

November-December 2008

# IAVI Report

A large, stylized microscopic image of cells, likely immune cells, is the background of the cover. The cells are rendered in vibrant red and blue colors, with some showing internal structures and spiky protrusions. The overall effect is a high-contrast, scientific visualization of cellular activity.

The Publication on AIDS Vaccine Research

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**Will a pill a day  
keep HIV at bay?**

**Pulling back the  
covers on HIV  
transmission**

**Considerations  
for the future of  
mucosal vaccines**

***PLUS:***

**Alan Bernstein:  
Commander of the Enterprise**

## EDITOR'S LETTER

ONE OF THE MAJOR THEMES in the recently held US presidential election was change. President-elect Barack Obama declared, "Change is coming to America." Meanwhile, here at *IAVI Report*, we've been working on some changes of our own. And with this issue, we are very pleased to introduce readers to our newly designed publication. As you read through this issue you will find several new graphic and design elements meant to enhance the look of *IAVI Report*. We are especially excited to be printing full-page cover images, kindly provided by researchers to showcase their work. Over the coming months, more changes are on the way. Early next year we will launch an improved version of the [iavireport.org](http://iavireport.org) website with additional features not available in the print publication. And for the first time, *IAVI Report* will also be available as an e-newsletter, so if you prefer to receive the publication by email, please just let us know.

These changes are based largely on feedback we received from the *IAVI Report* reader survey, and I would again like to thank everyone who took the time to provide their opinions and ideas about how to improve this publication.

Our primary goal, as always, is to provide up-to-date, accurate, engaging, and comprehensive coverage of AIDS vaccine research and development. In the future you can expect even more emphasis on the latest research trends, as well as the themes emerging from scientific conferences and meetings. It has been an interesting year in AIDS vaccine research—change is not only on the march in politics—and as the field moves ahead, the *IAVI Report* team will continue to track shifting research priorities, chronicle advances, and bring these stories to our readers.



KRISTEN JILL KRESGE



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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](http://www.iavi.org).

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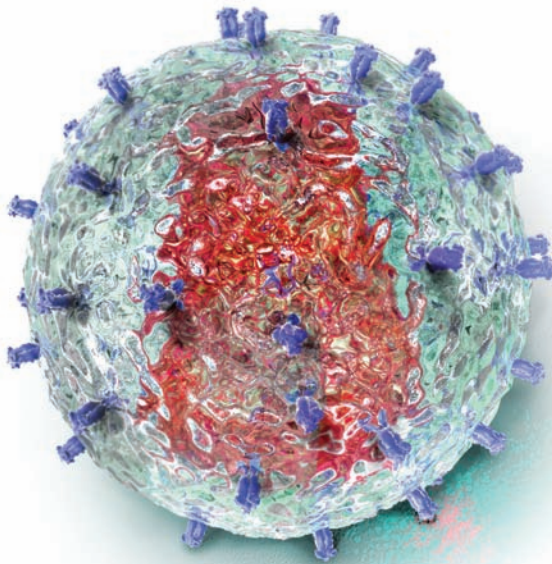
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# IAVIReport

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## [ ON THE COVER ]

The image on the front cover shows an infectious synapse where a human antigen-presenting dendritic cell (the larger cell) is passing HIV virions (green) to target CD4<sup>+</sup> T cells (the two smaller cells). The dendritic cell can also be seen extending projections, which are grabbing the CD4<sup>+</sup> T cells. Red: Actin; Blue: DNA.

*Courtesy of Dave McDonald and Tom Hope, Northwestern University.*

# *HIV Transmission:* THE GENETIC BOTTLENECK

Researchers are finally getting a handle on the nature of the virus that is transmitted and establishes infection, offering promising hints for AIDS vaccine development

By Simon Noble

**P**LENTY OF SCIENTIFIC QUANDARIES cause AIDS vaccine researchers restless nights, but one overriding challenge has always trumped them all and is a source of nightmares—the astonishing degree of genetic diversity that HIV presents. To put it in perspective, the genetic diversity of HIV within a single infected individual after six years of infection is roughly equivalent to the entire global diversity of influenza A virus in a year. With an estimated 33 million people around the world currently infected with HIV, that makes for a mind-boggling degree of genetic diversity.

Designing and developing an AIDS vaccine to tackle that degree of diversity can seem an overwhelming prospect. So any indication that the virus that is transmitted and establishes a new infection is less diverse would be welcome news. Evidence has been accumulating for the past decade suggesting that the dominant virus during primary infection is relatively homogeneous because of a genetic bottleneck during transmission, which effectively limits the degree of variation. And more recently, technical advances, the formation of large-scale consortia, plus a growing realization that the very earliest events of HIV infection are crucial, have given researchers a better look at the

enemy—the virus that a preventive AIDS vaccine would have to vanquish.

