to translate basic research findings into potential AIDS-prevention strategies for women. While NCRR's nonhuman primate resources are helping to identify and evaluate promising microbicides, the RCMI and patient-oriented resources stand ready to facilitate clinical investigations. Through these efforts, NCRR is assisting NIH's broad efforts to provide women with an effective agent that they can easily and safely use to achieve protection from HIV infection. ■

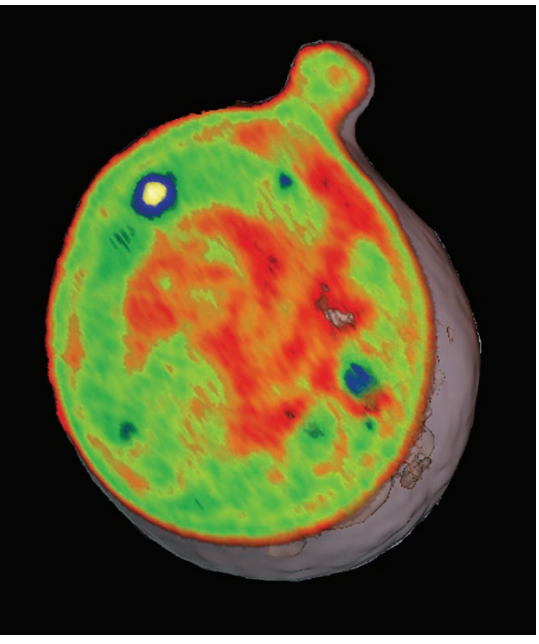
PHOTO COURTESY OF MEHARRY MEDICAL COLLEGE

X-Ray Microscope Scans Cellular Machinery

At the National Center for X-ray Tomography, scientists have built a transmission X-ray microscope that can produce meticulous 3-D images of cells. Just as CT (computed tomography) scans provide a detailed view within the human body, X-ray tomography can generate high-resolution images of the internal structures of cells.

The X-ray microscope, located at the Lawrence Berkeley National Laboratory in California, uses X-rays created by a synchrotron—a circular particle accelerator that produces X-ray beams many orders of magnitude brighter than those from laboratory X-ray generators. The high intensity allows researchers to collect a complete data set for the 3-D image of a 10-micron-thick cell in a matter of minutes, compared to days or weeks for electron microscopes. This quick turnaround will allow scientists to accumulate a statistically significant volume of data within a relatively short time. Also, the high penetrating power of these bright X-rays, coupled with a near absence of refraction, makes them an ideal probe for determining the locations of labeled proteins in cells.

"A protein can have different functions depending on where it's spatially located inside a cell," says cell biologist Carolyn Larabell, the principal investigator for the new center. "Knowing where a protein is located throughout the cell cycle can be very important in determining its role." The new microscope offers the possibility of generating "tomographic atlases" that chart with high precision the locations of individual proteins at every state in the cell cycle, thus providing unique insights into the architecture and workings of cells.



■ This 3-D image, showing a yeast cell during cell division, was created using a high-powered X-ray microscope. X-ray microscopes produce high-fidelity images quickly and can help scientists study protein locations within a cell.

X-ray tomography helps to bridge the "gap" between light and electron microscopy. Traditional microscopy uses visible light and is preferred by many biologists, because it enables them to examine living cells in their natural state. Resolution, however, is limited to a wavelength of 200 nanometers, slightly shorter than the wavelength of visible light. Electron microscopy provides better resolution but requires dehydration of cells and elaborate preparation and staining—a technique that potentially alters the structure of cells. X-ray tomography is an emerging technology that combines some of the best features of light and electron microscopy by allowing whole-cell visualization at a sharper resolution of nearly 35 nanometers, without the elaborate specimen preparations. Future improvements may eventually sharpen the resolution even further to nearly 15 nanometers.

The National Center for X-ray Tomography was officially dedicated on October 11, 2006, and will undergo further testing in January 2007 before opening to the biomedical community. The center was developed with support from NCRR and the U.S. Department of Energy (DOE). Larabell received initial funding from the Office of Biological & Environmental Research at DOE and the National Institute of General Medical Sciences to develop the X-ray microscope technology.

The National Center for X-ray Tomography is one of 52 NCRR-funded Biomedical Technology Resource Centers around the nation. These centers provide scientists with access to a broad spectrum of technologies, techniques, and methodologies, including computational tools, optical and spectroscopic technologies, and advanced microscopy.

"The National Center for X-ray Tomography is the only resource of its kind in the United States," says Gerry McDermott, a research biophysicist at the center. "We believe it will offer a completely new way to explore cellular structure and function."

