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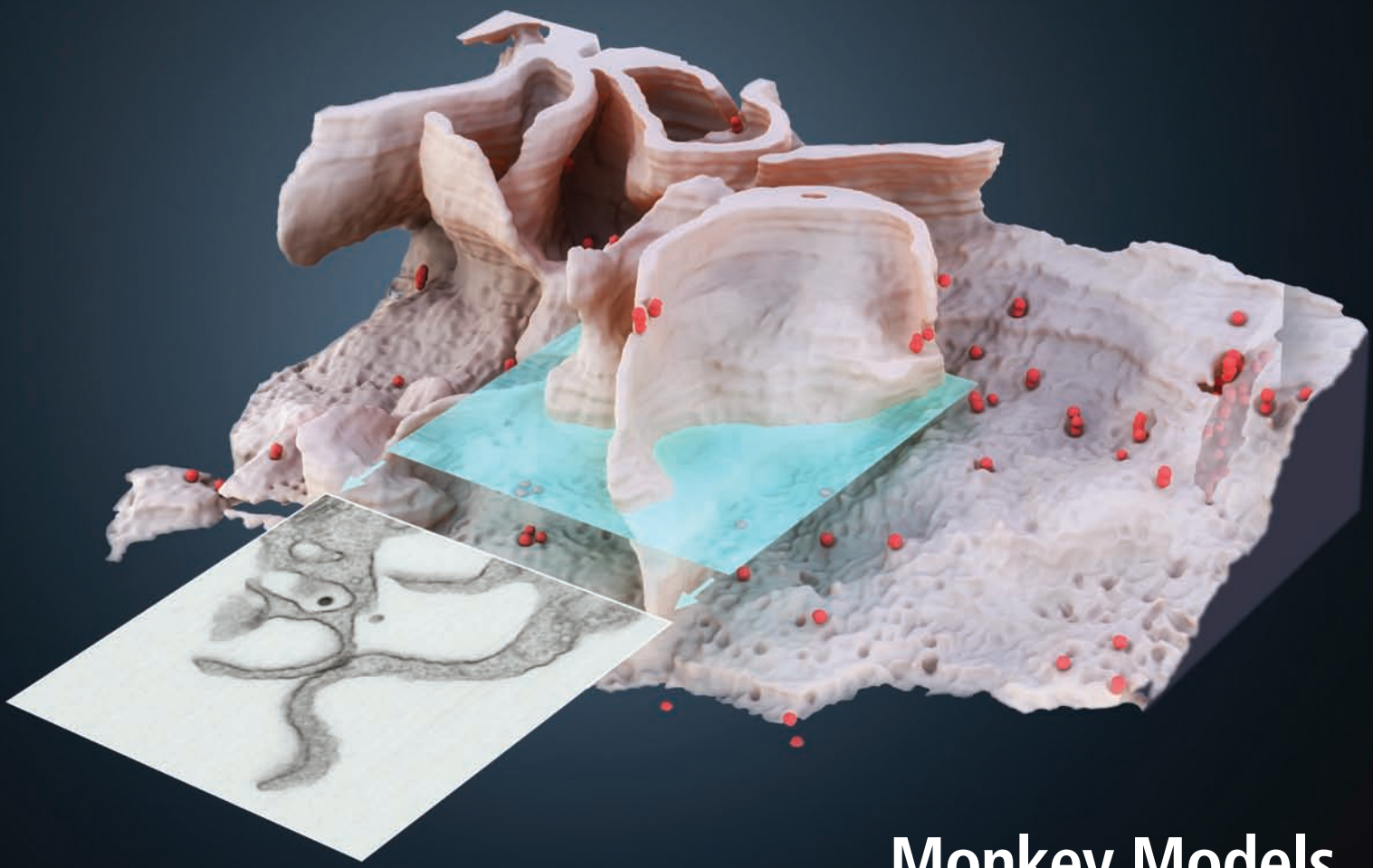
PROFILE: SRIRAM SUBRAMANIAM

Visualizing HIV



Monkey Models

Highlights from the meeting on nonhuman primates in AIDS research

EDITOR'S LETTER

As 2009—what has been referred to as a banner year in AIDS vaccine research and development—draws to a close, there are many exciting advances to highlight and reflect upon. Not the least of these was the first hint of vaccine-induced protection against HIV infection to emerge from a clinical trial. The 16,000-person RV144 trial in Thailand brought unexpected results—the prime-boost regimen many researchers in the field had all but written off, provided a modest but statistically significant 31% protection against HIV infection. Although this is just one step on the road to the development of a safe and effective vaccine, it was a positive signal, both scientifically and symbolically. These results have energized the field and reinforced the importance of clinical evaluation in the vaccine development process. At the same time, however, researchers continue to refine and enhance non-human primate models, which will likely contribute to development of improved vaccine candidates (see *Monkey Models: Far from Extinct*, page 4). The surprising results of RV144 also show that protection against HIV infection, what seemed a difficult goal to reach without the induction of neutralizing antibodies, may be possible.

Another highlight of 2009 was the discovery of five new broadly neutralizing antibodies against HIV, the first additions to the antibody armamentarium in a decade. Some of these potent new antibodies have unique binding sites that represent vulnerable spots on the virus, which could be exploited by researchers in an effort to design vaccine immunogens.

With all of these developments, there is much to capitalize on in the coming years. The *IAVI Report* team looks forward to tracking the latest research and bringing these evolving stories to our readers. This includes the hunt for possible correlates of protection from RV144, other trials that are underway that may also provide useful insights, and steady advances in basic research. We will continue to find new ways of bringing this work to life, including profiles, like the one featured in this issue of Sriram Subramaniam, whose innovative approach to studying HIV yields both stunning and detailed images of the virus within cells (see *The Beauty Behind the Beasts*, page 9). Additionally, we will continue to monitor and report the latest news on other HIV prevention strategies (see *A Cut Above the Rest*, page 13, and *Microbicide Candidate Fails in Phase III Trial*, page 16), and policy and economic developments that may impact the field (see *Update on Pandemic Shows New HIV Infections Steadily Declining*, page 17, and *Progress and Promise*, page 18).

Here's to a banner year and an even brighter future.

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 24 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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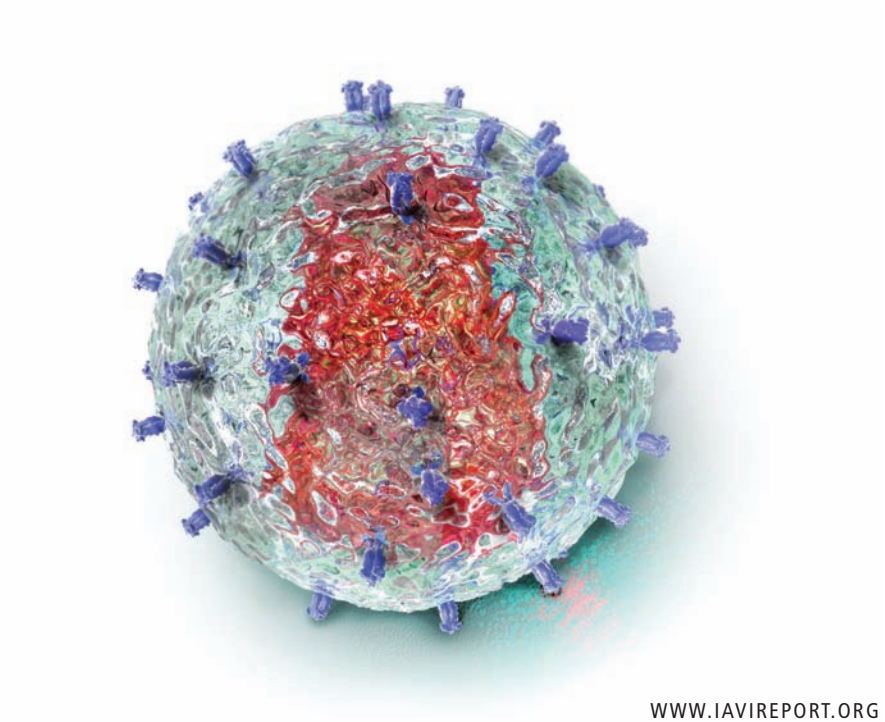

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[ON THE COVER]

Representation of the surface and interior of an HIV-infected macrophage obtained with newly developed tools for 3D imaging using ion-abrasion scanning electron microscopy. Sections that would appear to contain "filopodia" when imaged by transmission electron microscopy of individual sections can actually correspond to large wavelike membrane processes as illustrated by the cut-away view of a central slice. The membrane protrusions may potentially fold back to the surface of the cell, creating viral compartments (viruses shown in red) by trapping the contents of the aqueous environment within the invaginated folds of the membrane.

Image courtesy of Donald Bliss and Sriram Subramaniam, NIH, as published in Bennett et al., PLoS Pathog. ***5****, e1000591, 2009.*

MONKEY MODELS: Far from Extinct

A recent meeting on nonhuman primate models underscores their central role in AIDS research

By Andreas von Bubnoff

Nonhuman primate (NHP) studies have been and will be a central part of HIV/AIDS research, National Institute of Allergy and Infectious Diseases (NIAID) Director Anthony Fauci said at the opening of the 27th Annual Symposium on Nonhuman Primate Models for AIDS, which took place from October 28-31 in Boston. "The advances over the last 28 years are really astounding, [and] a lot of the things that have opened up doors in pathogenesis and vaccine [development] particularly come from NHP studies," he said, ending his remarks to the about 250 attendees of the meeting by saying, "I think you are here to stay."

