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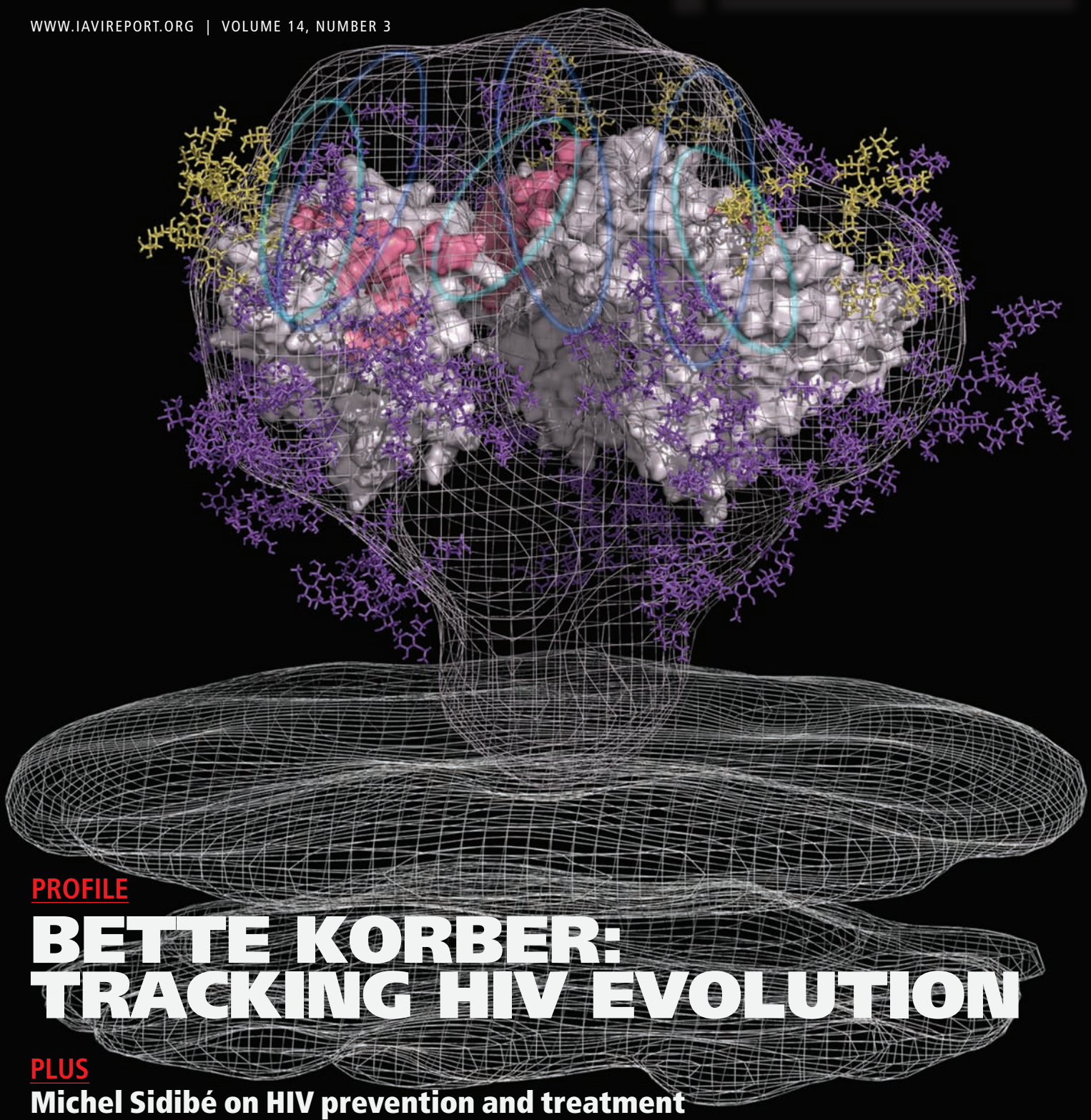

PROFILE

BETTE KORBER: TRACKING HIV EVOLUTION

PLUS

Michel Sidibé on HIV prevention and treatment

EDITOR'S LETTER

In just a few weeks, more than 20,000 delegates are expected to gather in Vienna, Austria, for the XVIII International AIDS Conference (IAC), the behemoth biannual meeting sponsored by the International AIDS Society.

A decade ago, in Durban, South Africa, the IAC marked a sea change in the HIV/AIDS pandemic. Following the meeting, there was a new emphasis on delivering life-saving antiretrovirals to millions of HIV-infected people in developing countries. Since then, significant progress has been made on this front.

In Vienna, universal access to HIV treatment will likely be one of the resounding themes. The world is far from meeting this goal—only about 30% of people in need are currently receiving antiretrovirals, and with many countries still gripped by the economic downturn, the prospects of increasing, or even maintaining, current HIV/AIDS funding levels, seem dim. In this issue, Regina McEnery asks Michel Sidibé, executive director of the Joint United Nations Programme on HIV/AIDS (UNAIDS), for his opinions on these issues (see *An Interview with Michel Sidibé*, page 12).

Other themes to emerge in Vienna will likely be the burgeoning HIV epidemic in Eastern Europe and Central Asia, human rights issues related to injection drug use, and the ongoing efforts to develop new prevention strategies.

Vaccine research will likely not take center stage at the IAC, even though there is a flurry of research activity. Efforts to try to decipher the modest protection afforded by a prime-boost regimen in the RV144 trial, which provided the first evidence for protection against HIV by a vaccine candidate, are underway (see *Meeting of the Minds on Mucosal Transmission*, page 10). And plans for several follow-up trials intended to improve upon the RV144 results are also in development (see *Researchers Unveil Plans for Follow-up Trials to RV144*, page 18).

Meanwhile, Bette Korber, the well-respected steward of the Los Alamos National Laboratory HIV Sequence Database, who is profiled in this issue, is collaborating with other researchers to develop mosaic vaccine antigens designed to overcome the obstacle of HIV variation (see *Tracking HIV Evolution*, page 4). The first clinical trial of this mosaic vaccine approach is slated to begin soon.

We'll be constructing our own mosaic, article not vaccine, out of highlights from the IAC, so stay tuned for the next issue.

KRISTEN JILL KRESGE

The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 25 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, India, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government, the European Union as well as The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see www.iavi.org.

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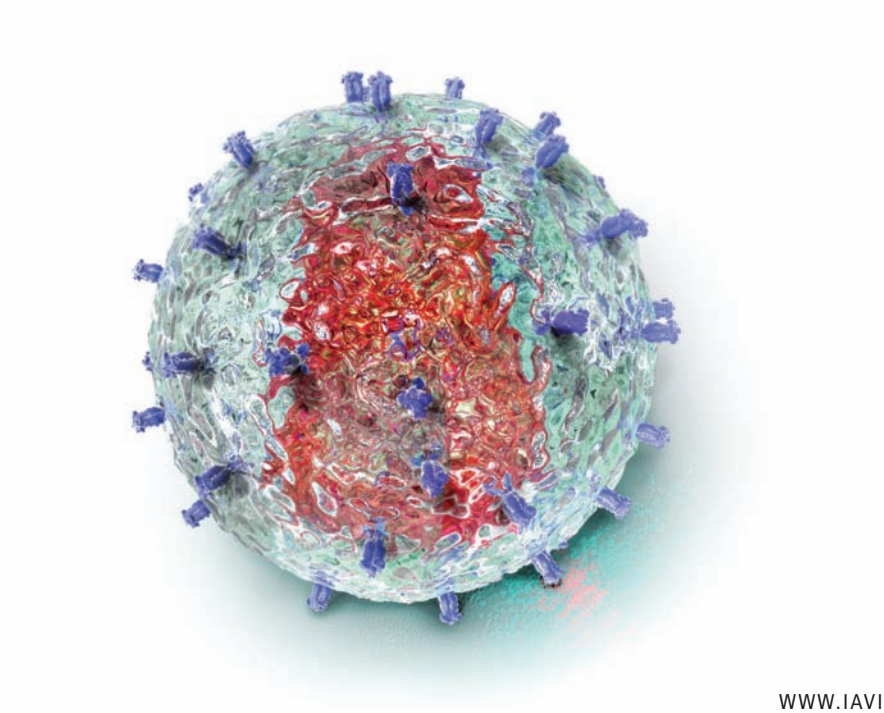