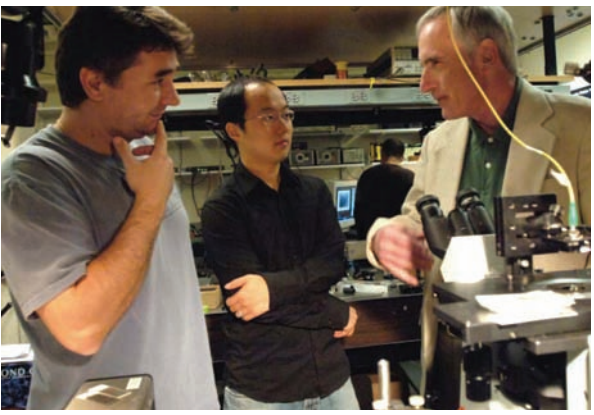
—AL STAROPOLI

TO GAIN ACCESS: The National Center for X-ray Tomography performs research in biological and biomedical imaging and cell biology. It houses the first soft X-ray microscope in the world designed specifically for biological and biomedical applications. The center is a joint program between the University of California, San Francisco, and Lawrence Berkeley National Laboratory. The National Center for X-ray Tomography is expected to become available to qualified biomedical researchers throughout the nation in Spring 2007. Researchers can gain access to the microscope and other resources by submitting a proposal to the center. To learn more or to submit a proposal, visit http://ncxt.lbl.gov.

IMAGE BY CAROLYN LARABELL AND MARK LE GROS, LAWRENCE BERKELEY NATIONAL LABORATORY AND UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Unique Partnerships Move Spectroscopy From Lab to Clinic

New laser-based instruments and techniques for visualizing biological tissues often show great promise in laboratory settings, but transferring these advances to the clinic can be an arduous process. Michael S. Feld, director of the Massachusetts Institute of Technology's George R. Harrison Spectroscopy Laboratory, has found the key to making the transfer possible: engaging physicians in a two-way street of education and collaboration. "A lot of clinicians are enthusiastic at first, but then a weeding out process occurs and only those who understand the research process remain," explains Feld. "The most successful collaborations have been with clinicians who are willing to roll up their sleeves and work with the staff scientists to determine jointly how to improve an instrument."



■ Michael Feld (right) at the MIT Laser Biomedical Research Center routinely collaborates with physicians to develop new imaging instruments.

Through the NCRR-funded Laser Biomedical Research Center (LBRC), which Feld founded in 1985 to exploit forefront applications of lasers, light, and spectroscopy to biology and medicine, he has enlisted a wide range of physicians—in specialties from pathology to cardiology and gastroenterology—to learn about the technical challenges they face in detecting the early stages of disease. At the same time, clinicians often seek out Feld's expertise to consider whether LBRC research may solve a clinical problem. "He selects collaborators who are scientists at heart, as well as physicians," says Maryann Fitzmaurice, associate professor of pathology, Case Western Reserve University, who is working with LBRC to develop a system to diagnose breast cancer.

The payoff is new instruments that improve patient care. One recent example is a diagnostic tool based on trimodal spectroscopy, a technique that combines three different methods to gather information, based on how light interacts with living matter. By coupling the technique with a tiny probe that shines laser light into patients' tissues through an endoscope, the new tool enables physicians to detect dysplasia, or precancerous cell changes, in the esophagus, mouth, colon, and cervix.

With input from the LBRC advisory committee, Feld and his LBRC colleagues choose which projects to pursue, based on whether a project's needs are compatible with the center's resources and how well a project fits the center's long-term goals. In some cases, ideas are so compelling that they lead to new goals. For example, although initial research at the center focused on coronary artery disease, it expanded its interests in 1989 when both staff and collaborators wanted to develop systems for detecting cancer. "Cancer detection, particularly the diagnosis of precancerous changes invisible to the eye, was seen to be a challenging scientific problem with important potential clinical applications," says Feld.

Once the LBRC team decides to take on a project, the collaborators begin an intense mutual education into both the fundamental science underpinning the project and the medical needs it will solve. Clinicians have input into every aspect of the project, including how instruments should be designed and used, the number and types of tissues or patients to study, how data are analyzed, and where and how clinical studies are performed. And when an instrument or technique is ready for clinical evaluation, which can take anywhere from several months to several years, Feld and his team piggyback on patient studies run by the clinician. "We're able to bring our instruments into a cath lab or endoscopy suite and integrate ourselves into the setting without getting in the way," he says.

Techniques conceived at LBRC have also enjoyed commercial success. An autofluorescence technique Feld's group developed to image and diagnose colon polyps became the basis for a high-resolution videoendoscope that detects subtle changes in lung tissue associated with the onset of lung cancer. Pentax Corporation, a major manufacturer of flexible endoscopes, began selling the instrument in Europe in 2006. Other potential products, including novel imaging systems and new types of microscopes, are in the pipeline. "Commercialization of our instruments and techniques is essential to getting them into widespread use, a step which is very important to us for improving patient care," explains Feld.

—SUSAN M. REISS

TO GAIN ACCESS: Outside projects can be initiated by contacting Ramachandra Dasari, Associate Director of the Spectroscopy Laboratory. Once the scope of the project is defined, a Research Project Application must be filled out. There is no charge for using the facilities or equipment.