The meeting featured research updates on a wide range of topics, including the characterization of existing challenge stocks, the development of new challenge stocks to better mimic human HIV infection, and studies of the role of bacterial translocation in the gut in the development of AIDS.

Optimizing challenge strains

One goal when developing NHP models for AIDS is selection of a challenge virus that mimics HIV transmission in humans. At the meeting, researchers discussed some efforts that focus on developing challenge stocks that mimic recently transmitted and not chronically replicating HIV, because the two appear to differ biologically.

Fauci addressed these differences between early and late viruses when he discussed the recent results from RV144, a 16,000-person efficacy trial of a prime-boost regimen in Thailand that showed the first hint of vaccine-induced protection against HIV infection. He said the effect the RV144 prime-boost regimen had on acquisition, but not on viral load (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009), suggests a difference between the immune response that blocks HIV acquisition and the one that blocks virus replication. This is consistent with biological differences between the transmitted virus and the late, chronically replicating virus. For example, compared to the chronically replicating virus, the transmitted virus is less glycosylated and therefore easier to neutralize. "It may be when we are looking at the effect of a vaccine we are matching it against a virus that it doesn't have to neutralize because the transmitting virus is very different from that," Fauci added.

Ruth Ruprecht, a professor of medicine at Harvard Medical School, welcomed Fauci's remarks. "That's what I have been saying for years," she said. Her lab has been developing SIV/HIV hybrid or SHIV strains that contain *env* from early, recently transmitted HIV clade C strains to better reflect the biology of the virus that is transmitted. "There is something special about the biology of the recently transmitted [clade C] virus," Ruprecht said, adding that it is more sensitive to neutralizing antibodies and has shorter variable loops of gp120 than the other strains that predominate in the infected donor.

At the meeting, Nagadenahalli Siddappa from Ruprecht's lab reported progress in the development of a SHIV strain with a clade C *env* gene that was isolated from a six-month-old child from a mother-infant cohort in Zambia (see *Looking for the Perfect Challenge*, *IAVI Report*, July-Aug. 2009). Clade C viruses are responsible for about 56%-60% of all HIV/AIDS cases in the world, according to Ruprecht. In unpublished work, Siddappa and colleagues have used this *env* to develop a SHIV challenge strain that is easy to neutralize, replicates reproducibly, and causes high peak viremia levels. This is the first R5 clade C SHIV that is tier 1, which means that it has a high sensitivity to neutralization, according to Ruprecht. "We have already titrated it so that we can do multiple low-dose [challenges]," Ruprecht said.

According to Ruprecht, having challenge viruses that are easy to neutralize is important in initial challenge studies, because otherwise the bar may be too high to see any protective effect of a candidate vaccine. "If you are going to need to enlist the humoral arm of the immune system, you have to have a challenge system that lets you score for that," she said.

Some of the current SIV challenge strains such as SIVmac251 are thought to be hard to neutralize, suggesting they may not be ideal for testing vaccine candidates, said Wendy Yeh, an instructor in medicine at Harvard Medical School who works at Beth Israel Deaconess Medical Center in Boston. But just how hard it is to neutralize SIVmac251 challenge strains compared to HIV-1 has not been rigorously tested. So Yeh and colleagues determined if serum of macaques infected previously with SIVmac251 in a repeat low-dose rectal infection experiment (*J. Virol.* **81**, 12368, 2007) could neutralize the exact virus variants that caused the infection.

Because SIVmac251 is a swarm of many different virus variants, Yeh and colleagues used single genome amplification (SGA) to isolate the transmitted founder viruses that caused productive infection. They found that a single transmitted founder virus was responsible for infection in each animal, and then made an SIV pseudovirion with the Env protein from these transmitted founder viruses. They then tested if sera taken from the animals at different time points after infection could neutralize the pseudovirion with the Env protein from the matching (autologous) transmitted founder virus.

Yeh reported that the sera from the animals could only neutralize the autologous pseudovirions between five and eight months after infection. This suggests that SIVmac251 induced neutralizing antibody (NAb) responses later than in HIV-infected humans, which develop responses two to three months after infection, Yeh said. In addition, the animal serum contained lower NAb titers than what is observed in humans. Even though the NAb response had a low titer and developed late, Yeh still found that it exerted selective pressure on the virus, causing the virus to develop escape mutations. It took several months before the infected animals developed NAbs to these escape mutants after they appeared.

"Compared to HIV-1, SIVmac251 appears to be even more resistant to antibody neutralization in that the antibodies did not appear until later in the course of infection, and even when they were present they were present at very low titers compared to what has been observed with HIV-1," Yeh concluded. This suggests that SIVmac251 may not be an ideal model to test candidate vaccines for human clinical trials. "Our data suggest that SIVmac251 may not accurately estimate the ability of vaccine candidates to elicit neutralizing antibodies that can protect against infection in a nonhuman primate model," Yeh said.

Brandon Keele, a senior scientist at the National Cancer Institute and Science Applications International Corporation (SAIC) in Frederick, Maryland, used SGA of full length *env* genes to analyze the sequence diversity of SIVmac251 and SIVsmE660, another challenge stock that is a swarm of many different variants. He found that these two challenge stocks had an overall sequence diversity of about 1%-3%, which is similar to the approximately 1% diversity of HIV variants in a single individual who has been infected for months to years. The same analysis showed there is approximately 20% maximum diversity between SIVmac251 and SIVsmE660 *env* sequences, compared with at most a 15% difference of HIV subtype B taken from different infected individuals. This suggests that a 251/660 heterologous challenge in animals is a good approximation of intra-subtype variation of HIV, Keele said.

Keele also used SGA to analyze the inoculum that was used to infect rhesus macaques intrarectally with the SIVmac251 stock generated by Ron Desrosiers at the New England Primate Research Center. He found that the transmitted founder virus often reflected the most common variants present in the inoculum. However, in some animals, rare variants from the inoculum established infection in the host. Keele is currently cloning these rare variants to identify the biological characteristics that enable them to get preferentially transmitted.

> A lot of the things that have opened up doors in pathogenesis and vaccine [development] particularly come from NHP studies.
>
> *– Anthony Fauci*

Keele also compared the effects of different doses of virus used to infect the animals via different transmission routes. When SIVmac251 was transmitted rectally, the number of transmitted founder viruses was typically lower when the challenge stock was more diluted. However, this dose dependence was less obvious when animals were infected vaginally. With vaginal infection, Keele said, "you have these huge differences where ten viruses get through once [and] one virus gets through in the next animal at the exact same dilution." One possible reason for this variation might be different availability of local target cells due to anatomical differences in the vagina, Keele said, perhaps because female rhesus macaques may be in different stages of their vaginal menstrual cycle when they are infected with the challenge virus.

Theodora Hatziioannou, an assistant professor at the Aaron Diamond AIDS Research Center, reported progress in using a modified version of HIV to infect pigtail macaques. Directly infecting them with HIV is not possible because of host restriction factors including the APOBEC3 proteins. But Hatziioannou and colleagues are developing an HIV-1 derived challenge virus called simian tropic HIV-1 (stHIV-1) that can infect pigtail macaques. stHIV-1 differs from HIV-1 in that its *vif* gene comes from SIVmac239 or HIV-2. This *vif* gene encodes a Vif protein that can destroy the APOBEC3 proteins in pigtail macaques, enabling stHIV-1 to replicate (*Proc. Natl. Acad. Sci.* **106**, 4425, 2009; see *Looking for the Perfect Challenge*, *IAVI Report*, July-Aug. 2009).

However, while SIVmac239 can cause disease in pigtail macaques, stHIV-1 cannot. So Hatziioannou reasoned that the pigtail macaque host cells must have additional factors that inhibit stHIV-1 replication more than SIVmac239 replication. At the meeting, she reported that peripheral blood mononuclear cells (PBMCs) cultured from pigtail macaques only inhibited stHIV-1 replication more than SIVmac239 replication in the presence of interferon (IFN)-α. This suggests that IFN-α induces factors in the pigtail macaque PBMCs that keep stHIV-1 from replicating.

Hatziioannou found that one of these factors is tetherin, a host restriction factor that inhibits the release of HIV particles from infected host cells. Tetherin could indeed be one of the reasons why stHIV-1 does not make pigtail macaques sick. Although stHIV-1 contains a gene encoding human Vpu which can overcome human tetherin, it is inactive against pigtail macaque tetherin.

Therefore, one way to alter stHIV-1 so that it can cause disease in pigtail macaques is to enable it to overcome tetherin. Hatziioannou said this could be achieved by introducing the *nef* gene from SIVmac239 into stHIV-1, because SIVmac239, which does not have a *vpu* gene, uses *nef* instead to overcome tetherin in macaque host cells (*Cell Host Microbe* **6**, 54, 2009). Another possible approach is to replace the stHIV-1 *vpu* gene with *vpu* genes from certain NHPs that can also overcome macaque tetherin, such as SIVgsn, the natural host of which are greater spot-nosed monkeys (*Cell Host Microbe* **6**, 409, 2009). However, that still leaves another limitation of stHIV-1—its Env protein is primarily X4 tropic, and therefore infects different target cells than most HIV-1 currently in circulation. But Hatziioannou said she will deal with that later. "I don't want to change too many things [at once] in my virus," she said.