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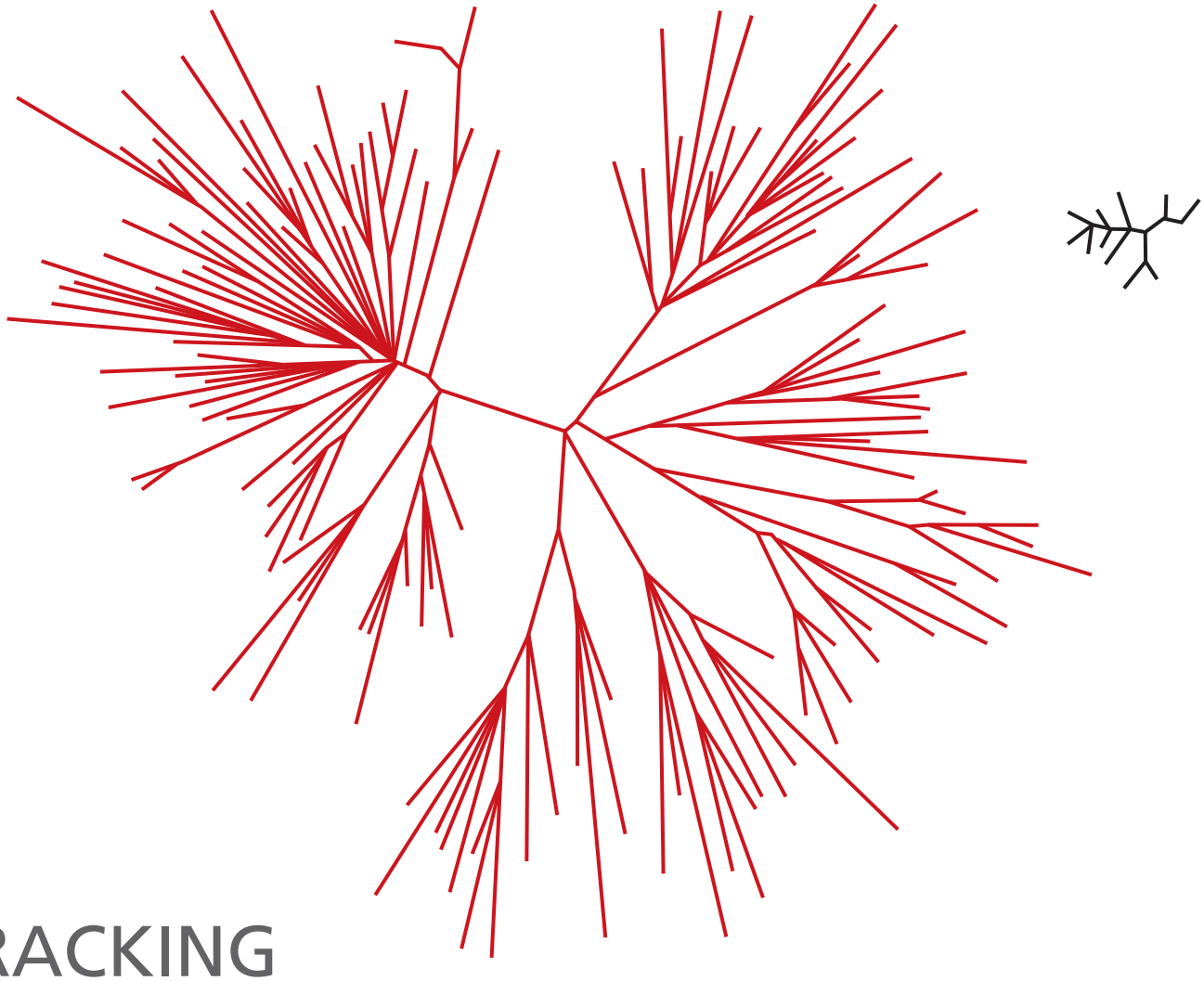
[ON THE COVER]

Image adapted from a cryoelectron tomographic structure of the native HIV Envelope trimer (outer shell; *Nature* **455**, 109, 2008), filled with the crystal structure of the b12-bound monomeric gp120 core (*Nature* **445**, 732, 2007). PG9 and PG16 are thought to bind to the V1/V2 and V3 loops (shown as dark blue and teal ovals, respectively), whose structure is unknown. Carbohydrate chains are shown in purple, and the oligomannose cluster targeted by 2G12 is shown in yellow.

Image courtesy of Christina Corbaci, The Scripps Research Institute, and Bill Schief, University of Washington. A similar version of the image also appeared in *Curr. Opin. Immunol.* **22**, 358, 2010.



TRACKING HIV EVOLUTION

Theoretical biologist Bette Korber's career has been devoted to classifying one of the most variable viruses ever identified

By Regina McEnery

Los Alamos, New Mexico, consists of a series of rust-colored mesas that form a picture-postcard setting: snow-capped Jemez Mountains in the distance and vast swathes of undisturbed wilderness that belie its history-making role in US defense.

Los Alamos is, of course, the place where 30 scientists gathered in 1943 to build the world's first atomic bomb. Physicists recruited to work at the Los Alamos National Laboratory (LANL) with nuclear physicist J. Robert Oppenheimer, scientific director of the Manhattan Project, worked feverishly in the race to develop a nuclear weapon before the Germans. Their top-secret crusade transformed this bucolic southwest community and its acres of pine trees into a nerve center for US military weapons research.

Seven decades later, the LANL campus still looks a bit like a frontier outpost. Single-story modular units—like those found at construction sites—are laid out like a maze and are surrounded by acres of federally-owned forest that remain largely off-limits to the public.

It is here that theoretical biologist Bette Korber and her team of 13 multi-disciplinary scientists track, with exquisite detail, the

▲ The red phylogenetic tree represents the genetic variability of HIV-1 V2-C5 in the Democratic Republic of the Congo in 1996. The black phylogenetic tree represents the genetic variability of global influenza A virus in the same year. The size depicts the extent of variation.

evolution of one of the most diverse and peripatetic viruses ever identified. They do this with a vast network of super-computer systems—some occupying a space equivalent to half a football field—that can crunch data at speeds of up to 10^{15} or a quadrillion calculations per second, roughly in the blink of an eye.

The work of tracking HIV began in earnest in 1986 with the creation of the HIV Database and Analysis Project. The US National Institute of Allergy and Infectious Diseases (NIAID), which funds the project through an agreement with the Department of Energy, hoped the formation of the database would accelerate the development of better drugs, as well as a vaccine to protect against HIV/AIDS.

Since its inception, the main goal of the HIV database project has been to collect, curate, and annotate HIV genetic material, and provide the data to scientists in an open-access environment to try to encourage collaboration within the field. Relying on data from an earlier LANL effort called GenBank, a public database set up in 1982 to store laboratory samples of previously sequenced organisms, the HIV database took the effort to a new level.

To date, the HIV database contains published genetic sequences of DNA from 250,000 different viruses obtained from HIV-infected individuals around the world. Although not the only database of its kind—Stanford University also has a database of about 100,000 viral sequences that it uses to identify drug-resistant HIV mutations—it is by the far the largest and most utilized by HIV researchers. And the project's scope and mission hasn't ended there. LANL scientists have also cooked up dozens of software tools to assist scientists in their research, including programs that help identify the specific subtype or clade of the HIV sequence. More recently, researchers used the HIV database at LANL to engineer vaccine candidates designed to provide greater coverage against the diverse strains of HIV in circulation—which Korber hopes will finally provide sufficient breadth to cope with the problem of viral diversity in HIV vaccine development.

LANL researchers have also established three additional databases. A molecular immunology database provides a comprehensive listing of defined HIV epitopes, including epitope alignments, epitope maps, and reference information for cytotoxic T-cell (CTL) and helper T-cell epitopes, as well as antibody-binding sites. Another database tracks drug-resistant HIV mutations, while another tracks HIV vaccine trials being conducted in nonhuman primates (NHPs).

◀ BETTE KORBER
Theoretical biologist Bette Korber, who oversees the HIV Database and Analysis Project at Los Alamos National Laboratory, has been tracking the peripatetic virus her entire scientific career.

The HIV database, overseen by Korber, is an incredibly important tool for HIV scientists, judging by the number of citations and acknowledgements to LANL, as well as the numerous plaudits from leading AIDS researchers. "They have supported everyone's research over the past 15-20 years and are the central repository not only for sequences but meticulous annotations of those sequences," says Barton Haynes, director of the Duke Human Vaccine Institute and the Center for HIV/AIDS Vaccine Immunology (CHAVI) at Duke University in North Carolina, who has collaborated with LANL researchers on a number of vaccine-related projects.

The spectrum of research studies that have benefited from the HIV databases has been huge, and ironic, given that its founder Gerald Myers, an LANL scientist, initially thought the HIV sequencing project would only last about a year. But from its inception, the project was flooded with database entries that reflected the incredible genetic variation of HIV strains circulating globally. Myers soon realized the tremendous challenges viral diversity posed in the development of an effective AIDS vaccine and pushed NIAID to escalate funding and expand its contract.

Much of the credit for the project's credibility today goes to Korber, whose aversion to doing experiments—or as she phrases it, the tedious

cycle of pipetting, pipetting, pipetting—drove her toward mathematics, and ultimately theoretical biology, when she was working toward her doctoral degree in immunology at the California Institute of Technology (Caltech) in the 1980s. "I like thinking and puzzling better. That also takes great care and hard work, but it's just the nature of the work that I like better," says Korber, when interviewed at her office on the eve of the annual Keystone Symposia on HIV Biology and Pathogenesis in Santa Fe, New Mexico.

As the largest repository of information about the mind-boggling diversity of HIV, it's no surprise that over the years, the HIV database project, and Korber as well, has been drawn into thorny, sometimes contentious, debates about who discovered HIV, the origin of the virus, and even the widely publicized case of a Florida woman who claimed she had been infected with HIV by her dentist.

Using math to tackle HIV

Theoretical biologists use a variety of analytic tools, from mathematical and computational models to systems biology and bioinformatics, to better understand biological systems and predict how they will evolve. This partly explains why Korber accepted a position at LANL—with its nascent database project and access to some of the best computer hardware in the world—after completing a post-doctoral fellowship in molecular epidemiology of human retroviruses at Harvard University in 1990.