PHOTO BY DONNA COVENEY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Triple Killer

Physicians create new cells to stave off common viruses that threaten transplant patients. **BY AL STAROPOLI**

After seeing many of her bone marrow transplant patients become seriously ill, physician Catherine Bollard decided to fashion a new immune cell to fight common viruses.

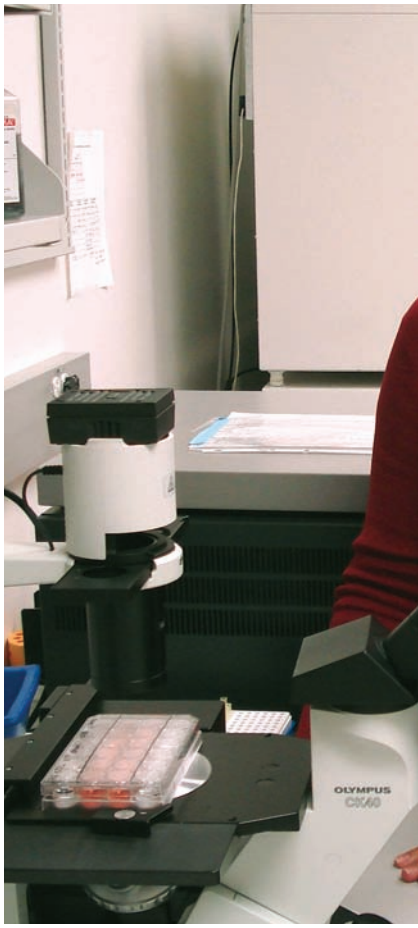
"We see viral infection in about 70 percent of our patients after transplant," says Bollard, a pediatric hematologist at the Texas Children's Cancer Center. The viruses, which are generally benign to healthy patients, can be life-threatening to transplant patients and others with compromised immune systems.

Transplant patients have traditionally been treated with antiviral drugs to prevent infections, but these drugs are expensive, have many toxic side effects, and need to be administered intravenously every day for approximately four months. In addition, when these medicines are stopped, patients are still prone to viral infections.

Instead of using antiviral drugs, Bollard decided to try a different strategy—one that employs the body's own cells to fight off infection. To accomplish this, she and her colleagues developed killer T cells that, when infused into patients, could protect against three of the most common causes of post-transplant infection: Epstein-Barr virus (EBV), cytomegalovirus (CMV), and adenovirus.

Epstein-Barr virus, which causes mononucleosis, and CMV are commonplace among adults. By age 40, up to 95 percent of adults have been infected with EBV. In most people, EBV causes mild, flu-like symptoms. Thereafter, EBV becomes dormant but can re-emerge in transplant patients, causing serious illness or death. CMV similarly infects and becomes dormant in many people, retaining the potential to cause serious infections—usually affecting the lungs and causing severe pneumonia—in patients with weakened immune systems.

To give patients a better chance for recovery, Bollard partnered with Malcom Brenner, who directs the National Gene Vector Laboratory (NGVL) at Baylor College of Medicine. Brenner used the NCRR-funded laboratory to engineer an adenovirus—a common virus that can infect many different types of cells—to produce proteins from CMV, in addition to adenovirus proteins. This hybrid virus was then used to infect immune cells, called B cells, that were already harboring EBV. When infected, B cells are known to stimulate the growth of killer T cells in tissue culture, which can in turn destroy virus-containing cells.



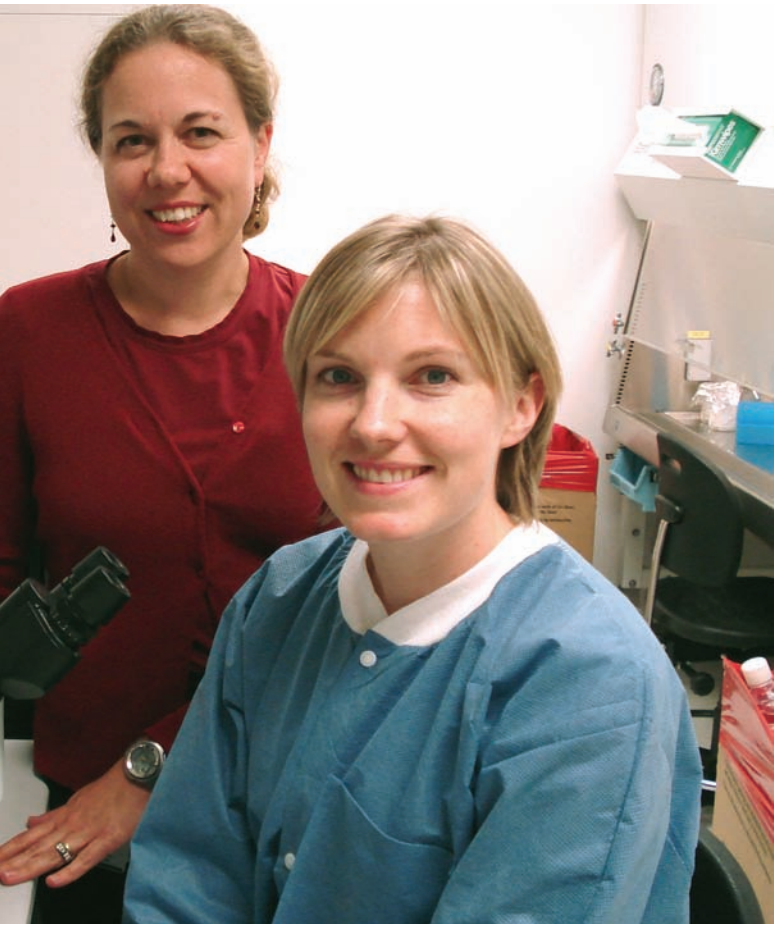
Catherine Bollard (back) and colleague Ann Leen developed a new type of killer T cell that can fight viral infections in bone marrow transplant patients.