Jim Smith, a senior service fellow in the Division of HIV/AIDS Prevention at the Centers for Disease Control and Prevention in Atlanta, also reported on the development of a new challenge strain, called RT-Env SHIV, which contains the genes for HIV-1 reverse transcriptase (RT) as well as Env. To construct the strain, Smith and colleagues combined two SHIV strains that are known to replicate well in macaques: RT-SHIV, which contains the HIV-1 RT gene, and SHIV162P3, which encodes an R5-tropic clade B HIV-1 Env protein. "We took two things that had a very good track record of replicating in macaques and we put those two together," Smith said.

Chinese rhesus macaques infected with RT-Env SHIV showed reasonably high viral loads of about 600,000 particles/ml, which is close to the peak viral loads typically observed in pigtail macaques challenged with RT-SHIV, Smith said. The new RT-Env SHIV strain will make it possible to test topical vaginal or rectal microbicides that not only contain RT inhibitors or fusion/entry inhibitors separately, but also combinations of them. Testing such combinations is important, Smith said, because of the anticipated resistance to these drugs. "We are trying to stay ahead of the curve," he said. Next, Smith wants to start infecting pigtail macaques with RT-Env SHIV to be able to do challenge studies of topical microbicides in pigtail macaques because they are more similar to humans than rhesus macaques in that they have a monthly menstrual cycle. He has also given the strain to the National Institutes of Health (NIH), where it is being propagated in Indian rhesus macaques so that it can be used in these animals as well.

MHC: Heterozygous is better

One hypothesis as to why the adenovirus serotype-5 based vaccine candidate tested in the Phase IIb STEP trial did not have any protective effect is the poor breadth of the cellular immune responses it induced, said Shelby O'Connor, an associate scientist at the University of Wisconsin-Madison. That raises the question as to whether the breadth of the CD8+ T-cell response correlates with control of viral load, she said. She presented evidence from Mauritian cynomolgus macaques that indeed, the breadth of the cellular CD8+ T-lymphocyte response appears to play a key role in suppressing viremia.

She found that SIVmac239-infected Mauritian cynomolgus macaques that are heterozygous for major histocompatibility complex (MHC) class I genes had about an 80-times lower average chronic phase viral load than those that are homozygous. These genes encode MHC class I receptors used by infected, antigen-presenting cells to present peptides to CD8+ T cells, which are then activated and kill the infected cells. She also showed sequences of viral escape variants from the cellular immune response in these animals, which suggested that the CD8+ T-lymphocyte responses in the heterozygous animals were a composite of the CD8+ T-lymphocyte responses in each of the homozygous groups of animals. In addition, MHC heterozygous animals had about twice as many distinct MHC molecules than MHC homozygous animals, and therefore have the potential to present twice as many different sets of peptides to CD8+ T cells. This means that in heterozygous animals, about twice as many different CD8+ T-lymphocyte responses might be induced as in homozygous animals.

Mauritian cynomolgus macaques are ideal for such studies because their genetic background is much more homogeneous than that of rhesus macaques, O'Connor said. In 99% of Mauritian cynomolgus macaques, seven MHC class I haplotypes can explain the MHC genetic diversity, she said.

"This data provides provocative evidence for us that an MHC heterozygote advantage exists in Mauritian cynomolgus macaques infected with SIVmac239. The data supports our hypothesis that a CD8 T-lymphocyte response directed at a maximally diverse set of epitopes is advantageous."

Mary Carrington, a senior investigator at the National Cancer Institute and SAIC in Frederick, said that the situation is similar in humans. Genome-wide association studies show that the MHC is the single most important locus in the control of viral load. Using data from the international HIV controllers cohort, which is organized by Bruce Walker, director of the Ragon Institute, she reported that individuals who control viral load to be below 2,000 copies/ml of plasma show a greater trend to be heterozygous in their HLA loci than non-controllers. But she added that the effect was not very strong, in part because in humans these loci are very polymorphic. O'Connor's data from cynomolgus macaques are much more obvious, she said, because these animals are genetically less diverse at the MHC loci. "I think this is one case where the nonhuman primate data really is essential to determine whether these effects are actually real," Carrington said. "I think [the cynomolgus macaque] data actually puts the nail in the coffin in terms of a heterozygous effect in a way that the [human] HLA data just cannot do." These observations add to the previous observation that people who are homozygous in their HLA loci progress to AIDS faster than people who are heterozygous in their HLA loci (*Science* **283**, 1748, 1999).

Focus on NK cells

While much of the focus was on antibodies and cellular immune responses, the pillars of the adaptive immune system, some speakers also discussed the role of natural killer (NK) cells, which are considered a part of the innate immune system. However, Ulrich von Andrian, a professor of pathology at Harvard Medical School, presented data that suggest that they also carry features of the adaptive immune system.

Previously, von Andrian and colleagues had shown that in mice without T and B cells, NK cells are both required and sufficient for a contact hypersensitivity response in their skin to hapten stimuli they were exposed to for a second time (*Nat. Immunol.* 7, 507, 2006). This response had been thought to be only B- and T-cell dependent.

Von Andrian said that this NK cell-dependent contact hypersensitivity response is similar to T- and B-cell immune responses in that it is an antigen-specific memory response. He now showed that mice without B and T cells could develop a stimulus-specific memory response to hapten stimuli to their skin as long as four months after the initial stimulus. "An adaptive response in the absence of T and B cells was very much against text books," von Andrian said. He also said that the mice appear to be able to develop such an NK-dependent response even to novel stimuli they could not possibly have encountered before, such as HIV. While the mechanism of this adaptive memory response of NK cells

Continued on page 14

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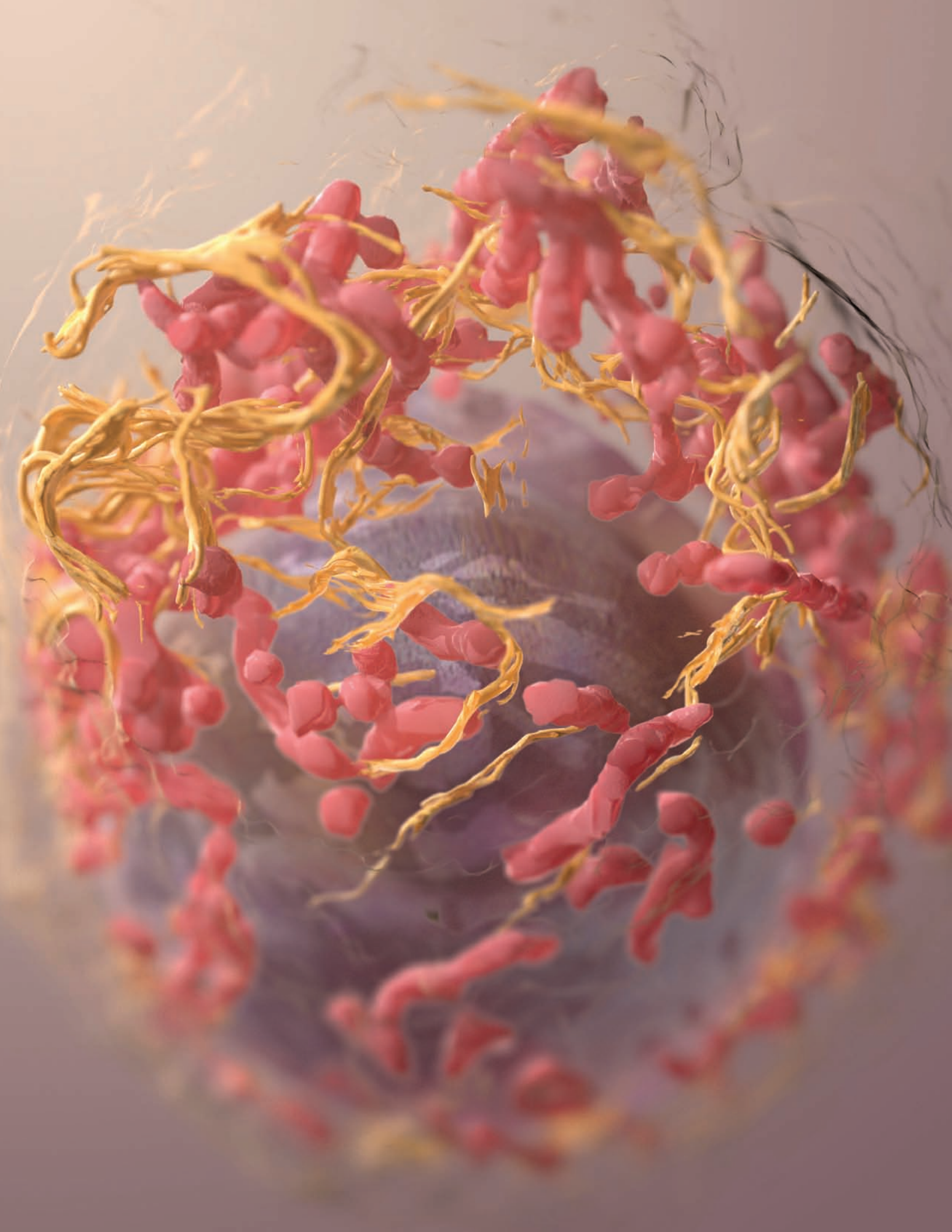
>
> *– Ulrich von Andrian*



THE Beauty BEHIND THE Beasts

Andreas von Bubnoff visits with **Sriram Subramaniam** and discusses his work studying the structure of HIV and visualizing HIV-infected cells

In 1987, Harden McConnell, a professor at Stanford University, was out of the country when Sriram Subramaniam, an Indian-born graduate student in his lab, wanted to tell him that there was no longer any need to send a recommendation letter to H. Gobind Khorana at the Massachusetts Institute of Technology (MIT), where he had applied for a postdoctoral position.