But there were also very deep, personal reasons why Korber decided to focus her attention on HIV. In the early 1980s, when Korber and her fiancé James Theiler were studying at Caltech, they became close friends and housemates with a physicist from the UK. The bond was so close that when Korber and Theiler decided to get married in 1988, their friend received training as a lay minister so he could marry them at a ceremony by a stream in the mountains above Pasadena, California. "He was just a wonderful, brilliant man," Korber says of her housemate.

Their friend was also, unfortunately, one of the earliest reported individuals to be infected with HIV in Pasadena. His struggle with the virus had a profound effect on Korber's life. It was still early days in the escalating epidemic, long before highly active antiretroviral therapy (HAART) began rescuing HIV-infected individuals from the brink of death. "We learned a lot about HIV while he was sick," says Korber. "But there was no treatment for him and he died in 1991. I decided when I graduated from my PhD program that I wanted to work on HIV."

Specifically, her friend's battle with HIV propelled Korber to commit her life to finding an AIDS vaccine. "I *hate* HIV," she says, her voice rising with emotion. "I lost a couple friends to it. HIV kills in horrible ways. I think of what the epidemic has done to Africa and it motivates me."

Korber spent her first few months at LANL getting used to "playing" on the computer, a transition made easier, she says, because her mentor, Myers, was patient and gave her space. Eventually, Korber suggested to Myers that LANL add the Molecular Immunology Database, which like the HIV Sequence Database, was the first database of its kind dedicated to a single pathogen.

The goal of the immunology database was to provide a comprehensive listing of defined HIV and SIV epitopes associated with sequences previously published in scientific literature and submitted to the HIV database, and then make the searchable collection available to the general scientific community. Launched in 1995, it now contains more than 1,200 HIV epitopes, with at least 275 of them considered "A-list" because they have been characterized with a high degree of detail, according to *HIV Molecular Immunology*, which provides annual updates and reviews of the database. Over time, the development of large cohorts of individuals known as long-term nonprogressors, who have demonstrated an unusual ability to control HIV infection without treatment, and an evolving war chest of gene sequencing and data analysis tools has enabled researchers to assess different HIV epitopes for their potential role in controlling or preventing HIV infection, the authors of the compendium noted in its 2009 review.

"The HIV Database project took on the issue of the interface of the virus with the host, compiling not only viral sequences but immunological epitopes recognized by B cells, $CD4^+$ and $CD8^+$ T cells, and antibodies, then laid out the foundation for a relational database that they made available to the field. They emphasized the need for collaboration early on in the AIDS epidemic," says Haynes.

In most cases, the information about each epitope includes the protein fragment's published name, the specific protein that it is associated with, the location on the protein within a region

Continued on page 8

A Computer Powerhouse

The HIV Database and Analysis Project at the Los Alamos National Laboratory (LANL) catalogs and analyzes a dizzying array of HIV fragments and isolates. The ability to track one of the most variable viruses in history comes from an evolving stable of supercomputers and state-of-the-art genotyping tools that researchers at LANL can access. Here are three key examples of how computational technology has informed AIDS research.

• About a decade ago, scientists at LANL turned to what was then the fastest unclassified supercomputer in the world, a system known as Nirvana, to construct phylogenetic trees that ultimately helped them trace HIV back to its most common recent ancestor (*Science* **288**, 1789, 2000). The scientific analysis conducted by theoretical biologist Bette Korber and other members of the LANL team showed that the HIV pandemic likely began between 1915 and 1930.

This was not just an interesting development for the biological history books, it directly challenged a controversial hypothesis that the virus had sprung up in humans in the late 1950s because batches of oral polio vaccine cultured in primate cells were contaminated with simian immunodeficiency virus (SIV), the monkey equivalent of HIV. Developers of the vaccine denied that chimp tissue had been used to make the polio vaccine, but the theory persisted, in large part, due to circumstantial evidence laid out in the book "The River," by British journalist Edward Hooper. The LANL research, with the help of Nirvana, provided the strongest evidence to counter that theory.

▲ **ROADRUNNER.** The Roadrunner supercomputer, developed by IBM, operates at speeds exceeding one petaflop, or a thousand trillion calculations per second, roughly equivalent to the combined computing power of 100,000 of the world's fastest laptop computers.

The Nirvana system, capable of making one trillion calculations per second, enabled scientists to analyze very large sets of HIV Envelope sequences derived from blood samples of about 160 individuals infected with HIV-1, and then apply these sequences to sophisticated evolutionary models. This type of work would have been impossible using previous computer systems.

Nearly a decade later, Michael Worobey, an evolutionary biologist at the University of Arizona, built on the LANL findings. Using more advanced technological tools, he estimated that the HIV pandemic likely began between 1884 and 1924, based on the amplification and sequencing of a wax-embedded lymph-node specimen obtained in 1960 from an adult female from what is now the Democratic Republic of the Congo (*Nature* **455**, 661, 2008).

• Last year, through a unique arrangement that allowed a handful of scientists access to LANL's latest supercomputer, the Roadrunner, before it was moved to a classified computing network, Korber, computer scientist Marcus Daniels, and physicist Tanmoy Bhattacharya compared the evolutionary history of more than 10,000 genetic sequences from more than 400 HIV-infected individuals to try and identify common features of the virus that is transmitted and establishes infection. This work was done in collaboration with the Center for HIV/AIDS Vaccine Immunology (CHAVI), of which Korber is an investigator. CHAVI collected the samples from both acutely and chronically HIV-infected individuals from around the world. The samples were used to construct the world's largest phylogenetic tree, with the end goal of identifying similarities in HIV sequences from samples taken during acute and chronic infection. A single HIV-infected person can have 100,000 different variants of the virus circulating throughout their body, so understanding how these variants branch off from the initially transmitted virus is important for the development of vaccine candidates. To build such a tree, LANL researchers needed Roadrunner's processing capability. Roadrunner does 1.042 petaflops, or a quadrillion calculations per second, using 122,400 processors. To gauge the power of Roadrunner, consider this: It took a single week to run a calculation on Roadrunner that the fastest supercomputer a decade ago needed 20 years to complete.

• LANL researchers and their collaborators at Duke University and the University of Alabama-Birmingham have also applied a next-generation genotyping tool to track the evolution of HIV immune escape during acute infection, allowing researchers to identify rare viral variants that would not have been detectable using conventional sequencing technologies. The 454 sequencing technology, developed by Roche spinoff 454 Life Sciences, is being used increasingly by AIDS researchers to study viral diversity because it requires fewer cloning steps and produces unprecedented quantities of sequencing data. This sequencing tool can obtain more than one million DNA base pairs per run.

Korber and her collaborators recently used 454 sequencing to look at early cytotoxic T-cell escape in four epitopes from three HIV-infected individuals during acute infection. The first sample from each individual was taken during acute infection, prior to an observed immune response, with two additional samples over the course of several weeks. The number of sequences obtained ranged from a few thousand to more than 100,000 per sample, and reflected a much higher level of diversity generated by immune escape than was expected. Korber, who directs the HIV database project, says the level of detail and clarity provided by the genotyping tool is enlightening. "It reminded me of when I was 14 and I got my first pair of glasses," says Korber. "Before that, I saw trees as great green blobs. When I got my glasses, I could for the first time see the leaves." *–RM*

Continued from page 6

of 21 amino acids or less, the viral subtype, and the host species.

A more in-depth search of each epitope will show the country where the circulating virus was identified, assays used to test the immune response, the major histocompatability complex/human leukocyte antigen (MHC/HLA) of the infected donor, and how many different epitopes are linked to the particular HIV sequence in question. Each epitope entry in the HIV Molecular Immunology Database also includes annotated footnotes that summarize information about the immune responses measured, such as cross-reactivity patterns, escape mutations, and antibody sequences that overlap with an epitope, as well as a link to studies measuring the epitope response in human and animal studies.

She's extremely careful, meticulous, and passionate about digging through to the truth behind the phenomenon we are observing.
– Bruce Walker

By documenting all the known epitopes of every DNA sequence published in the HIV literature, the HIV Immunology Database offers researchers an unprecedented way of studying HIV's diversity. "What we did was really unique," says Korber.

Bruce Walker, director of the Ragon Institute, first met Korber when she was doing her post-doc at Harvard and the two are now collaborators on various projects. Like many scientists in the field, he has found the LANL HIV database a uniquely valuable resource, and gives Korber high marks for her oversight of the project.

"I think she's extremely careful, meticulous, and passionate about digging through to the truth behind the phenomenon we are observing," says Walker. "She's been a fantastic steward for this repository because she puts so much effort into making sure that what is in there is accurate. I can't express that enough. A database is only as good as the data put into it. This is a resource you can completely count on and it has been an enormous benefit for the field."

Korber, along with Myers, also helped shepherd in an at first controversial policy for journals in the early 1990s that ended the practice of allowing researchers to publish papers about viral sequences without submitting the sequences to the public repository. Sometimes researchers would not take the time to make the sequences public, closing the door on other researchers trying to replicate the findings and missing the opportunity to build on the collective body of sequencing information. "We had to fight for this," says Korber reflecting on the new policy, which was eventually adopted by major scientific/medical journals. "It will be interesting to see how curation and data sharing unfold in the years ahead with the advent of new sequencing technologies."