In the laboratory, Bollard's team developed 15 lines of killer T cells from bone marrow donors. Of these lines, 14 responded to EBV, CMV, and adenoviruses. The newly fashioned T cells were tested in a Phase I clinical trial conducted in Houston at the Methodist Hospital and the Texas Children's Hospital. The T cells were administered intravenously to 11 patients who had received bone marrow transplants.

Each patient in the Phase I clinical trial received an initial cell infusion after their bone marrow transplantation. Following the infusion, immunosuppression was discontinued in about half of the patients and conventional antiviral therapy was discontinued in all of the patients. None of the patients developed graft-versus-host-disease—a condition in which the newly transplanted cells attack the patient—or other toxicities in over three months of safety monitoring after infusion.

Other teams of investigators also have evaluated similar types of T cell therapies in the past, but their cells were not "stimulated" to fight as many viruses as those used by Bollard and her colleagues. The other therapies showed some evidence of controlling antiviral activity, but they sometimes led to an increase of graft-versus-host disease.

Although Bollard's Phase I trial was designed to evaluate safety



of the T cell therapy, the researchers also found evidence of virus-fighting potential. Patients who had active infections with any of the three viruses at the start of therapy showed rapid improvements in viral titer and disease symptoms, and none developed new viral infections after the killer T cells were infused.

"We treated three patients who had a lot of problems with CMV reactivation prior to infusion. They had to be on antiviral agents for weeks after the transplantation. But once the T cells were given, none of them had to receive antiviral agents," says Bollard.

"None of the patients developed subsequent adenovirus infections," adds Brenner. "This is the first time we've described a cell culture that can protect against adenoviruses, which is a major problem in immunocompromised patients," he adds.

While the frequency of T cells specific to EBV and CMV rose, an increase in adenovirus-specific T cells was seen only in patients with a previous adenoviral infection. Only five individuals tested positive for adenovirus infection before infusion, and it was exclusively in these individuals that a maximal increase in adenovirus-specific T cells was seen. In all of these patients, the adenoviral infections eventually subsided.

"One of our marrow transplant patients had adenovirus pneumonia and was also on a ventilator. This condition has an incredibly high mortality rate, and most people did not think he was going to pull through. But this patient had a dramatic clinical response after receiving the therapy," says Bollard.

The Phase I trial was supported in part by the NCRR-funded General Clinical Research Center, which provided the needed medical care and follow up for patients. The NGVL, also funded by NCRR, was critical in the development of the cells. "We helped Dr. Bollard by finding the vector that would work best for her application. We also developed the vector and tested it. It was a smooth process," says Brenner.

The NGVL also helped Bollard to develop the preclinical information needed for the application to the Food and Drug Administration. Since 1995, the NGVL network has helped to create new therapies by supporting the development of new vectors at no cost to researchers. These vectors can be designed to infect and specifically modify other cells.

Ongoing clinical trials are evaluating the potential of Bollard's therapy, but Brenner sees future possibilities. "There's no reason for limiting this therapy to only three viruses. We could protect against other problematic viruses and perhaps even fungi," he says. The new killer T cells proved effective and safe in all 11 patients studied. Unlike drugs, which control only viruses, the cellular infusions addressed the underlying problem of creating a stronger immune system, without generating toxic effects.

The research described in this article was funded in part by NCRR and the National Heart, Lung, and Blood Institute. For more information on the GCRCs and NGVLs, visit www.ncrr.nih.gov/scientific_rsrcs.asp.

ADDITIONAL READING: Leen, A. M., Myers, G. D., Sili, U., et al., Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. *Nature Medicine* 12:1160-1166, 2006.