## Sampling advances

Although HIV was first identified over 25 years ago, researchers are just beginning to unravel the earliest events in HIV infection, the crucial window of opportunity that a vaccine needs to exploit. Within the first two weeks, HIV is disseminated throughout the body and rapidly devastates the reservoir of CD4<sup>+</sup> T cells, firmly establishing infection. A detailed molecular understanding of the transmission and the early evolution of HIV, including a precise description of the transmitted or early founder virus, would seem to be critical steps in the development of an effective AIDS vaccine. But one of the major reasons for the lack of insight until recently is pragmatic; HIV is, most often, a sexually transmitted infection, so for obvious reasons it's extremely difficult to identify and study the actual infectious event.

That's where improved sampling has paid off. Many efforts to identify newly infected individuals have come about through the various research programs that have been established in recent years. Susan Allen of Emory University and director of the Rwanda Zambia HIV Research Group

has established cohorts of discordant couples—in which one partner is HIV infected and the other is not—in Rwanda and Zambia with support from IAVI (see *Serodiscordant couples: Africa's largest HIV at-risk group*, IAVI Report, May-Aug. 2004). The development of these cohorts entails screening huge numbers of couples, identifying those that are HIV discordant, and then persuading those couples to come back on a regular basis to undergo sampling and counseling. The nature of the cohort allows researchers to identify newly infected individuals when they are viral antigen (p24) positive but antibody negative.

Discordant couple cohorts uniquely enable both the study of the virus that establishes infection and the virus population in the chronically infected partner, and in numbers of individuals that would be impossible in other cohorts. “Having the viral quasispecies that the transmitted virus originated from, you can ask was it a dominant or minor variant, was it enriched in the genital compartment, what’s the history of the transmitted virus?” says Cynthia Derdeyn, assistant professor of pathology and laboratory medicine at Emory University, who studies the samples from these cohorts. Traditionally, cohort members have returned to the clinic at three-month intervals, but recently they’ve been asked to return for monthly visits. This allows for more frequent sampling and also helps reinforce counseling messages about condom use among couples.

Other recent efforts by the Center for HIV/AIDS Vaccine Immunology (CHAVI) to identify newly HIV-infected individuals have required keen detective work to track down historical serial plasma specimens.

### The genetic bottleneck

When a study appeared in 2004 suggesting that the virus that establishes HIV infection goes through a severe genetic bottleneck, and might be more sensitive to antibody neutralization, it caused quite a stir (*Science* 303, 2019, 2004). Eric Hunter, a professor at Emory University, and colleagues, including Derdeyn, studied eight heterosexual transmission pairs from a discordant couple cohort in Zambia, four male-to-female (M-F) and four female-to-male (F-M) subtype C HIV transmissions. Viral *env* sequences, specifically the region spanning the V1-V4 loops, were studied from peripheral blood mononuclear cells (PBMCs) and plasma. They

found that an extreme bottleneck occurred in all the transmissions, which they interpreted as the transmission or outgrowth of a single sequence from the donor quasispecies. They also found that the transmitted virus tended to have shorter V1-V4 regions, which meant it had fewer glycosylation sites than the donor virus. A likely functional consequence of the fewer glycosylation sites is greater exposure of the CD4 binding domain, which often results in an augmented susceptibility to antibody neutralization. Indeed, the authors found that the recipient viruses were up to 10 times more sensitive to neutralization by antibody present in the plasma from the donor.

But much of the excitement that ensued was due to misinterpretation, because even though the founder viruses seemed to be more sensitive to antibody in the donor plasma, there was no statistical difference in their sensitivity to pooled plasma from subtype C infection. “It didn’t appear that the founder viruses were globally more sensitive to neutralization,” says Hunter. “We were at pains to convey that the neutralization sensitivity was not absolute, only in relation to the antibodies present in the donor plasma.” Rather, Hunter says the founder viruses “seemed as if they had lost some of the protection that had developed in the majority of circulating viruses in the chronically infected partner.”