Her many passions

Korber's work schedule is grueling. She usually rises at 4 a.m. and is often firing off emails to colleagues as the clock approaches midnight in her Los Alamos-area home. "I can vouch for that," says Mark Muldoon, a long-time friend and colleague from the UK, who was visiting Korber's lab while he was in Santa Fe for the January Keystone Conference.

But HIV research is not her sole passion. Korber and her husband, a physicist at LANL's Space and Remote Sensing Sciences Division, both love to hike, and Korber holds a black belt in Tae Kwon Do. Korber also jams regularly with a Celtic band called Roaring Jelly, named for the blasting gelatin used more than a century ago for mining operations. Korber plays the bodhran, an Irish hand-held drum about twice the size of a tambourine, and the Irish whistle. Her 17-year-old son, Sky Korber, plays a "hot fiddle" in the band, says Korber, referring to her son's musical prowess. Korber's 21-year-old son, Max Theiler, attends the University of California in Santa Cruz.

Korber has also taken a keen interest in helping people and regions disproportionately impacted by the HIV pandemic. Four years ago, Korber used US$50,000 in prize winnings from the prestigious E.O. Lawrence Award—the Department of Energy's highest honor for scientific achievement—to help establish, along with family and friends, an orphanage in South Africa for 500 AIDS orphans. The orphanage was created under the auspices of Nurturing Orphans of AIDS for Humanity (NOAH). Korber is also trying to help initiate use of portable, maintenance-free gardening systems known as Earth Boxes, which have been placed at various orphanages, clinics, and schools in Africa.

In addition to leading an eclectic group of molecular biologists, sequence analysts, and

computer technicians at LANL, Korber is also on the faculty of the Santa Fe Institute, a 26-year-old research and education non-profit organization that encourages collaboration among scientists across different disciplines to solve complex problems of the day. Her research portfolio also includes hepatitis and she recently received a $1.5 million grant to study the interactions between tuberculosis and HIV.

But Korber's main research endeavor, from the start, has been driving toward the development of the elusive AIDS vaccine, and specifically, how a vaccine could overcome HIV's diversity, one of the most potentially vexing obstacles to the development of a vaccine. Although recent findings from complete genome sequence analyses of transmitted founder viruses suggest a single viral variant usually initiates infection in heterosexual transmission cases, the infecting strains are still unique and distinctive.

Designing a vaccine capable of overcoming such genetic variation has been daunting. One approach being explored by Korber, along with collaborators at Beth Israel Deaconess Medical Center in Boston, the University of Manchester, NIAID's Vaccine Research Center, the University of Alabama, and Duke University, is to use various computational methods to determine the most common amino acids in the Envelopes of multiple variants of HIV from different clades, and then develop antigens based on these Env proteins, which are referred to as consensus sequences.

When a vaccine candidate containing a computationally derived, global consensus Envelope sequence was evaluated in rhesus macaques, it generated cellular immune responses to three- to four-fold more HIV epitopes of Env proteins across clades A, C, and G than a clade B immunogen from a naturally occurring Envelope sequence from a single individual did against clades A, C, and G. Moreover, the T-cell responses stimulated by the consensus immunogen within clade B was comparable with those stimulated by the naturally occurring clade B immunogen (*Proc. Natl. Acad. Sci.* **105**, 10489, 2008).

More recently, Korber and her collaborators also created what are referred to as mosaic vaccine antigens, which are assembled from natural sequences and optimized to achieve coverage of the many different versions of HIV proteins that are circulating. These mosaic vaccine candidates triggered strong cross-reactive immune responses in rhesus macaques in two separate studies (*Nat. Med.* **16**, 319, 2010; *Nat. Med.* **16**, 324, 2010).

One study led by Norman Letvin, a professor of medicine at Beth Israel Deaconess Medical Center, showed that the $CD8^+$ T-cell responses in rhesus macaques vaccinated with a prime-boost regimen of a DNA plasmid followed by a recombinant vaccinia virus vector were stronger if the vaccine constructs expressed mosaic immunogens compared to those expressing consensus immunogens (*Nat. Med.* **16**, 324, 2010). "This increased breadth and depth of epitope recognition could contribute to protection against infection by genetically diverse viruses and, in some instances, may block the emergence of common variant viruses," the study's authors noted.

A second animal study led by Dan Barouch, also of Beth Israel Deaconess Medical Center, evaluated mosaic Gag, Pol, and Env antigens expressed by recombinant, replication-incompetent adenovirus serotype 26 (rAd26) vectors. The team immunized 27 rhesus macaques with a single injection of the rAd26 vectors expressing mosaic antigens, consensus antigens, combined clade B and clade C antigens, or naturally occurring clade C Gag, Pol, and Env antigens. The Ad26 vector expressing mosaic antigens induced $CD8^+$ T cells that recognized more epitopes, as well as more variants within an epitope, than Ad26 vectors expressing consensus or natural sequence antigens (*Nat. Med.* **16**, 319, 2010). Overall, mosaic antigens provided a four-fold improvement in the breadth of the immune response.

Taken together, these NHP studies suggest that mosaic antigens could both broaden the range of recognized epitopes and increase responses to high-frequency HIV variants, although it remains to be seen if this approach will work as well in humans. A Phase I trial to compare the safety and immunogenicity of mosaic Envelope antigens with antigens that express either a global consensus Env sequence or a natural *env* gene, is scheduled to begin later this year and will involve about 100 volunteers. The HIV Vaccine Trials Network is conducting the trial in collaboration with CHAVI, the European Vaccine Effort Against HIV/AIDS, and the Bill & Melinda Gates Foundation.

"I am really hopeful," says Korber, who confesses she "thinks about sequences and HIV diversity all the time. We have to deal with the diversity issue. If we don't, we will never have a vaccine that works." ■

We have to deal with the diversity issue. If we don't, we will never have a vaccine that works.
— Bette Korber

Meeting of the Minds on MUCOSAL TRANSMISSION

Researchers underscore the possible role of non-neutralizing antibodies in protection against HIV and discuss ways to optimize animal models to study mucosal transmission

By Andreas von Bubnoff

HIV IS MOST OFTEN SPREAD through sexual transmission and therefore primarily enters the body through mucosal surfaces at the genitals or the rectum. At a recent meeting on "New Insights into Mucosal Transmission of HIV/SIV and its Prevention by Vaccines and other Modalities," which was held at the US National Institutes of Health June 3-4, researchers discussed efforts to better understand mucosal transmission of HIV in clinical trials, non-human primate models, and *in vitro* studies.

Now that there is the first evidence of vaccine-induced protection against HIV, many researchers are focused on trying to find an explanation for these results. RV144, an HIV vaccine efficacy trial conducted in Thailand involving more than 16,000 volunteers, showed that a canarypox vector-based candidate, ALVAC-HIV, administered in a prime-boost combination with an engineered HIV gp120 protein, AIDSVAX B/E, provided a modest 31% protection against HIV infection. While the candidate vaccine regimen used in RV144 does not seem to induce much of a neutralizing antibody response, it did induce antibodies capable of binding to gp120 in the majority of vaccinees. Some researchers believe it is possible that non-neutralizing, gp120-binding antibodies may have contributed to the modest protection, perhaps by inhibiting HIV at one or several points along its entry path through mucosal tissues.

At the meeting, Barton Haynes, a professor of medicine and immunology at Duke University Medical Center who chairs the scientific steering committee for RV144 analysis and follow-up studies, presented plans designed to test this hypothesis. In the absence of mucosal samples from RV144 vaccinees, researchers plan to conduct assays that measure whether antibodies taken from blood samples from RV144 vaccinees who remained HIV uninfected can inhibit HIV on its way into the body through the mucosa better than antibodies from vaccinees who were infected with HIV during the trial. "The assays are designed to look at every stage in the initial mucosal transmission event to make sure that we have assays that could pick up blocking at each one of those stages before traditional neutralization," Haynes said.

For example, some assays will measure the ability of the vaccine-induced antibodies to slow the movement of virions through the mucus that

covers the mucosal surfaces of the female genital tract. Other assays will measure whether these antibodies can inhibit transcytosis, which is one way HIV is thought to cross the epithelial layer of the mucosa. Inhibition of transcytosis can be tested *in vitro* using a cultured epithelial layer. Other assays will test for other antibody activities, including antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell-mediated virus inhibition (ADCVI; see *Antibodies: Beyond Neutralization, IAVI Report*, Jan.-Feb. 2010).