PHOTO BY CRYSTAL SILVA-LENTZ

Virus Anatomists

John E. Johnson and his colleagues are peering into the tiny machinery of a virus to understand how it works. They are trying to figure out how a virus packs its DNA, a key process for the replication of some viruses. Johnson, a professor of molecular biology at The Scripps Research Institute, says his detailed studies of viral structure can help locate potential drug targets to keep a virus from replicating.

A focus of Johnson's research is the bacteriophage P22, which infects the food-borne pathogen *Salmonella*. The P22 consists of little more than a sphere-like shell, called a capsid, its DNA, and a tail used to attach to *Salmonella* cells prior to infection.

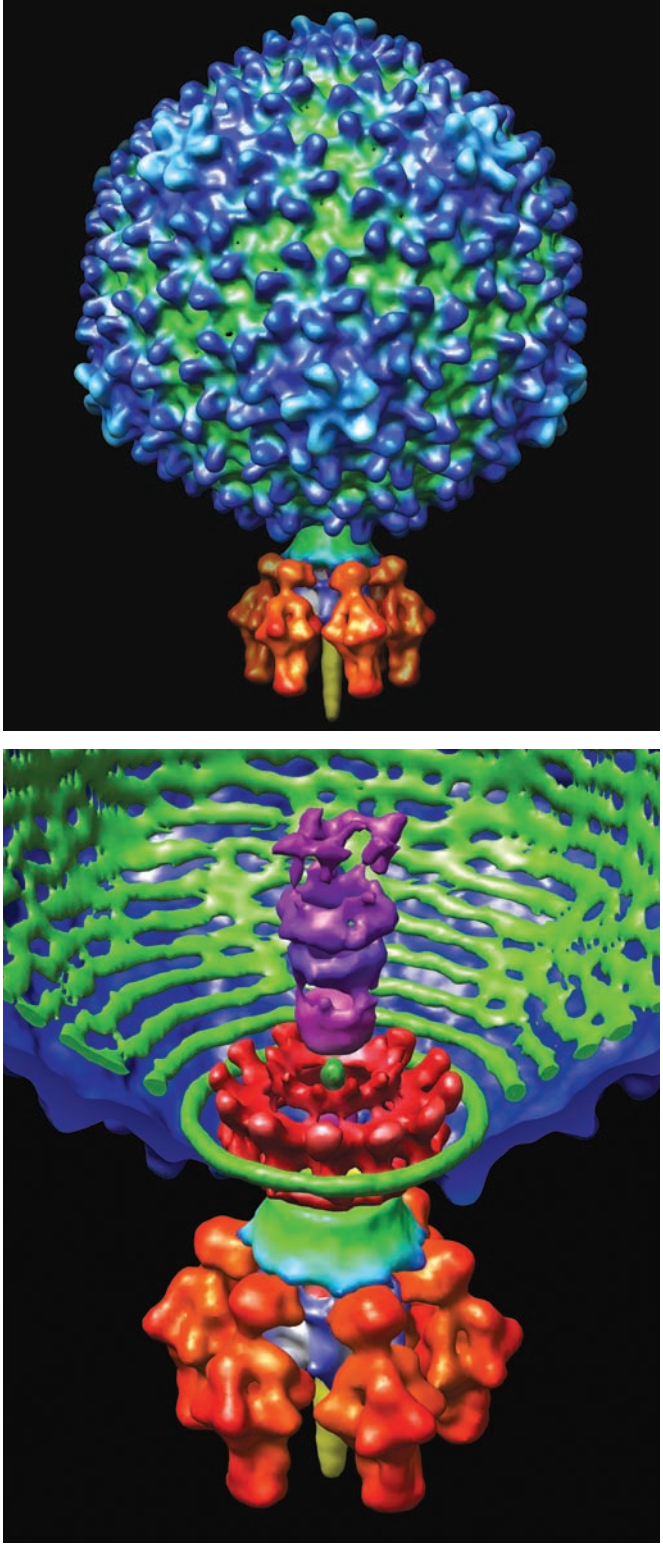
By using a cryo-electron microscope (cryo-EM), Johnson, graduate student Gabriel Lander, and their colleagues have determined the method by which the P22 switches off the process of packing its DNA into the capsid. "A structure in the interior of the virus acts as a pressure sensor that tells the virus when it's full of DNA," says Johnson. When the capsid is full, the molecular configuration changes at the opening of the capsid's base, which triggers a halt to the loading of DNA into the capsid. Assembly of the tail then begins, completing the virus.

> "If the cryo-EM were not automated, it would have taken many months to obtain all the necessary data."

Understanding how the P22 replicates was possible through use of the cryo-EM at the National Resource for Automated Molecular Microscopy (NRAMM) in La Jolla, California. Over the past three years, the NCRR-funded NRAMM has developed innovative tools that automate the process of image collection, speeding results and reducing labor for scientists. "Using cryo-EM typically requires a repetitive task of acquiring and processing thousands of images from different viruses in order to average them into one 3-D image," says Bridget Carragher, director of the NRAMM. "We have developed technology that performs many of the tasks a microscopist would do, including image selection, to automate this process."