Subramaniam had just received a letter of rejection from Khorana. But McConnell could not be reached, and sent the letter to Khorana anyway. Once Khorana read it, he changed his mind and decided to invite Subramaniam for an interview. "[McConnell] must have said nice things [in his recommendation letter]," remembers Subramaniam, now chief of the biophysics section in the laboratory of cell biology at the National Cancer Institute (NCI) in Bethesda. "I went to the interview and then immediately he offered me the position."

The incident was one of many lucky breaks that he has had in his academic life, Subramaniam says. "[It] was a complete accident." It would also be the first of several times Subramaniam would switch fields.

In McConnell's lab, Subramaniam was trained as a physical chemist, studying lipid monolayers at an air water interface. But when his application for a position at Bell labs to continue this type of research had been unsuccessful, he decided to switch to biology. In part, that decision may have been influenced by McConnell, who had done the same. "He was a physicist's physicist for the things that he did," Subramaniam says, "but he also became interested in immunology." Subramaniam chose to apply to work in Khorana's lab because he realized that research in biological membranes was related to his previous work in lipid monolayers.

Once in Khorana's lab, he studied bacteriorhodopsin, a light-activated transmembrane protein that bacteria use to pump protons across the membrane to store energy. His non-biological background was quite unusual there, he remembers. "I had never done anything biological," Subramaniam says. "I was the only physical chemist when I joined [Khorana's] lab. I think he was very suspicious at first."

But he had experience in building instruments and that turned out to be quite useful. In Khorana's lab, Subramaniam spent two years building an instrument to measure the effect light of different wavelengths had on the ability of bacteriorhodopsin to pump protons.

Perhaps his broad background in physics and biology contributed to Subramaniam's next lucky break. In 1989, Richard Henderson, a structural biologist at the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK, sent the manuscript of a paper he was about to send out for publication to Khorana for comments. The paper used electron microscopy (EM) to reveal the first atomic-resolution structure of bacteriorhodopsin (*J. Mol. Biol.* **213**, 899, 1990). Subramaniam was the only person in Khorana's lab who responded with comments. "Only he was sufficiently broad minded and interested to reply with some con-

◀ Ion-abrasion scanning electron microscopy (IA-SEM) image of a human melanoma cell. Mitochondria, endoplasmic reticulum and nucleus are shown in red, yellow, and dark purple, respectively. Courtesy of Donald Bliss and Sriram Subramaniam, NIH.

structive comments on our manuscript," remembers Henderson. "It was clear he appreciated what we were doing."

About a year later, Henderson and Gebhard Schertler, another postdoc from his group, spent several days talking to Subramaniam at a Gordon conference and realized "with more certainty that Sriram was quite a capable person," Henderson remembers. So he tried to persuade Subramaniam to join his group in Cambridge for a second postdoc. Even though Subramaniam had already accepted a position as an assistant professor at Johns Hopkins University in Baltimore, Maryland, he decided to go to Cambridge for six months in 1992. "[Henderson] said he would teach me everything I needed to know about EM in two days," Subramaniam says. "[It] seemed like a good deal."

In Cambridge, Subramaniam and Henderson shock-froze bacteriorhodopsin in its "open state," while it was pumping a proton across the bacterial membrane. They then used the electron beam of an electron microscope to analyze two-dimensional crystals of the protein, in a method called electron crystallography. This resulted in the first study that described the structure of the "open state" (*EMBO J.* **12**, 1, 1993). "[It] was the first structural description of a proton pump caught in action," Subramaniam says. It was also Subramaniam's first experience with EM, which he is still using today—in a different way—to study the structure of the Envelope protein spike of HIV.

After six months in Cambridge, Subramaniam became an assistant professor at Johns Hopkins University after all, to study visual pigments in fruit flies. Again, the project involved something he had never done before: Growing fruit flies in the dark. Not being a biologist, he remembers, "I was very squeamish even to touch a fly, [let] alone growing it in the dark, [but] we figured it out." And again, his ability to tinker with and build instruments came in handy, this time to build an instrument that could measure the absorption of different wavelengths of light inside mixtures of ground up fly eyes. This work led to the elucidation of the mechanism that allows fruit flies to recycle their light pigments after they have been exposed to bright light (*Science* **266**, 1369, 1994).

Eventually, Subramaniam lost interest in this biochemical approach and decided he wanted to resume his structural work, so he called Henderson to ask if he could rejoin his lab.

Henderson said yes, and in 1997, Subramaniam again went to Cambridge, where he used EM to further refine the open structure of bacteriorhodopsin he and Henderson had published in 1993.

Again, Subramaniam and Henderson used electron crystallography to analyze two-dimensional crystals of the bacteriorhodopsin open state. But this time, they analyzed the crystals at varying angles, each time measuring the diffraction pattern from the electron beam. Since each time the electron beam destroyed the protein, the challenge was to use computers to merge the information of thousands of individual data sets, Subramaniam says. In other words, the approach averages, or merges, a large number of data that come from the way the electron beam in an electron microscope is scattered when it goes through a biological sample. If this is done with a large number of the same proteins, it becomes possible to deduce a common structure at a relatively high resolution. This work resulted in the first three-dimensional (3D) atomic model of bacteriorhodopsin captured in the process of proton pumping done completely by EM (*Nature* **406**, 653, 2000).

Later that year, Subramaniam moved to the NCI, his current home. Initially, he continued to use electron crystallography to study other crystallized membrane proteins. But later, he realized that it should be possible to use EM to also understand the structure of proteins that are too variable to form two-dimensional crystals, such as the HIV envelope spike.

This approach, called electron tomography, also analyzes the scattering of electron beams through proteins, but in this case, the proteins don't have to be crystallized. "We cannot crystallize HIV because each virus is different," Subramaniam says. "That's why tomography became the natural choice." The approach requires the merging and analysis of thousands of data sets from the same protein to get a high-resolution image of the protein.

Last year, this work resulted in the analysis of the structure of the HIV Envelope spike with electron tomography, at a higher resolution than previous studies (*Nature* **455**, 109, 2008; see Figure 1, this page). "[The paper] is generally considered to be the best paper on the HIV trimeric spike structure," Henderson says.

Next, Subramaniam plans to use electron tomography to analyze the structure of Envelope spikes of different HIV variants, such as ones that are more or less difficult to neutralize, or of

FIGURE 1

3D Structure of the Trimeric Glycoprotein Spike on Native HIV-1

Electron tomographic analysis of the native, undecorated Env trimer shows the general shape and arrangement of gp120 monomers in the native spike (*Nature* **455**, 109, 2008).

Courtesy of Sriram Subramaniam, US National Institutes of Health

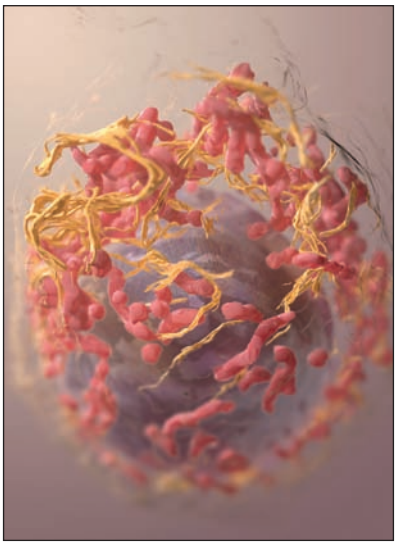
transmitted founder viruses that are responsible for establishing infection. He also wants to take a closer look at the way broadly neutralizing antibodies bind to the HIV Envelope spike.

Slice and view

Electron tomography isn't the only approach Subramaniam has been using to study biological structures. In 2003, another method that uses EM caught his attention. He realized that ion-abrasion scanning electron microscopy (IA-SEM) could be used to understand the structure of relatively thick biological samples, such as cells, at a high resolution. IA-SEM uses a beam of gallium ions to take off layers of the surface of a sample. That surface can then be analyzed by scanning EM.