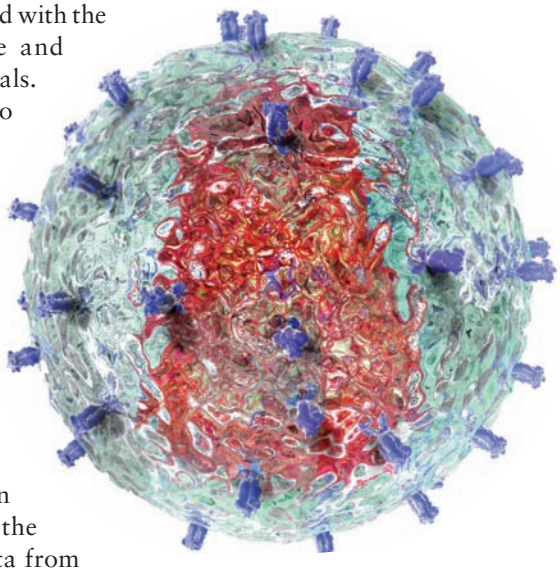
[The] data from our nonhuman primate studies support a role for non-neutralizing antibodies in protection. *—Marjorie Robert-Guroff*

Before analyzing the most valuable samples from the 51 vaccinated volunteers in RV144 who subsequently became HIV infected, researchers from many different labs will test different assays in a pilot phase that will help them decide which are the best and most reliable assays to use. Haynes said the pilot phase has been delayed because of the political unrest in Thailand. "Hopefully it's abating," he said.

The assays that give the most solid and consistent results will then be used to compare, in a case-control study, the antibody activities of HIV-infected and uninfected vaccinees, Haynes said, adding that he expects to have first results in the fall. "Hopefully we will have something to say by the time of the vaccine meetings in October," Haynes said. "That's pushing it, but that's the current timeline."

Haynes said his lab will also test if antibodies in the serum of the RV144 vaccinees that specifically bind the Env proteins contained in AIDSVAX B/E, the protein boost used in RV144, can protect rhesus macaques in passive infusion experiments. To determine this, researchers will use specific probes with the exact Env variants used in AIDSVAX B/E to isolate memory B cells from the serum of RV144 vaccinees. They will then use the antibody genes in these cells to synthesize the antibodies, and then infuse the antibodies into rhesus macaques. The macaques will then be challenged with an R5-tropic simian immunodeficiency virus (SIV)/HIV hybrid known as SHIV that is recognized by the antibody, if one is available.

The role non-neutralizing antibody activities may play in protection against HIV was also discussed in other talks at the meeting. Marjorie Robert-Guroff, the chief of the immune biology of retroviral infection section of the vaccine branch at the National Cancer Institute (NCI), presented evidence that non-neutralizing antibodies do play a role in protection in rhesus macaque experiments. Guroff and colleagues recently reevaluated the serum from animals that had been primed twice with a replicating adenovirus serotype 5 vector expressing SIVmac239 *gag* and *nef* and HIV gp140 *env,* followed by two gp140 Env protein boosts. When the animals were challenged with SHIV89.6P that expressed the same genes contained in the vaccine regimen, they showed reduced acute and chronic viral load even though they did not have neutralizing antibodies until four weeks after challenge (*Virology* **374**, 322, 2008). When Guroff and colleagues explored whether non-neutralizing antibody activity could account for the reduction in viral load, they found that ADCC and ADCVI mediated by serum antibodies from these animals correlated with the observed reduction of acute and chronic viral load in these animals.



For the first time, they also showed that inhibition of transcytosis by antibodies in rectal secretions correlated with reduced chronic viral load, Guroff said (*J. Virol.* 2010, doi:10.1128/JVI.00410-10). "The hypothesis [is] that non-neutralizing antibody contributed to the protection seen in the RV144 clinical trial," said Guroff, who is one of the researchers involved in measuring ADCC activity in the Thai trial samples. "[The] data from our nonhuman primate studies support a role for non-neutralizing antibodies in protection."

Another possible mechanism that could help explain the protection in RV144 is that antibodies might inhibit HIV movement in vaginal mucus. Tom Hope, a professor of cell and molecular biology at Northwestern University, presented evidence for this. He isolated cervicovaginal mucus from women and measured HIV movement with and without anti-HIV antibod-

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An Interview with MICHEL SIDIBÉ

The executive director of UNAIDS talks about AIDS funding, his hope for a vaccine and a cure, and a sustainable approach to treatment

By Regina McEnery

What do you see as some of the most important aspects of HIV prevention?

First off, it is critical to think about all the issues around primary prevention. We have 1.2 billion young people who need sexual education, who need to be educated about HIV prevention, and who need to be prepared to negotiate their sexuality differently. That is certainly one objective. The second objective, from my point of view, is to make sure that we continue to push for a cure for HIV and a vaccine. If we drop this from the agenda we will regret it because we need a mechanism to help us reduce the long-term costs of AIDS. The best way to accomplish this, in the long run, is to find a vaccine or a cure. In the meantime, we are facing a major challenge in treatment. We have around five million people on treatment and 10 million people waiting for treatment and there is a very important role for treatment in supporting prevention.

How would you characterize the long-term funding forecast for HIV prevention research and antiretroviral treatment programs?

We are moving from a period of opportunity to an era of scarcity that is characterized by many crises, particularly the global financial crisis. Despite the global financial crisis, this is not the time to scale back, but the time to scale up. We have been making progress. We need to fully fund AIDS research and continue to scale up the programs we have today. But we need to make sure that as we mobilize resources we become smarter, that we make the money work better, that we make the programs more cost-effective, and we must try to bring more integration to health systems. I think that is critical. For example, we know that there are more than 800,000 maternal deaths linked to HIV, so we definitely need to look at ways to integrate HIV services into maternal health programs.

So are you in favor of more integrative approaches to funding global health, including the US$63 billion Global Health Initiative launched by US President Barack Obama's administration?

Integrating HIV services is the only way we are going to be able to provide comprehensive services to people living with HIV. I think we should be looking for opportunities to leverage resources from AIDS programs to produce broader health and development outcomes. But what we should not lose is perspective. We welcome the integrated approach of the Global Health Initiative, but the effort should be to

MICHEL SIDIBÉ

On December 1, 2008, World AIDS Day, Michel Sidibé was named executive director of the Joint United Nations Programme on HIV/AIDS (UNAIDS), an agency that was established in 1994 to lead a coordinated global response to the AIDS pandemic. Sidibé holds a dual appointment as Under-Secretary General of the United Nations (UN) and is only the second person to lead UNAIDS—Peter Piot, who is now director of the London School of Hygiene and Tropical Medicine, was the first. Since his appointment, Sidibé has laid out an ambitious agenda that includes eliminating mother-to-child transmission of HIV and halving the number of tuberculosis-related deaths among HIV-infected individuals by 2015.

Recently, he called for the creation of a UN high-level commission on HIV prevention and a collaborative effort among scientists, AIDS advocates, countries, and drug manufacturers to find a comprehensive and sustainable approach to administering antiretrovirals in developing countries, an effort named Treatment 2.0.

Sidibé's three decades of experience in international public health and development include 23 years of service with the UN, the last nine with UNAIDS. Sidibé was born in 1952, is a citizen of the West African country of Mali, and hopes his World Cup soccer favorite, Ghana, advances to the finals.

scale up AIDS programs, not scale down programs. We don't know exactly how we can make sure that the 10 million people waiting for treatment in Africa will have access. For the majority of people in the world on antiretroviral therapy, decisions about an optimal first-line, second-line, or third-line [treatment regimen] are made based on cost rather than the drug itself. That is why I called for Treatment 2.0—which will explore a new generation of treatment options—to see how we can best provide long-term comprehensive treatment in a cost-effective manner. We need to develop a sustainable approach to HIV treatment.

What was your reaction to the results of the RV144 AIDS vaccine trial and for the prospects of a partially effective vaccine?

Well, it was the first time that an HIV vaccine reported some efficacy in human beings. That was very important. But what was also important for me was the fact that the vaccine was specifically designed for Thailand. I was glad to see a vaccine being explored in another part of the world. It is very important to view this not as a failure, but as a beginning that we can build on to make sure we can have a vaccine one day. In a nutshell, we also need to continue to explore other medical tools which can be used for HIV prevention. If we can combine a vaccine with other tools, such as male circumcision, perhaps a microbicide one day, and pre-exposure prophylaxis (PrEP), we can probably make a difference.

Are you confident that a vaccine for HIV/AIDS will be developed?

I am completely positive on that one. I think that RV144 has been creating a lot of energy and mobilizing the research field. The definitive answer at the end of the road is to find a vaccine. When will we have a vaccine; that is the question. It will be a pity if in the 21st century we fail to have a vaccine.

You have called for the formation of a high-level commission on HIV prevention. What specifically will this commission do and will it involve AIDS vaccine research and development?

The aim is not to have a new global commission that will produce 80 different recommendations. What we want is to make a political case for a prevention revolution. The commission will be guided by a scientific advisory panel, headed by Laurie Garrett [senior fellow for Global Health at the Council on Foreign Relations], and the prospects of an HIV vaccine would be part of the commission's agenda. We want to know what is happening around HIV incidence—how are we measuring it, are we making progress in driving HIV prevention around the world, and how can we measure progress. We want to bring a revolution in terms of existing knowledge and existing scientific evidence. You know if you look back during the last few years, we have not been able to push prevention to the level it should be.

What are your views on test-and-treat and other ARV-based prevention strategies?

There is an important role for treatment-supported prevention. I am not disagreeing with that. But there are 10 million people who need antiviral treatment today and only around five million receiving it. It is so critical for us to be able to reach the maximum number of people who are in need of treatment today. It should be treatment [for these individuals] before anything else, in my point of view.

The growth of the HIV epidemic in Eastern Europe will be a topic at the International AIDS Conference in Vienna. What is the current status of the epidemic there?