"If the cryo-EM were not automated, it would have taken many months to obtain all the necessary data. With the automated system it took only a week," says Lander. "This opens the possibility of doing studies that would previously not be feasible."

Lander hopes this research will open the door to future clinical applications on similar viruses that affect humans, such as the herpesvirus, which causes oral and genital herpes, chicken pox, and mononucleosis. "If we could manufacture a drug that targets the pressure sensor during assembly, the herpesvirus



The P22 virus in full view (top). A cross section (below) reveals the mechanism that packs DNA into the virus. Targeting this mechanism could stop the virus from replicating.

IMAGES BY GABRIEL LANDER, THE SCRIPPS RESEARCH INSTITUTE

would not package its DNA properly, rendering the virus unable to infect cells," he says. **—AL STAROPOLI**

NCRR RESOURCES: The cryo-EM at the National Resource for Automated Molecular Microscopy has helped to conduct more than 50 studies to date and is open for use by external scientists. Researchers can request use of the cryo-EM resource by completing an online application found at http://nramm.scripps.edu/resource/resource.php. Applications are accepted year-round and reviewed within one month of receipt.

Fertility Clues

Trying to understand the causes of infertility is the job of biologist Diana Chu, assistant professor at San Francisco State University. Chu and her colleagues hope to shed some light on male infertility by examining proteins associated with sperm production in the tiny worm *Caenorhabditis elegans*. By studying sperm from worms obtained through the NCRR-funded Caenorhabditis Genetics Center, Chu has discovered dozens of proteins that could be associated with various aspects of infertility.

"These findings could have strong implications on human fertility," says Barbara Meyer, who led Chu as she began her postdoctoral research. Meyer is a professor of genetics and development at the University of California, Berkeley.

Chu suspected that some of the hundreds of proteins found in sperm would have essential functions in fertility, but conducting detailed studies of so many proteins was not practical. To trim down the number proteins to a critical few, Chu and her colleagues designed an ingenious multiphase experiment.

In the first phase, Chu used proteomics to identify only those proteins likely to play a role in spermatogenesis. To assist this effort, Meyer facilitated a collaboration between Chu and two proteomics experts—John Yates and research fellow Hongbin Liu—at The Scripps Research Institute. Yates and Liu drew on the technology of the Yeast Resource Center at the University of Washington to perform detailed mass spectrometry analysis on Chu's worm samples. At this NCRR-funded resource, Liu used a type of mass spectrometry analysis called MudPIT (multidimensional protein identification technology), coupled with custom software, to identify proteins specific only to sperm or eggs. The analysis revealed more than 1,000 potential proteins to study.

Certain proteins, such as the ones stained in blue in the *C. elegans* worm (below), are linked to male infertility. Similar proteins are also found in humans.

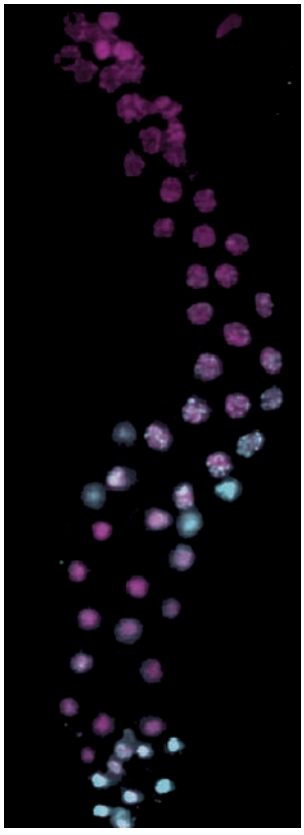


IMAGE BY DIANA CHU, SAN FRANCISCO STATE UNIVERSITY AND JESS GUNTHER, UNIVERSITY OF CALIFORNIA, BERKELEY

Thinking that more important proteins also would be more abundant, Chu and Liu searched for proteins that appeared frequently in samples. Only 132 proteins were found to be abundant in and unique to sperm. This small group of proteins was more likely to have key functions in spermatogenesis, Chu suggests.

In the second phase of the experiment, Chu worked with Meyer's lab to perform RNA interference and eliminate the expression of each of these 132 proteins, thus determining their effect on fertility. Their resulting effects were assessed by worm brood counts, level of sterility, embryo death, and abnormalities in chromosomes or sex glands.

Of the 132 proteins studied, RNA interference determined that sterility or embryonic lethality was related to blocking protein production in 50 genes. In addition, 70 proteins in *C. elegans* were found to have human homologues that have not yet been tested for their fertility function. "Our hope is to supply scientists with a short list of proteins to determine if their counterparts in humans or mammals have a role in fertility," says Chu.

Chu and Meyer say that analyzing additional homologous proteins in humans may aid in the development of diagnostic tests to assess causes of male infertility, sperm competence, or human reproductive potential. "There's a wealth of proteins to be explored that could have implications," says Meyer.