Subramaniam says he first heard about such an instrument when visiting a microscopy manufacturer. At the time, the semiconductor industry used these instruments to cut silicon wafers to see if they contained the right circuits. But Subramaniam realized that the approach could be modified into a "slice and view" strategy to get three-dimensional images of cells, by taking SEM images each time the gallium beam had taken off additional layers of the sample. The images could then be recombined to give a 3D image. "When I learned about the existence of the focused ion beam, it was fairly obvious to me that it could be used iteratively to not just look at it once, [but to] keep looking at it multiple times," Subramaniam says. "It occurred to me that we could use it to look inside cells."

Subramaniam says the microscope manufacturer FEI had had the same idea, and so he collaborated with FEI to show that IA-SEM could be used to study biological samples such as yeast cells and tumor tissue (*J. Struct. Biol.* **155**, 63, 2006). Subramaniam hopes the approach will be able to



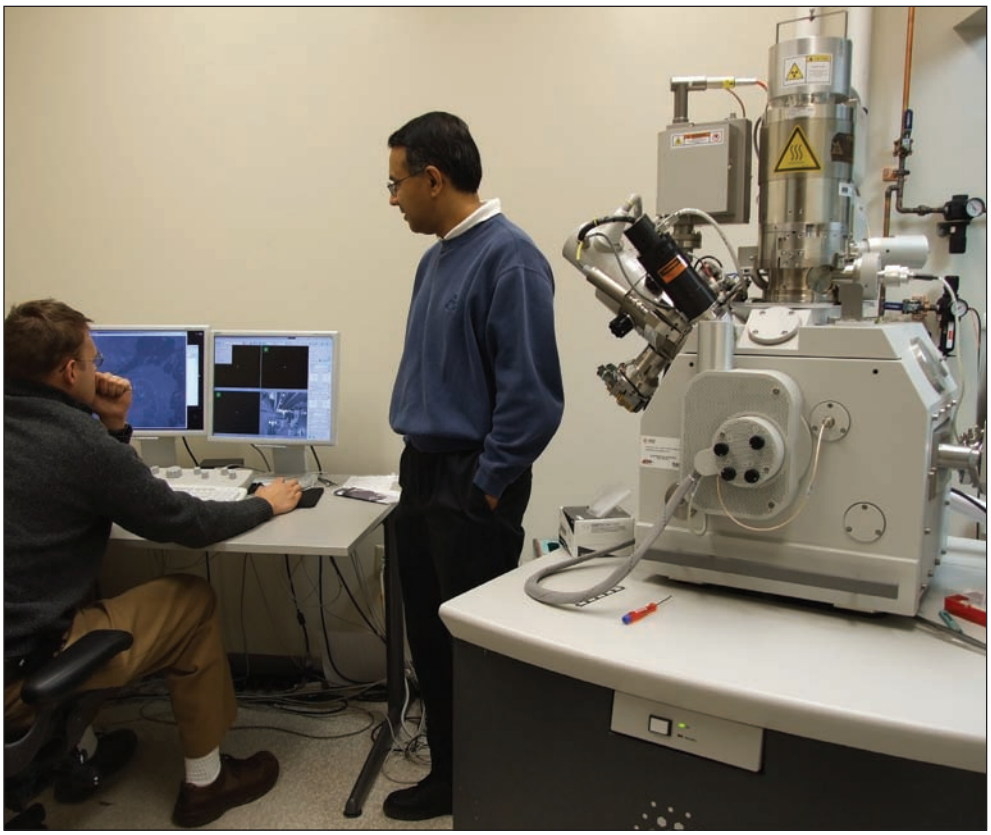


Photo by Andreas von Bubnoff

[VISUALIZING CELLS IN 3D]

Describing and modeling the hierarchical organization of molecules, molecular machines, and organelles within the cellular interior is a challenge of fundamental interest in cell biology. To bridge this gap in imaging, Subramaniam and colleagues have been developing ion-abrasion scanning electron microscopy (IA-SEM), a strategy for three-dimensional (3D) imaging of biological specimens that utilizes a focused ion beam to remove material from the surface, followed by a scanning electron beam to image the newly exposed surface. These steps can be iterated to "walk into" the cell at step sizes of ~20 nm, resulting in a stack of 2D-surface images that can be combined to visualize the cell in 3D. This is illustrated here with the segmented rendering of a human melanoma cell (mitochondria, endoplasmic reticulum, and nucleus are shown in red, yellow, and dark purple, respectively). Pictured on the right is Sriram Subramaniam (center) discussing IA-SEM measurements with Gavin Murphy, a postdoc in his lab. The ion-abrasion scanning electron microscope is on the right.

provide 3D images with a higher resolution than other approaches, bridging the gap between light and electron microscopy. In addition, the work results in beautiful images. In 2008, one image of a human melanoma cell won him an honorable mention in the International Science & Engineering Visualization Challenge, a competition of efforts to visualize scientific data sponsored by *Science* magazine and the US National Science Foundation (see page 8).

But IA-SEM can yield more than stunning images. Earlier this year, Subramaniam used IA-SEM to show HIV-filled compartments deep inside macrophages that are connected to the surface through channels that HIV particles appear to use to move to the surface (*PLoS Pathog.* **5**, e1000591, 2009; see cover image). He is also using IA-SEM to study virological synapses, the contacts HIV uses to move from infected to uninfected cells. This work, he hopes, will provide clues about how to prevent HIV from moving to a $CD4^+$ T cell and infecting it. "The driving force for the work is [to understand] how HIV [is] delivered to the synapse," Subramaniam says, adding that cell-bound HIV transfer through a virological synapse is over a thousand times more effective than transfer of cell-free virus. "We want to know why."

"I think that Subramaniam does great work," says Tom Hope, a professor of cell and molecular biology at Northwestern University, who also visualizes HIV. "The ion-abrasion work will bring new insights into the 3D structure of cells and how HIV takes advantage of these structures during viral replication."

Subramaniam's frequent moves between different fields and techniques make him quite unique, says Henderson. "He is very flexible and very willing to move into new areas and try new things," Henderson says. "He is fearless about using new techniques." But for now, Subramaniam plans to stay where he is. "There is a lot to be done," says Subramaniam, who heads a lab of about a dozen researchers, over half of whom are working on HIV. "I am just beginning to get into HIV. We have barely scratched the surface."

He works in an office where the door is never closed, right next to his wife Jacqueline Milne, whom he met at Johns Hopkins University. Milne, an associate scientist at NCI, is a cell biologist by training, but now also uses electron microscopic methods to study biological structures. The two don't have a TV at home and "talk about science as much as we can," she says. Recently, when Richard Henderson came from Cambridge to visit, they talked about science until the wee hours of the morning. Milne says she went to bed at 2:30 a.m., but her husband and Henderson "kept on cutting fruit cake and drinking coffee until 4:30 in the morning."

The fact that many conversations revolve around science even appears to affect the words they use in everyday situations. "I am diffusing around," Subramaniam said during a recent visit, referring to the fact that he can rarely be found at his desk. "He is sort of osmosing," Milne said.

And with two daughters, age two and 10, they are truly busy. "It's been busy since our younger daughter was born," Milne says. "We are still sort of trying to get highly efficient." One thing that allows Subramaniam to be more efficient is the fact that he can remotely control the electron microscope he uses to analyze HIV particles from anywhere in the world. "[When I] was in India to see my mother," he recalls, "I had this wireless card. I was doing my email and logging onto the microscope—from a taxi in India!" ■

Photo by Andreas von Bubnoff

Sriram Subramaniam and his wife, Jacqueline Milne, at their desks.

A Cut Above *The Rest*

A progress report on the promotion of adult male circumcision as an HIV prevention strategy

By Jonathan Grund

It has been two years since *TIME* magazine voted male circumcision for HIV prevention the top medical breakthrough of the year. This followed results from three clinical trials in Kenya, Uganda, and South Africa, which showed that circumcised men are approximately 60% less likely to acquire HIV from heterosexual sex (see *Cutting HIV Transmission*, *IAVI Report*, July-Aug. 2005). Since then, this intervention has slowly started to become an integral part of HIV prevention efforts in several African countries severely affected by HIV.

In Swaziland, a tiny African kingdom known for having the highest adult HIV prevalence in the world (26.2%), male circumcision services are being scaled up in an effort to help curb the country's epidemic. Currently, the country's circumcision rate is quite low, with only 8% of adult men having already undergone the surgical procedure. Population Services International (PSI), a nonprofit organization with an office in Swaziland, recently opened a new men's clinic called Litsemba Letfu, which means "our hope" in siSwati, the local Swazi language. This men's clinic is situated between Mbabane and Manzini, the commercial center of the country, and is designed to meet some of the country's demand for circumcision services.

Several studies based on statistical modeling indicate that sustained roll-out of male circumcision could have a substantial impact on the HIV epidemic in countries, like Swaziland, that have high HIV prevalence and low circumcision prevalence. The World Health Organization has identified 13 priority countries where male circumcision could have the most significant impact in averting new HIV infections (see Table 1, page 14). Mathematical models have shown that if Swaziland could circumcise 50% of males aged 15-49 by the end of 2020, one HIV infection could be averted for every four circumcisions performed.

But the plans for Swaziland are even more ambitious. "The goal is to circumcise 80% of men and adolescent young men in Swaziland in five years," or slightly more than 100,000 males, says Jessica Greene, technical services director of PSI-Swaziland, whose work is supported by the US President's Emergency Plan for AIDS Relief (PEPFAR) and the Bill & Melinda Gates Foundation. And based on several studies among adult Swazi men, it seems that the surgical procedure should be widely accepted. "There have been a number of surveys done in Swaziland indicating that the intention to circumcise is generally 60-80%," adds Greene.