While the HIV epidemic has slowed down globally, it is accelerating at an alarming pace in Eastern Europe and Central Asia. We are not seeing anything like it in other regions of the world. There are 1.5 million people living with HIV/AIDS in Eastern Europe and Central Asia. The main cause of the epidemic is injection drug use, and about two-thirds of new diagnoses are occurring in regions where injection drug users have no access to services because they are considered criminals. We need social justice. We can't stop the epidemic if we don't have approaches that deal with this population. Universal access will never be achieved if we are basing our strategy on exclusion rather than inclusion. People who use drugs have rights, too. ■

Continued from page 11

ies present. He found that both non-neutralizing Env-binding antibodies and broadly neutralizing antibodies such as b12 slowed HIV movement in the mucus. HIV movement was also slower in mucus from HIV-infected women, suggesting HIV-specific antibodies were present in their mucus. "We think this could be another mechanism through which antibodies binding to virus could influence transmission," Hope said.

Refining animal models

Other work related to mucosal transmission involves fine-tuning animal models. In humans, most productive clinical HIV infections can be traced back to a single transmitted founder virus (see *HIV Transmission: The Genetic Bottleneck, IAVI Report,* Nov.-Dec. 2008). And, ideally, researchers would want to be able to reproduce these same transmission dynamics in nonhuman primate models so that they could mimic the human situation as closely as possible.

Brandon Keele, a senior scientist at NCI and the Science Applications International Corporation in Frederick, Maryland (SAIC-Frederick/NCI), analyzed the number and sequence of SIV variants that establish productive infection after infecting rhesus macaques intra-rectally with SIVmac251 stocks grown by different labs. He found that diluting the same 251 challenge stock led to a reduced number of transmitted founder viruses, eventually resulting in animals that were infected by a single SIV variant.

Guroff has used a repeat low-dose rectal challenge with a 1:500 dilution of an SIVmac251 stock that was grown in the lab of Ronald Desrosiers at the New England Primate Research Center at Harvard Medical School and provided by the Division of AIDS at NIAID. In this infection model, up to nine challenges were needed to infect all naive macaques, and Keele found that eight of nine animals became infected by a single viral variant (the ninth was infected with two variants). "We think that's a pretty reasonable approach to recapitulate the single variant infection we find in humans," Keele said.

Genoveffa Franchini, chief of the animal models and retroviral vaccine section at NCI, said she plans to use Guroff's intra-rectal challenge regimen in her efforts to develop an animal model that could recapitulate the results of RV144, when the vaccine regimen is tested in rhesus macaques. "[So far], we have used a dose that gives us a lot of variants," Franchini said. "Now we want to use a dose that gives us one variant."

Meanwhile, Keele is also studying other transmission routes. He finds intra-vaginal infection to be more variable than intra-rectal infection in terms of the number of transmitted founder viruses after infection with the same dose. In collaboration with Jake Estes, a senior scientist at SAIC-Frederick/NCI, Keele is now studying the location and sequence of individual SIV-infected cells in rhesus macaques that were infected vaginally to better characterize where transmitted founder viruses first take hold in the animals. He showed that it is possible to infect an animal vaginally and then use a laser to isolate SIV-infected cells from histological sections of the cervix of the infected animal. The integrated proviral SIV from these infected cells can then be sequenced. Along with Chris Miller, a professor at the School of Veterinary Medicine at the University of California in Davis, Keele has also started to analyze the number of transmitted founder viruses in male rhesus macaques whose penises were exposed to SIVmac251. ■

Fighting Viruses with Bacteria

One topic covered at the recent meeting on "New Insights into Mucosal Transmission of HIV/SIV and its Prevention by Vaccines and other Modalities," which was held at the US National Institutes of Health from June 3-4, was the development of novel approaches to prevent mucosal HIV transmission in humans. Laurel Lagenaur, a senior scientist at the California-based company Osel Inc., presented an update of efforts to develop a live vaginal protein-based microbicide by introducing the gene for Cyanovirin into Lactobacilli, bacteria that normally live in the vaginal mucosa (see *Mucosal Vaccines: Insights from Different Fields*, *IAVI Report*, Nov.-Dec. 2008).

Cyanovirin is a protein that binds to HIV gp120 with a very high affinity. Lagenaur showed that in rhesus macaques, the Lactobacilli expressed Cyanovirin protein for up to six weeks after the animals had been inoculated with the transgenic bacteria.

To see if the transgenic bacteria could protect from challenge, the researchers inoculated macaques with the bacteria every week. Each weekly inoculation was followed 24 hours later by a low-dose vaginal challenge with a simian immunodeficiency virus (SIV)/HIV hybrid, known as SHIV. More weekly inoculation/challenge cycles were necessary to infect the animals with the Cyanovirin-expressing Lactobacilli than control animals. According to Lagenaur, this translated into a 62% reduction of the SHIV transmission rate in the animals with the Cyanovirin-expressing Lactobacilli. "This is the first successful demonstration of a live microbicide," said Lagenaur. She thinks it might also be interesting to express some of the recently isolated broadly neutralizing antibodies, such as PG16 or VRC01 (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009), in the Lactobacilli in future experiments. —*AvB*

Research BRIEFS

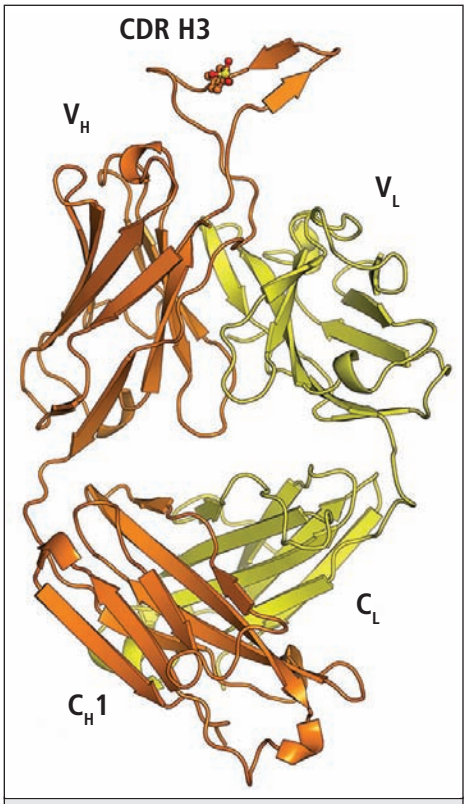
Crystal Structure of PG16 Antigen-Binding Portion Reveals Unusual Features

The recent isolation of several new broadly neutralizing antibodies was good news for researchers working to design AIDS vaccine candidates that could induce antibodies capable of neutralizing many of the viral variants currently in circulation (see *Adding to the Armamentarium of Broadly Neutralizing Antibodies, Research Briefs, IAVI Report*, Jan.-Feb. 2010). Last September, IAVI researchers, in collaboration with researchers at The Scripps Research Institute (TSRI), announced the isolation of two of these antibodies, dubbed PG9 and PG16.

Now, two research teams, one led by Ian A. Wilson and Dennis Burton at TSRI, the other by John Mascola and Peter Kwong at the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID), have identified the crystal structure of the Fab portion of PG16, which is the part of the antibody involved in antigen binding (*Proc. Natl. Acad. Sci.* **107**, 11483, 2010; *J. Virol.* 2010, doi:10.1128/JVI.00966-10). Because PG16 binds best to the native HIV Env trimer, which researchers have so far been unable to crystallize, both teams determined the structure of PG16's Fab in an unbound state.

One of the most unusual features of PG16, the studies found, is the structure of its extremely long (28 amino acids) CDR H3, which is one of the most variable parts of the antigen-binding region of an antibody and known to often be important for antigen binding. "There has never been a crystal structure of an antibody with a [CDR H3] loop that long," says Robert Pejchal, a postdoctoral researcher in Wilson's lab and the first author of the *PNAS* study.

"[The CDR H3] forms a mini domain that sort of towers above the rest of the antibody," adds Wilson, Hansen professor of structural biology at TSRI and one of the lead authors of the *PNAS* study. "We have called it a hammerhead [because it looks] like a hammerhead shark." This shape suggests that the antibody may use this domain to access an occluded epitope on the Env trimer that would not ordinarily be easy to access, says Pejchal.



PG16 Fab structure with variable (V) and constant (C) parts of the heavy chain in orange and the variable and constant parts of the light chain in yellow. The CDR H3 can be seen on top, with the sulfate group on one of its tyrosine residues shown in yellow (S) and red (O). This is a ribbon diagram that outlines the protein backbone and shows beta strands as arrows and random coils as thin tubes.

Courtesy of Robert Pejchal at The Scripps Research Institute; also published in *Proc. Natl. Acad. Sci.* **107**, 11483, 2010.

Peter Kwong's group at the VRC found the CDR H3 loop had the same shape, which they call an axe, but they also found that in some cases, the CDR H3 is disordered. "Even though it can form this axe or hammerhead structure, it doesn't necessarily have a fixed structure. You can deform it relatively easily," says Kwong, who is chief of the structural biology section at the VRC. "The amount [it] deforms might be important for recognizing its particular epitope. If it was totally fixed it might not be able to get into the little crevice that it might have to get to."