Chu agrees. "Men who face infertility often have few options," she says. "Looking at the human counterparts of the identified worm proteins can help determine the causes of male infertility. Identifying the problem can help to eventually define new options for treatment." (*Nature* 443:101-105, 2006.)

—AL STAROPOLI

NCRR RESOURCES: The Yeast Resource Center at the University of Washington is one of 52 Biomedical Technology Resource Centers supported by NCRR around the nation. The center offers access to five advanced technologies: mass spectrometry, yeast two-hybrid assays, deconvolution fluorescence microscopy, protein structure prediction, and computational biology. For more information or to submit a proposal, visit http://depts.washington.edu/yeastrc/pages/contact.html.

The Caenorhabditis Genetics Center (CGC), located at the University of Minnesota, is responsible for collecting, maintaining, and distributing stocks of *C. elegans*. The center also coordinates genetic nomenclature and maintains a *C. elegans* bibliography, genetic map, and Web server. For more information or to request a *C. elegans* strain, visit www.cbs.umn.edu/CGC.

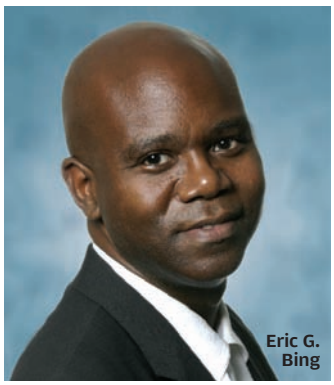
NEWS FROM NCRR

People, Awards, Grants, and New Developments

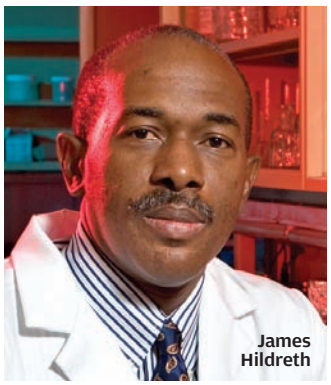
Researchers Named Health Ambassadors

NCRR-funded researchers **Eric G. Bing** and **James E. K. Hildreth** were among the 27 prominent scientists named as Ambassadors by the Paul G. Rogers Society for Global Health Research. The Rogers Society, which advocates for greater U.S. investment in global health research, announced its inaugural group of Ambassadors in November. These prominent scientists will be involved in public outreach and advocacy to convey the importance of global health research to the nation.

Bing is an assistant professor of psychiatry at Charles R. Drew University of Medicine and Science. He is also an epidemiologist with extensive expertise in HIV and the director of the Drew Center for AIDS Research, Education, and Services. The Center develops domestic and international programs to combat and treat HIV. Through the Center, Bing oversees programs in Angola, Namibia, and Rwanda aimed at preventing and treating HIV. Bing's research focuses on developing and evaluating interventions that aim to improve the health care for disadvantaged populations, particularly those affected by alcohol and drug problems, HIV, and mental illness.



Eric G. Bing



James Hildreth

Hildreth is the director of the Meharry Center for Health Disparities Research in HIV at Meharry Medical College. The Center examines biological factors that may explain racial disparities in HIV infection and studies behavioral factors that place African Americans at a higher risk for the disease. Hildreth's research focuses on the relationship between HIV and cholesterol, a necessary component of HIV transmission. He is developing vaginal creams that can extract cholesterol from HIV thus blocking infection during sexual intercourse. (See "Exploring the Potential of HIV Microbicides" on page 4.) Hildreth is the former chief of the division of research at the National Center on Minority Health and Health Disparities at NIH.

The Paul G. Rogers Society for Global Health Research was established in July 2006 by Research!America.

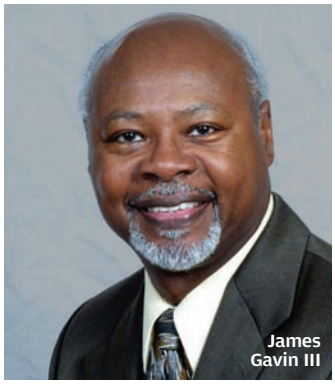
Investigators Receive Greenwood Award

A scientist studying diabetes and one whose work focuses on mental health were the winners of the 2006 Greenwood Awards. The awards are presented biennially through the NCRR-funded Research Centers in Minority Institutions (RCMI) Program in recognition of research excellence involving minority health issues or long-time meritorious service to minority institutions.

Margarita Alegría was recognized for her research on disparities in mental health and substance abuse services for Latinos and other minority populations. **James R. Gavin, III** was honored for his commitment to improving the management of chronic diseases, especially diabetes, in underserved groups.