Even more ambitious than Swaziland's circumcision campaign is one that recently was initiated in Kenya. The Kenyan government, with the support of PEPFAR and the Gates Foundation, has already implemented programs that have circumcised approximately 50,000 males to date. Starting in

TABLE 1

HIV and Male Circumcision Prevalence in Priority Countries*

Country	HIV Prevalence	MC Prevalence
Botswana	17.6%	11.2%
Kenya	7% nationally; 15.3% Nyanza Province	84% nationally; 40% Nyanza Province**
Lesotho	23.2%	48%
Malawi	12%	21%
Mozambique	12.5%	60%
Namibia	18%	21%
Rwanda	2.8%	15%
South Africa	18.1%	35%
Swaziland	26%	8%
Tanzania	5.7%	70%
Uganda	6.4%	25%
Zambia	14.3%	13.1%
Zimbabwe	15.6%	10%

*Data from: Progress in Male Circumcision (MC) Scale-up Country Implementation Update, July 2009; www.malecircumcision.org/documents/Country_Update_July09.pdf

**Ministry of Health. Policy on Male Circumcision in Kenya; September 2007.

November 2009, the country has also embarked on an aggressive campaign to circumcise 30,000 additional men in seven weeks in Nyanza Province. Nyanza has nearly half of the country's 1.2 million uncircumcised men, and its HIV prevalence is more than double the national average. With the support of the Prime Minister, this campaign is utilizing mobile clinics to provide circumcision services. At this time, there are approximately 1,200 men undergoing male circumcision daily.

Other countries, like Botswana, have also recently begun promoting male circumcision as an HIV prevention strategy. The country plans to conduct almost 500,000 male circumcisions in the next five years, which would raise the circumcision prevalence from its current level of 11% to 80%. Public health campaigns emphasizing the benefits of circumcision have utilized soccer themes to draw on some of the excitement surrounding next year's World Cup, which is the first to take place on African soil.

As countries begin promoting adult male circumcision more aggressively, they have also had to respond to the paucity of trained healthcare professionals capable of performing the surgical procedure. In Orange Farm, South Africa—the township outside of Johannesburg where one of the clinical trials of adult male circumcision for HIV prevention took place—healthcare providers are trying to increase their capacity to conduct adult male circumcision through highly coordinated sharing of clinical responsibilities among healthcare provider teams. This model "improves efficiency while taking advantage of the key skills of each provider to provide safe circumcisions," according to Catherine Hankins, chief scientific adviser at the Joint United Nations Programme on HIV/AIDS (UNAIDS). Other approaches to improve the efficiency of male circumcision have also been explored, including having nurses and other clinical officers perform the procedure.

Meanwhile, research on adult male circumcision is still ongoing. Researchers are currently exploring the potential HIV prevention benefits circumcision may afford in men who have sex with men, as well as innovative surgical techniques that improve the efficiency of the procedure, lower complication rates, and reduce post-operative healing time. Hankins describes the overall global progress as "slow but steady," though she says the progress is "never as fast as you'd want it to be." ■

Jonathan Grund is a contributing writer based in Atlanta, Georgia.

Continued from page 7

isn't known, this suggests that vaccinologists should keep an open mind as to the kind of immune responses a vaccine might induce, von Andrian said. "It's possible that current vaccines induce some level of NK cell memory," he said. "It's just not really been well explored so far."

NK cells are also involved in antibody dependent cytotoxicity (ADCC), which some researchers have suggested may be partly responsible for the modest efficacy of the vaccine regimen in the recently completed RV144 efficacy trial in Thailand. In ADCC, the Fc regions of antibodies bound to HIV-infected cells bind to Fc receptors of innate immune cells such as NK cells. This binding can activate the NK cell, which then kills the target cell with the antibody bound to it.

At the meeting, Michael Alpert, a graduate student in the lab of David Evans at Harvard Medical School, reported the development of a new rapid, standardized assay to measure the ability of antibodies from rhesus macaques to direct ADCC against SIV-infected cells. To avoid variability, the assay uses cell lines for both the target cells and effector NK cells. The target cell line is a $CD4^+$ T-cell line with a *luciferase* reporter gene that is expressed when the cell is infected by SIV. This Luciferase expression gets lost as soon as these SIV-infected target cells are killed by the effector cells, an NK cell line that expresses an Fc receptor.

The third component of the assay is the serum, which contains the antibodies that bind to SIV on the infected $CD4^+$ target cell line. This activates the NK cells once their Fc receptors bind to the Fc regions of the antibodies. Alpert said that the assay uses serial dilutions of serum that are added to wells that each contain the same number of effector and

target cells. "This will [then tell] you what percentage of Luciferase activity is still present in the various wells after an eight hour incubation in [the] presence of plasma and NK cells," Alpert said. Previous assays looked at just one dilution, whereas the new assay looks at a titration curve over several orders of magnitude, which leads to cleaner data, according to Alpert. In addition, previous ADCC or antibody dependent cellular virus inhibition (ADCVI) assays are more variable than the new assay because they rely on fresh cell preparations every time, he said. This could lead to differences in the killing efficiency of the NK cells, for example. "You can't set up the same assay twice," Alpert said. "Meanwhile, we have cell lines you can just take out of an incubator and use for the assay." He is also working on a version of the assay that could be used to measure ADCC in human samples, which might be useful for analyzing samples from the Thai trial.

Bacterial translocation

Bacterial translocation through the gut is a possible cause of the chronic immune activation that leads to the development of AIDS. However, so far the evidence that this translocation causes immune activation has been indirect, coming from correlative studies that show an association of increased bacterial lipopolysaccharides (LPS) in plasma and markers of immune activation. Now, Jacob Estes, a senior scientist at the National Cancer Institute and SAIC in Frederick, and colleagues have found more direct evidence for bacterial translocation in detailed analyses of tissue sections of the large bowel and lymph nodes in rhesus macaques acutely or chronically infected with SIVmac239, SIVmac251, or SIVsmE660. They also quantified the relative amount of LPS in the lamina propria, the layer underneath the gut epithelium, and the lymph nodes.

Estes showed that chronically infected animals with end-stage AIDS have a severe breakdown of the epithelial barriers in the gut. This resulted in a large amount of bacterial products such as LPS in the mucosal tissues of the gut and in the gut-draining and peripheral lymph nodes. Chronically infected non-end-stage animals had an intermediate to severe degree of gut epithelial damage and bacterial products in these sites, whereas uninfected animals had no epithelial damage, and bacterial products remained in the gut lumen. On the other hand, SIV-infected animals showed more signs of dividing cells in the gut epithelium than uninfected animals, indicating that infected animals were trying to regenerate the damaged epithelial barrier.

In contrast, chronically SIVsmm infected sooty mangabeys did not show evidence of bacterial translocation in the large bowel or peripheral lymph nodes, which is consistent with the fact that they don't have signs of chronic immune activation and don't get sick from SIV infection. "This data suggested to us that the damage to the epithelial lining really plays a key role in microbes [and microbial products] getting across into the host," Estes said.

He also showed that chronically SIV-infected animals had many bacterial products in the gut that were not associated with macrophages, even though many macrophages were present in the area, suggesting a possible defect in the ability of macrophages to clear translocated bacterial products.

In a related talk, Sieghart Sopper, a group leader at the German Primate Center, challenged the notion that microbial translocation is the major driving force of the immune activation that leads to AIDS. He said that so far there is no evidence for microbial translocation during acute viremia in humans, and that recent studies did not find a correlation between microbial translocation and disease progression.

Sopper found that in SIV-infected rhesus macaques, viral load correlated better with immune activation and disease progression than bacterial translocation did. He did a retrospective analysis of 37 Indian rhesus macaques, half of which were infected with SIVmac239, the other half with SIVmac251. When he analyzed a group of 18 rapid progressors (animals that developed AIDS earlier than 15 weeks after infection) and 19 slow progressors, he found that LPS levels did not differ between the two groups.

In addition, LPS levels correlated only weakly with viral load or survival, and only at one time point late after infection. In contrast, viral replication and immune activation correlated very well with disease progression and survival at all time points after infection. "In contrast to the model that viral replication leads to microbial seepage and activation of the immune system, we think that viral replication can directly activate the [innate] immune system which then leads to the production of cytokines or other mediators, which leads to the impairment of the mucosa and the subsequent seepage of microbial products," Sopper concluded. "We think that the interplay between immune activation and viral replication is more important than the effect of the microbial translocation for the development of AIDS." ■

We think that the interplay between immune activation and viral replication is more important than the effect of the microbial translocation for the development of AIDS.

– Sieghart Sopper

Vaccine BRIEFS

Microbicide Candidate Fails in Phase III Trial

The field of microbicide research was dealt another blow this month when a candidate known as PRO 2000, which had shown some promise in an earlier study, failed to have any effect in preventing HIV infection in a Phase III efficacy trial involving 9,385 women in the UK, Tanzania, South Africa, Zambia, and Uganda.