The CDR H3 loop appears to be necessary for neutralization because mutating parts of it greatly reduced or even eliminated the ability of PG16 to neutralize HIV, the authors of the *PNAS* study found. "We think that this hammerhead is probably what's mediating the interaction between PG16 and the HIV trimer," says Laura Walker, a graduate student in Dennis Burton's lab, who did the mutation analysis for the *PNAS* study.

The CDR H3s of PG9 and PG16 differ mostly in a stretch of seven amino acids, and, according to the *PNAS* study, exchanging the seven amino acid stretch of the two antibodies resulted in PG16 being able to neutralize certain HIV isolates with a similar potency to PG9 and vice versa. Kwong's group found a similar effect when they swapped the entire CDR H3 between PG9 and PG16.

The authors of the *PNAS* study found that another unusual feature of PG16 is that one of the tyrosine residues on the CDR H3 contains a sulfate group, the removal of which decreased the neutraliza-

tion potency of PG16 by about ten-fold, Walker says. "[The sulfate is] not required for neutralization but it enhances neutralization," says Walker.

Kwong says that PG16 also shows quite an extensive degree of affinity maturation—about 20% of the amino acids in the variable region of PG16 differ from its germ-line version. This is still less than the 30% affinity maturation reported for the variable region of the VRC01 antibody, but more than the 5-10% affinity maturation observed in most other antibodies (see *Antibody Fever, IAVI Report,* Mar.-Apr. 2010).

Kwong's group found that the extensive affinity maturation was important for the neutralization potency and breadth of PG9 and PG16. When the researchers made less affinity matured versions of these antibodies that had some, or all, of their variable gene portion reverted to germ line, they found that increased affinity maturation correlated with increased potency and breadth of neutralization of a panel of HIV isolates.

Still, somewhat surprisingly, a PG9 version that had its variable gene portion completely reverted to germ line could still neutralize one HIV isolate. This led Kwong and colleagues to suggest that a possible vaccine strategy to kick start the affinity maturation process would be to use Env proteins from the HIV isolate that can still be neutralized by the less affinity-matured version of the antibodies. This could then be followed by boosts with a cocktail of Env proteins from HIV variants that are neutralized by increasingly more affinity-matured versions of the antibodies. However, it's still unclear how the CDR H3 part of the antibody may be generated, and how it could be induced by a vaccine.

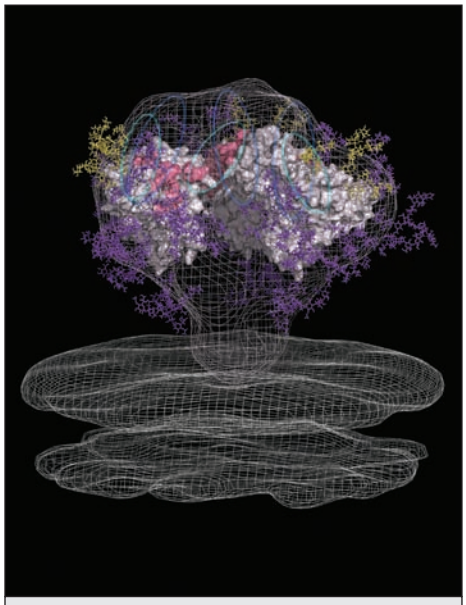


Image adapted from a cryoelectron tomographic structure of the native HIV Envelope trimer (outer shell), filled with the crystal structure of the b12-bound monomeric gp120 core. PG9 and PG16 are thought to bind to the V1/V2 and V3 loops (shown as dark blue and teal ovals, respectively), whose structure is unknown. Carbohydrate chains are shown in purple, and the oligomannose cluster targeted by 2G12 is shown in yellow.

Robert Doms, who chairs the department of microbiology at the University of Pennsylvania and was not connected to the study, says the findings fit a trend among broadly neutralizing antibodies. "Virtually all broadly neutralizing antibodies to date are structurally 'odd', in that they have one or more features that have either never been seen before, or that have been seen in other antibodies only rarely. Will unusual structural features be required for potent and broad neutralizing activity? If so, we know essentially nothing about how to elicit such antibodies."

Next, it will be important to solve the structure of the HIV Env trimer and PG16 bound to it, says Pejchal. "That will really reveal how these antibodies work," he says.

It won't be easy, however, because PG16 binds best to the native trimeric form of Env, which researchers have so far been unable to crystallize. "That's an enormously challenging undertaking," Wilson says. "The Envelope [trimer] is not very stable, it's very hard to produce and you cannot produce large quantities of it for structural studies. It's probably one of the most challenging things that still have to be done." —*Andreas von Bubnoff*

Possible Explanation for HLA-class I Associated Control of HIV Infection

Researchers have known for some time that elite controllers—HIV-infected individuals who, without antiretroviral therapy, can keep their viral load at a level that is undetectable by currently available commercial viral load assays—are more likely to have certain versions, or alleles, of genes that encode major histocompatibility complex (MHC) class I molecules, including one called *HLA B57* (in humans, MHC is called human leukocyte antigen, or HLA). This suggests that genes like *B57* are involved in the control of viral load in elite controllers.

Now, a research team led by Arup Chakraborty, a professor of chemistry, chemical engineering, and biological engineering at the Massachusetts Institute of Technology, and Bruce Walker, a professor of medicine at Harvard Medical School, have developed a model that suggests that people with *HLA B57* control viral load better in part because their T cells go through a less rigorous selection process in the thymus (*Nature* **465**, 350, 2010).

In the thymus, $CD8^+$ T cells encounter cells that present self peptides—small pieces of the body's own proteins—bound to MHC class I receptors. $CD8^+$ T cells whose T-cell receptors bind strongly to these self peptides die, which is important for avoiding autoimmune disorders.

In their model, Chakraborty and colleagues suggest that more protective versions of the MHC class I receptor, such as B57, bind and therefore present a smaller diversity of the body's own peptides to the $CD8^+$ T cells in the thymus than less protective versions. As a result, immature $CD8^+$ T cells in the thymus of people with *B57* have fewer opportunities to die as a result of binding strongly to self peptides,

making it more likely that CD8+ T cells survive that are more cross-reactive to point mutations of viral peptides. "[In] *B57* [positive] people, the T cells have to pass a less rigorous test because they have to avoid binding strongly to only a smaller diversity of self peptides," Chakraborty says. "Their T cells were educated in a thymus with fewer types of self peptides."

If this model is correct, it would suggest that people with *B57* can better control viral load in part because their CD8+ T cells are more likely to recognize a diverse array of HIV peptides presented by MHC class I molecules on the surface of HIV-infected cells, including peptides that come from mutants of HIV that arise during infection. As a result of this recognition, these CD8+ T cells will turn into cytotoxic T lymphocytes that kill the infected cell. Therefore, people with genes like *HLA B57* would be more likely to generate cytotoxic T cells that can kill cells infected with a wider array of HIV strains, particularly HIV mutants that arise during infection.

The first hint that led Chakraborty and colleagues to their model came when they calculated how many self peptides could be bound by the different MHC receptor types encoded by different HLA alleles. Using published data from binding experiments, they first tested whether computer algorithms developed to predict which peptides can bind to different versions of MHC class I receptors were accurate. They found that the most accurate algorithm predicted that B57 would bind fewer self peptides than other versions of the MHC class I receptor that are less protective to HIV. "[This] was striking since *HLA B57* is the allele most associated with HIV control," says Walker.

Using a computer model they had developed to simulate the thymic selection of T cells, they also found that T cells that survive after having been presented fewer self peptides in the thymus are more likely to bind to a diverse set of HIV peptides, particularly ones that come from HIV mutants that arise during infection. Using yet another computer model, they then showed that this would be expected to lead to better control of viral load.

Finally, the researchers tested these computer modeling predictions with viral load data from cohorts of about 1,100 controllers and 600 progressors. They found that HIV-infected people with the MHC class I versions B57 and B27, which are predicted to bind to fewer self peptides, are more likely to be HIV controllers, whereas HIV-infected people with B07 and B35, versions that are predicted to bind more self peptides, are more likely to be HIV progressors. "We have identified one contributing factor to why people with this [*B57*] allele may be more likely to control HIV," Chakraborty says.

> [In] *B57* [positive] people, the T cells have to pass a less rigorous test because they have to avoid binding strongly to only a smaller diversity of self peptides.
>
> — *Arup Chakraborty*

The model is consistent with observations that people with protective HLA alleles are more likely to have autoimmune and hypersensitivity reactions, for example the *HLA B57*-associated hypersensitivity reaction to the antiretroviral drug Abacavir, Chakraborty says.

Another prediction of the model is that the better cross-reactivity of CD8+ T cells in people with *B57* should also enable them to better control infections with other pathogens. "This [CD8+ T cell] cross reactivity would not be specific only for HIV," Chakraborty says. Unpublished data from Walker and colleagues indicate that HLA alleles such as *B57* are also good at controlling hepatitis C virus (HCV), another rapidly mutating RNA virus.

But Mark Connors, chief of NIAID's HIV-specific immunity section who was not involved in the study, says this doesn't necessarily fit with his observations. He found that *B57* positive HIV non-progressors who are also infected with cytomegalovirus (CMV) or HCV have only a few CMV and HCV peptides presented by B57 (*J. Virol.* **83**, 2728, 2009). "If this [mechanism] was operating by the way that the authors claim, you would expect that B57 would dominate all responses to all viruses because you are going to have more broadly reactive T cells," Connors says. "But it doesn't."