Alegría, a former RCMI investigator, is a psychology professor at Harvard Medical School and the director of the Center for Multicultural Mental Health Research at Cambridge Health Alliance. Her research focuses on conceptual and methodological issues involving minority populations, risk behaviors, disparities in service delivery, and health services research. Alegría also has been a council member of the American Public Health Association and has served as faculty for various

Margarita Alegría



James Gavin III

PHOTOS BY: PAUL ANTICO, CHARLES R. DREW UNIVERSITY OF MEDICINE AND SCIENCE (BING), MEHARRY MEDICAL COLLEGE (HILDRETH), THOMAS MCGUIRE (ALEGRÍA), MOREHOUSE SCHOOL OF MEDICINE (GAVIN)

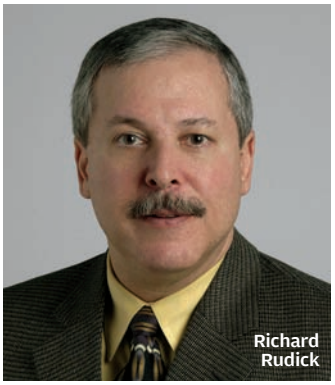
National Institute of Mental Health training programs.

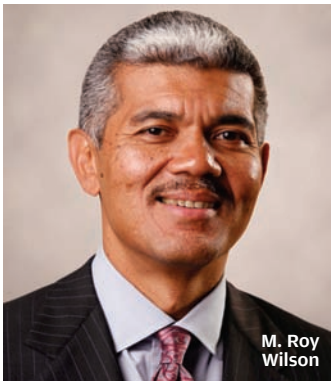
Gavin is a clinical professor of medicine at Emory University School of Medicine and president of MicroIslet, Inc., a company developing cell transplantation therapies for patients with diabetes. He also is the former president of the Morehouse School of Medicine in Atlanta. He has held numerous leadership positions. He is the former president of the American Diabetes Association, former scientific officer at the Howard Hughes Medical Institute, a member of Duke University's Board of Trustees, and a former member of the Board of Trustees for the Robert Wood Johnson Foundation. Gavin also has served on numerous NIH advisory committees including the National Diabetes Advisory Board.

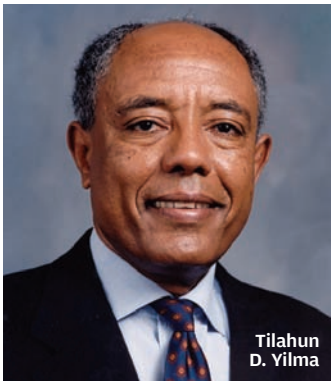
The awards are named in honor of the late researcher and administrator Frederick C. Greenwood, who did much to increase the ranks of minority scientists and promote research on health issues that affect ethnic minorities. They were presented at the Tenth RCMI International Symposium on Health Disparities, held in Puerto Rico in December 2006.


Kevin B. Johnson


Thomas J. Rosol


Richard Rudick


M. Roy Wilson


Tilahun D. Yilma

Five Members Appointed to NCRR Advisory Council

The National Advisory Research Resources Council, which advises NCRR on policies and programs and performs second-level peer review of grant applications, has five new members. The new appointees are:

Kevin B. Johnson, associate professor of pediatrics, vice chair and associate professor of biomedical informatics at Vanderbilt University. Johnson's research lies at the intersection of clinical informatics and medicine. His areas of study include developing and evaluating computer-based software to record patient encounters and electronic prescribing systems to assist with ambulatory order entry and clinical decision support.

Thomas J. Rosol, dean of the college of veterinary medicine at The Ohio State University. Rosol studies hormones and cytokines related to cancer. He is known for his discovery of parathyroid hormone-related protein in multiple animal cancers. He also has made contributions to the study of bone metastasis and the metabolism of calcium and bone, and he has developed mouse models of human and animal cancers.

Richard Rudick, director of the Mellen Center for Multiple Sclerosis Treatment and Research; chairman of the division of clinical research; and Hazel Prior Hostetler professor of neurology at the Cleveland Clinic Foundation. Rudick researches the pathogenesis and treatment of multiple sclerosis (MS). He is also involved in the study of new outcome measures for MS clinical trials.

M. Roy Wilson, chancellor of the University of Colorado at Denver and Health Sciences Center. Wilson is a skilled administrator in the health sciences field. He is the former president of the Texas Tech University Health Sciences Center and vice president for health sciences at Creighton University. His interests include glaucoma research, minority health, and health disparities.

Tilahun D. Yilma, distinguished professor of virology and director of the International Laboratory of Molecular Biology for Tropical Disease Agents at the University of California, Davis. Yilma's research focuses on the development of a vaccine against HIV as well as the development of recombinant vaccines for major diseases of livestock.

PHOTOS COURTESY OF VANDERBILT UNIVERSITY (JOHNSON), J. R. HARVEY, COLLEGE OF VETERINARY MEDICINE, OHIO STATE UNIVERSITY (ROSOL), THE CLEVELAND CLINIC (RUDICK), UNIVERSITY OF COLORADO AT DENVER (WILSON), UNIVERSITY OF CALIFORNIA, DAVIS (YILMA)

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