All women received condoms and regular HIV prevention counseling. At the conclusion of the MDP 301 trial—a randomized, double-blind, placebo-controlled study launched four years ago—130 HIV infections had occurred among women who received PRO 2000, compared to 123 infections among those who received a placebo gel. This analysis excluded HIV-infected women who became pregnant during the trial, as well as women whose HIV infection was detected a year after their first study visit.

Another analysis that included all HIV infections, regardless of pregnancy or time of infection, was equally disappointing: 145 HIV infections among women in the PRO 2000 group, compared to 143 in the placebo group. There was also no significant difference in efficacy between women who used the gel more consistently prior to sexual activity. A woman was determined to be a consistent gel user if she attended at least seven of the 13 scheduled visits over 12 months, reported using the gel prior to her most recent sex act at at least half of her site visits, and returned at least one used applicator at all visits when gel use was reported.

This should not be a time for despondency, we need to move on.

– Salim Abdool Karim

PRO 2000 is a water-based topical gel composed of 0.5% of a synthetic polyanionic polymer that was intended to bind nonspecifically to viruses and bacteria, thus preventing the pathogens from binding to and infecting cells. MDP 301 was originally designed to test two doses of the PRO 2000 gel, but investigators discontinued the higher 2% dose in February 2008, when the study's Independent Data Monitoring Committee concluded that the higher dose was unlikely to show any increased benefit in preventing acquisition of HIV compared to the 0.5% gel.

A year ago, researchers reported results from a Phase IIb test-of-concept trial involving 3,099 women in South Africa, Malawi, Zambia, Zimbabwe, and the US, which showed that women who received PRO 2000 gel along with condoms had 30% fewer HIV infections than those who received the placebo gel and condoms (see *Canvassing CROI*, *IAVI Report*, Jan.- Feb. 2009). The finding, though promising, was not statistically significant.

Salim Abdool Karim, a clinical infectious disease specialist who led the Phase IIb trial of PRO 2000 known as HPTN 035, said he was surprised by the MDP 301 findings and had no explanation for why the trials produced such different results. "It points to the complexity of human trials," says Karim. "Was HPTN 035 the result of a pure fluke, a pure chance finding for a product that has no benefit? I can't answer that."

PRO 2000 is the latest in a string of microbicide candidates that have been found ineffective in preventing HIV infection. Some candidates tested previously have even been associated with an increased susceptibility to HIV infection (see *Vaccine Briefs*, *IAVI Report*, March-April 2008).

The MDP 301 trial was conducted by the Microbicide Development Programme, a partnership of 16 African and European research institutions, and was primarily funded by the UK's Medical Research Council.

AIDS advocates responded to the results with disappointment, but said the field should continue to press forward in exploring microbicides that are gel formulations of existing antiretrovirals (ARVs). Topical forms of the ARV tenofovir, and a combination of tenofovir and emtricitabine known as Truvada, are currently being tested for efficacy in Southern Africa (see *PrEP Work*, *IAVI Report*, Nov.-Dec. 2008).

Sheena McCormack, principal investigator of MDP 301, said the results were disheartening, but she remains optimistic about the future. Karim says the future for microbicides is bright, singling out three areas of research. One is, of course, the development of ARV-based microbicides. But Karim also said there is research looking at how best to formulate the products, such as delivering the gels through a vaginal ring. Additionally, researchers are looking at new ways to address adherence. "The need for a microbicide is as great as ever," says Karim. "This should not be a time for despondency, we need to move on." —*Regina McEnery*

Update on Pandemic Shows New HIV Infections Steadily Declining

In its annual update on the status of the global epidemic, the Joint United Nations Programme on HIV/AIDS (UNAIDS) reported a 17% drop in the number of new HIV infections over the past eight years and suggested that the spread of HIV appears to have peaked in 1996, when 3.5 million new infections occurred (see Figure 2).

According to the report, which was released in November in advance of World AIDS Day, an estimated 2.7 million new HIV infections occurred in 2008. This brings the estimated number of people living with HIV to 33.4 million, slightly higher than in 2007 when 33 million were estimated to be living with the virus (see Figure 3). This is largely due to the life-prolonging effect of antiretrovirals (ARVs). UNAIDS estimates that there are now about four million people in low- and middle-income countries receiving ARVs—a 10-fold increase over the past five years. AIDS-related mortality peaked in 2004, when there were 2.2 million deaths. Last year, it is estimated that there were two million AIDS-related deaths.

The 2008 data reflect advances in software that have enabled epidemiologists to more reliably estimate HIV incidence using updated mathematical models. The more accurate accounting is expected to help countries more precisely define the scope of the epidemic in high-risk regions and populations. A dozen countries have used a model to analyze HIV incidence by the mode of transmission. This enables epidemiologists to predict where new infections are likely to occur, both generally and within pre-identified subgroups. This approach enabled Uganda to identify an estimated number of new infections that may occur among heterosexual couples considered at low risk of HIV infection.

The latest data also found dramatic variations in HIV prevalence within countries, a sign that prevention strategies need to be tailored to local needs and that national responses to the AIDS epidemic should be decentralized, according to UNAIDS. "The common failure to prioritize focused HIV prevention programs for key populations is especially apparent," according to the report. "Even though injecting drug users, men who have sex with men, sex workers, prisoners, and mobile workers are at higher risk of HIV infection, the level of resources directed toward focused prevention programs for these groups is typically quite low, even in concentrated epidemics."

The common failure to prioritize focused HIV prevention programs for key populations is especially apparent.

– UNAIDS Annual Report

For instance, although serodiscordant couples account for a substantial percentage of new infections in some African countries, HIV testing and counseling programs are seldom geared specifically toward this risk group, the report said. Similarly, many programs that have targeted adolescents fail to grasp some of the key determinants of their vulnerability to HIV, such as the high prevalence of intergenerational partnerships in many countries.

Timed to coincide with the release of its annual report, UNAIDS also rolled out a glossy, more reader-friendly magazine-style publication of its *UNAIDS Outlook*. A link to both the UNAIDS annual report and its new publication can be found at www.unaids.org.—*Regina McEnery*

Figure 2: Number of People Newly Infected with HIV

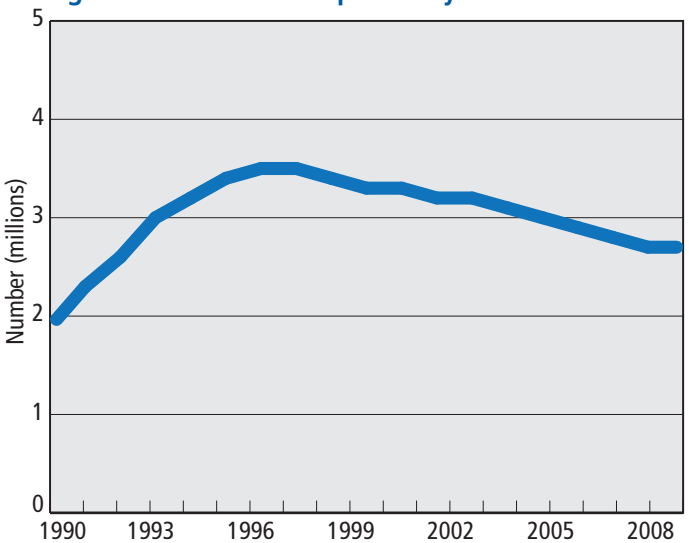
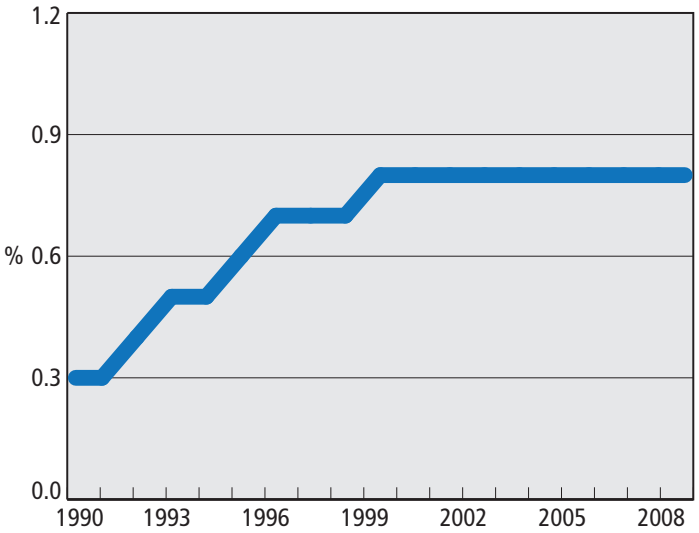


Figure 3: Adult (15-49) HIV Prevalence (%)



Progress and Promise

On World AIDS Day, scientists marked a promising year on both research and policy fronts

As it does every year, the global AIDS community marked World AIDS Day, December 1, with programs intended to raise awareness about the pandemic. Countries entered the year stuck in a global recession that has cast a shadow on funding for AIDS programs. But despite the economic uncertainty, 2009 turned out to be a promising year on both the research and policy fronts, with many of these developments highlighted, if not announced, on or around World AIDS Day.