Walker says that the different observations in Connors' study might be because Connors mostly studied people who are co-infected with HIV and CMV or HCV, whereas Walker's unpublished data mostly comes from people who were not co-infected. "We find that the strong correlation of *B57* with protective HCV responses is diminished for individuals co-infected with HCV and HIV," Walker says, "suggesting that co-infection may have unique aspects to it that impact immune control."

David Baltimore, a professor of biology at the California Institute of Technology who was not connected to the study, called it "a fascinating new interpretation of the effect of an MHC locus on a person's ability to fight off HIV."

However, Connors says, the study's authors could have experimentally validated their prediction that protective MHC class I receptor variants really bind fewer self peptides than non-protective variants. "You can do things like elute peptides and determine whether in fact B57 does bind more or fewer [different self peptides]," Connors says, adding that not all protective alleles seem to bind fewer self peptides.

But Chakraborty says that even though the effect might not be as strong for all alleles, that doesn't necessarily mean the model is wrong. "The new factor we have identified is not the only factor that contributes to virus control," he says, adding that this new mechanism should contribute the most for alleles that bind either very few or very many types of self peptides, according to his experimental data. "All effects that are identified should not be thought to be mutually exclusive." —*Andreas von Bubnoff*

Vaccine BRIEFS

Researchers Unveil Plans for Follow-up Trials to RV144

Less than a year after the RV144 trial in Thailand provided the first evidence of protection against HIV infection through vaccination, researchers are preparing to launch two new trials and are planning other future trials that may show whether tweaks to the prime-boost regimen tested in RV144 could increase the efficacy of this approach. Jerome Kim, deputy director of science at the US Military HIV Research Program (MHRP), a key collaborator in RV144, spoke about future plans to build on the modest efficacy results from RV144 at a recent HIV vaccine symposium at The New York Academy of Sciences in New York City on May 19.

More than 130 individuals attended the event, held the day after World AIDS Vaccine Day, which marks the day in 1997 that then-US President Bill Clinton delivered a speech calling for a renewed commitment to the development of an AIDS vaccine. The Global HIV Vaccine Enterprise and the Academy jointly sponsored the event.

The RV144 prime-boost regimen consisted of a canarypox vector-based candidate, ALVAC-HIV (vCP1521), and AIDSVAX B/E, a genetically engineered version of HIV's gp120 surface protein (see Figure 1 for dosing schedule).

> If you had a 60% efficacy at two years, it might be a reasonable vaccine to consider for licensure in places where the epidemic is substantial.
>
> *– Jerome Kim*

In a post-hoc analysis performed after the trial was unblinded, Kim said it appeared that the vaccine efficacy among vaccinated volunteers six months after the last injection may have been as high as 60%. Unfortunately, it is difficult to draw any conclusions from this observation because the study was not designed to measure whether a certain number of injections were effective or if the protective responses waned over time. "If you had a 60% efficacy at two years, it might be a reasonable vaccine to consider for licensure in places where the epidemic is substantial," said Kim.

But at the conclusion of the six-year RV144 study, the overall efficacy of the prime-boost regimen declined to 31.2%. "The question is, if we take the same regimen and the same population, but use a boost at 12 months, could we see a 60% efficacy at 24 months [post-vaccination]?" asked Kim. An initial series of trials are planned to examine what happens immunologically after boosting.

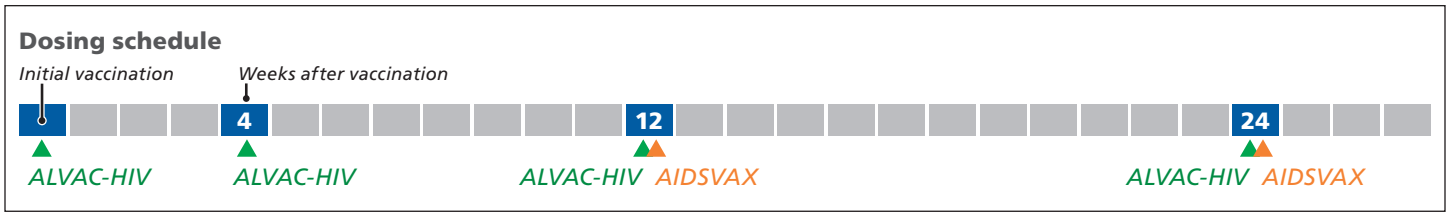
One trial, known as RV305, will measure the effect of administering two additional protein booster shots to 108 of the 16,000 vaccinated participants from RV144. The planned RV305 study will have three arms, each including 36 vaccinated volunteers from RV144, 30 of whom will receive the additional booster shots and six who will receive placebo. The first group will receive an ALVAC/AIDSVAX boost, the second will receive just ALVAC, and the third group will receive just AIDSVAX. The booster shots will be given at 48 and 60 months post follow-up of RV144. Kim said MHRP intends to seek regulatory approval from the US Food and Drug Administration soon and hopes to launch this trial by 2011.

Another follow-up trial, known as RV306, which is expected to begin in mid- to late-2011, will involve collection of more samples from vaccinated volunteers so that researchers can gather more information regarding the immunogenicity of the ALVAC/AIDSVAX prime-boost combination.

Plans for this trial aren't finalized but Kim said it may enroll about 500 HIV-uninfected individuals and include four study arms. Each arm would include 125 volunteers, 10 of whom will receive placebo. All four groups will receive the standard ALVAC/AIDSVAX regimen administered in RV144, but 48 weeks following the initial vaccination, one group of volunteers will receive another dose of ALVAC and AIDSVAX, a second group will receive just an AIDSVAX boost, a third group will receive only an ALVAC boost, and the fourth group will not receive any boost. Trial investigators are hoping to obtain regulatory approval early next year for this trial.

With the volunteer's consent, researchers will perform leukapheresis in a small number of subjects, a lengthy process that spins out white blood cells by first pumping a person's blood through a centrifuge. The procedure takes several hours

FIGURE 1



per volunteer, but ultimately enables investigators to better characterize immune responses following vaccination. Mucosal specimens, including gut tissue, will also be sampled. "Ultimately, we want to see whether there are changes induced by the late vaccination, which will help us determine which combinations are the ones we should use as a booster," said Kim.

Kim said another trial comparing a prime-boost regimen containing NYVAC—a poxvirus-based vector similar to ALVAC that was also developed by Sanofi Pasteur—to one containing ALVAC, is also being contemplated. This trial would have the same dosing schedule as RV144, but would also include an additional protein boost at 12 months. The collaborators are interested in exploring NYVAC because it may improve immune responses. The study population size for this trial hasn't yet been determined.

MHRP is also considering conducting larger efficacy trials of the ALVAC/AIDSVAX prime-boost regimen with an additional boost at 12 months. Researchers are considering a trial in 2,500 men who have sex with men in Thailand who are specifically at high risk of HIV infection. They have also discussed a larger trial in Thailand that would involve 27,500 volunteers at community risk, a population similar to that enrolled in RV144. But as opposed to the three and a half year follow-up of volunteers that occurred in RV144, investigators would plan to analyze the data from this trial after two years. However, Kim acknowledged that the field is divided about whether to commit to another "gigantic trial" at this time. A major barrier to moving forward with another large efficacy trial is funding. "We can't pay for all of it," said Kim, adding that MHRP would have to rely on industrial or governmental partners to be able to conduct a 27,500 person study. —*Regina McEnery*

US Government Singles Out Eight Countries for Pilot Program of Global Health Initiative

A YEAR AFTER UNVEILING a US$63 billion six-year Global Health Initiative (GHI), a more integrated strategy for tackling preventable diseases and improving maternal and child health and nutrition in more than 80 countries, the US government singled out eight countries that are slated to receive $200 million in additional funding to quickly implement this new approach.

The eight countries—collectively referred to as GHI Plus countries—are Bangladesh, Ethiopia, Guatemala, Kenya, Malawi, Mali, Nepal, and Rwanda. The US Agency for International Development (USAID), one of several US agencies involved in the implementation of the GHI, said the US government intends to add additional countries to the GHI Plus list in the future. What is learned from the GHI Plus pilot program will be used to improve service delivery in all countries that have GHI-related programs and inform decision making of the US government and its partners.

The GHI, which encompasses all existing global public health initiatives funded by the US government, seeks to build on nine target areas: HIV/AIDS, malaria, tuberculosis, maternal health, child health, nutrition, family planning and reproductive health, neglected tropical diseases, and health systems strengthening. The US President's Emergency Plan for AIDS Relief (PEPFAR) accounts for $51 billion of the GHI funding over six years. PEPFAR began under former President George W. Bush and was reauthorized in 2008 for five years to fight HIV, tuberculosis, and malaria in 31 countries.

Mark Harrington, executive director of the AIDS advocacy organization Treatment Action Group, says the GHI is a "great and timely idea," but he believes the initiative is not adequately funded, and therefore its impact will suffer. US President Barack Obama's administration has requested $9.6 billion in funding for GHI programs in 2011. Spending for GHI programs was $8.4 billion in 2009 and $8.8 in 2010.

For more information on the Global Health Initiative, go to http://www.usaid.gov/our_work/global_health/home/Publications/docs/ghi_consultation_document.pdf. —*Regina McEnery*

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