The biggest burst of news in HIV prevention this year emerged from the AIDS vaccine field in September. RV144, a 16,000-person trial conducted in Thailand, provided the first evidence of vaccine-induced protection against HIV. This finding, along with other scientific developments, helped energize the field. "This has been a banner year in the AIDS vaccine effort," said Wayne Koff, senior vice president of research and development at IAVI, during a World AIDS Day seminar in New York City about recent progress and future directions in AIDS vaccine research and development. The event was co-sponsored by IAVI, the AIDS Vaccine Advocacy Coalition (AVAC), and the Global HIV Vaccine Enterprise.

Magda Sobieszczyk, a Columbia University AIDS researcher who spoke at the seminar about recently completed and ongoing HIV prevention trials, said RV144 "piqued people's interest" and mobilized the field. The results of RV144 also took center stage at a World AIDS Day event in Washington, D.C., sponsored by more than a dozen organizations, including AVAC, IAVI, and the Vaccine Research Center at the US National Institute of Allergy and Infectious Diseases.

Other AIDS vaccine-related events included a two-day rally, seminar, and workshop at the Global Science Academy in Basti, India, and a presentation in Maryland by the Walter Reed Army Institute of Research and the US Military HIV Research Program, major collaborators on the RV144 trial. Elsewhere around the world, organizations pledged solidarity to the search for an AIDS vaccine by holding rallies, debates, lectures, sports events, and plays. The Desmond Tutu HIV Research Center in South Africa sponsored a soccer tournament for young people and used the event as a way to spread information about the importance of HIV testing and counseling and to encourage adolescents to inform their peers about how to reduce the spread of HIV. An event in Amsterdam sponsored by IAVI, AIDS Fonds, and Stop AIDS Now!, centered on new prevention technologies, while vaccine trial sites in the Dominican Republic held a video forum on vaccine research and sponsored a frank discussion about the commercial sex trade in Santo Domingo.

World AIDS Day also provided a stage for the announcement of several policy shifts. In South Africa, the epicenter of the AIDS pandemic, President Jacob Zuma announced that antiretrovirals (ARVs) would be made available to all HIV-infected pregnant women and infants, that HIV testing would be expanded, and that he was planning to get tested for HIV. Treatment will also be expanded to those with tuberculosis, the leading cause of death among South Africans infected with HIV.

Reflecting a change in treatment guidelines unveiled by the World Health Organization (WHO) the day before, Zuma said his country would also offer treatment sooner to all HIV-infected individuals. The WHO's previous recommendations called for treatment to be initiated when a person develops AIDS (as defined by having fewer than 200 $CD4^+$ T cells in a microliter of blood) or an AIDS-related illness. But on November 30, the WHO announced that it was raising the minimum threshold for initiation of treatment to 350 $CD4^+$ T cells. The WHO's updated guidelines are now in line with those of leading government health agencies in the US and Europe.

The new WHO guidelines also recommend the prolonged use of ARVs to reduce the risk of mother-to-child transmission of HIV. For the first time, the WHO recommends that HIV-infected mothers or their infants take ARVs while breastfeeding to prevent HIV transmission.

Zuma's policies stand in sharp contrast to those of his predecessor, Thabo Mbeki, whose administration was heavily criticized for its HIV/AIDS policies. Glenda Gray, executive director of the Perinatal HIV Research Unit at the University of the Witwatersrand in Soweto, South Africa, described the government's commitment to expanding access to treatment as "incredibly ambitious and incredibly right."

Gray says there are 1.4 million people in South Africa who need to be on ARVs. "So we basically need to double the amount of people on treatment by 2011," she says. "How to get there will be another challenge."

This will be a global challenge. There are an estimated four million HIV-infected individuals worldwide who are currently receiving ARVs. However, approximately five million HIV-infected individuals who were eligible for treatment based on the old WHO guidelines still do not have access to therapy. With the updated guidelines in place, the number of people eligible for therapy could potentially double, substantially increasing the demand for ARVs.

After announcing that it planned to lift a controversial policy that prevented HIV-infected individuals from entering the US beginning next year, the Obama administration announced on World AIDS Day that Washington, D.C., the nation's capital, would host the XIX International AIDS Conference in 2012. The International AIDS Society (IAS), which sponsors the biannual conference, had opposed the travel ban, which was instituted in 1987, and made it clear it would not hold the conference in the US until the ban was lifted.

"Everybody recognized that the US travel ban had no scientific merit and no public health merit," says IAS President Julio Montaner, noting that 14 other countries still have similar travel bans in place. "It was based on ignorance and discrimination, and persisted on the books for historical reasons. It was a serious infringement on the rights of people with HIV."

Montaner says the fact that the US capital—which has the

highest HIV/AIDS prevalence in the country—will be hosting the 2012 AIDS conference is significant. "We hope the conference will serve as a catalytic event in trying to rally the necessary forces around addressing the epidemic, not just in the inner city of D.C., but elsewhere," says Montaner. The last time the AIDS conference was held in the US was in 1990 in San Francisco.

The US government also unveiled a new five-year strategy for the US President's Emergency Plan for AIDS Relief (PEPFAR) on World AIDS Day. Notably, this new strategy signals a transition for PEPFAR from an emergency response to HIV/AIDS to the promotion of sustainable programs in individual countries. Prevention, care, and treatment services provided through PEPFAR will still be expanded, but efforts will also be made to integrate HIV/AIDS initiatives into broader global health and development programs to maximize the impact on health systems in developing countries. PEPFAR will now focus on strengthening capacity in its target countries to enable them to take the lead on their responses to AIDS and other health demands and improve service delivery.

US Global AIDS Coordinator Eric Goosby said the current economic realities are forcing changes in the way the government is approaching the program. The Obama administration is seeking to make PEPFAR part of a US$63 billion Global Health Initiative that will also focus on other major public health challenges such as nutrition and maternal health (see *Despite Recession, New Funding Stimulates Research*, *IAVI Report*, May-June 2009).

PEPFAR-funded programs are at work in more than 30 countries. In 2009, the program provided antiretroviral drugs to more than 2.4 million HIV-infected people and plans are to provide treatment to four million people by 2014. But AIDS advocates fear that the global recession and a shift in political priorities in the US could hinder the success of PEPFAR. Michel Kazatchkine, executive director of the Global Fund to Fight AIDS, Tuberculosis and Malaria, stressed the importance of remaining committed to the goals of universal access to treatment when he spoke at a World AIDS Day discussion about food security, HIV/AIDS, and maternal and child health sponsored by the World Bank and held in Washington, D.C. —*Regina McEnery*

PrEP Trial Unable to Meet Efficacy Endpoints

A Phase III trial originally designed to test the safety and efficacy of Truvada—a combination of the antiretroviral (ARV) drugs tenofovir and emtricitabine—in reducing the risk of HIV infection among 1,200 HIV-uninfected heterosexual men and women in Botswana will not be able to determine the efficacy of this drug combination because the HIV incidence rate among volunteers was lower than anticipated. To meet the pre-specified efficacy endpoint for this trial, investigators would have had to double enrollment. However, the clinical research centers participating in the trial had also encountered unanticipated problems in retaining volunteers, so instead trial investigators have decided to modify the protocol and collect only safety and behavioral data.

The study, known as TDF2, is among several large clinical trials investigating whether the delivery of ARVs prior to HIV exposure, an idea known as pre-exposure prophylaxis (PrEP), can prevent HIV transmission among individuals at risk of HIV infection. The trial began in 2005, testing tenofovir alone, but then switched to testing Truvada in early 2007. TDF2 is being conducted by BOTUSA, a partnership between the Botswana Ministry of Health and the US Centers for Disease Control and Prevention (CDC) in Atlanta. The amended trial protocol will be submitted for approval to the scientific and ethical review boards in Botswana and the US in January.

Lynn Paxton, team leader for the PrEP and Microbicides Team for the CDC's Division of HIV/AIDS Prevention, says initially the incidence data for men and women ages 18-29 in Botswana was estimated at around 10%. "We were conservative and halved that number but we subsequently found, over the course of the study, that the incidence was likely much lower than that." Paxton said researchers are still analyzing data collected from the trial and are unable to say what the observed HIV incidence rate was during the three-year study.

Paxton attributes the lower-than-anticipated HIV incidence to a number of factors, including government-sponsored education and prevention programs that target younger men and women. She says the availability of ARVs among HIV-infected people in Botswana may also play a role in driving down HIV incidence rates in the country.

The low retention rates in TDF2 were also due to many factors. Some enrollees moved out of the area or became pregnant, which made them ineligible to continue in the trial, while others found the time requirements for participation too great. Paxton said BOTUSA took steps to overcome these challenges, including expanding weekend clinic hours, increasing participant reimbursements, and strengthening participant education and retention procedures. While these improvements have made a difference, the trial organizers still weren't sure a valid efficacy endpoint could be determined.

Paxton says Botswana is known for having a very mobile population. "Also, our study population was young and working. That made it more difficult," she says.

The original TDF2 trial protocol called for volunteer participation for 12 months after the study was fully enrolled. The CDC says it may now be possible to shorten the follow-up time, while still securing the necessary data to address safety and adherence questions. Proposed plans are being discussed and finalized with the Botswana Ministry of Health, as well as with the trial's community advisory boards. —*Regina McEnery*

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