**We were at pains to convey that the neutralization sensitivity was not absolute, only in relation to the antibodies present in the donor plasma.**

– Eric Hunter

This genetic bottleneck at the point of HIV transmission was recently confirmed by George Shaw, a professor in the department of medicine at the University of Alabama at Birmingham, and colleagues working within CHAVI when they defined the *env* genes of transmitted subtype B HIV from 102 plasma donors who became newly infected with HIV, a study that Hunter calls “a tour de force in terms of numbers.” Shaw’s group employed some technical and theoretical insights, using a combination of single genome amplification (SGA) and direct sequencing to analyze viral RNA in plasma samples—virus in plasma has an extremely short lifespan and so reflects very

recent viral replication. From historical serial samples they were able to work back and identify the sample from each individual plasma donor closest to the infectious event and, using a mathematical model of random viral evolution, infer unambiguously the transmitted founder *env* sequence in 98 of 102 individuals (*Proc. Natl. Acad. Sci.* 105, 7552, 2008).

In the majority of transmissions (76%) in this cohort, a single virus was responsible for productive clinical infection, with the remainder showing evidence of infection by between two and five viruses. But Shaw is careful to define exactly what he means when he talks about the transmitted virus. “Our inference of the transmitted founder viral sequences obtained near peak viremia is, I think, well accepted in the field right now. If we qualify it by saying that these are transmitted founder sequences that are leading to productive clinical infection, people agree with that,” he says. If, as Shaw’s work suggests, limited viral evolution precedes peak viremia, it suggests that a vaccine would only need to be effective against a small inoculum. “In the first two to six days of infection, the extent of viral diversity is quite low,” he says.

**It’s important to know that it’s not going to be a magic antibody that’s going to block all these viruses, they’re all different.**

– Cynthia Derdeyn

Shaw and colleagues also reported some biological phenotypes of the transmitted founder viruses that are important for vaccine design. Invariably, they were R5 tropic, meaning they used CCR5 as a coreceptor to gain entry into cells. “The virus is not using X4 [CXCR4 as a coreceptor] and then being selected for R5 tropism,” says Shaw, “it’s R5 tropic at the moment of transmission.”

Also, the phenotype of the envelopes is typical for primary virus strains—R5 tropic, CD4 dependent, and both the coreceptor-binding surface of gp120 and the V3 sequences are effectively concealed. “If a vaccine were to be based either on CD4-induced epitopes or V3 epitopes, our data would suggest it’s likely to be ineffective against the transmitted founder virus,” says Shaw. He and his colleagues also determined that

the susceptibility of the founder envelopes to broadly neutralizing antibodies was similar to that of primary virus strains. “It’s important to know that it’s not going to be a magic antibody that’s going to block all these viruses, they’re all different,” says Derdeyn. In future studies, Shaw wants to investigate additional biological, immunological, and antigenic properties of transmitted HIV to determine how these viruses behave, their tissue/cell tropism, and to identify a possible Achilles’ heel that could be exploited by vaccine researchers.

Hunter has also confirmed the genetic bottleneck at HIV transmission in additional studies in the discordant couple cohorts, extending it to another subtype of HIV. In a paper currently in press, his group has now looked at a total of 20 transmission pairs—11 subtype C and 9 subtype A infections—and in 90% of transmissions they see a single genetic *env* variant initiating infection. His group also used the SGA and direct sequencing methodologies since they wanted to determine the frequency with which the genetic variant in the newly infected individual was present within the donor and determine whether it was the most frequent or a rare variant in donor plasma or PBMCs. They frequently see an identical or very closely related variant present in the donor, but in almost every case that variant is a minor species within the donor quasispecies. “We see a very homogeneous virus population early on, and can track that back to what we believe is the founder virus,” says Hunter. “The added value of our study is that we can relate that back to the virus in the donor.”

These studies are still ongoing but so far Hunter and colleagues have not yet been able to answer a perennial question in HIV research—whether the initial infection was caused by cell-associated virus or cell-free virus. “I think that’s still an open question in the field,” he says.

### Compartmentalization

To get an even clearer picture of the actual transmission events, Hunter’s group is now characterizing the virus in the genital fluids rather than the peripheral blood of the donor. In a presentation at the recent AIDS Vaccine 2008 Conference in Cape Town, Debi Boeras, an affiliate scientist in Hunter’s group, presented data indicating similar evidence of the genetic bottleneck with viruses that are present in the genital com-

partment of the donor. They studied five F-M transmissions and three M-F transmissions, and while there is compartmentalization of the virus in the donor genital fluids, “the variant that is most closely related to the founder virus in the recipient is not in those enriched populations at the time we’re looking,” says Hunter. It is not yet clear whether this is a real finding or another consequence of the practical barriers hindering the study of HIV transmission. “We obviously can’t be there at the time that transmission actually occurs, and we’re now trying to determine just how much virus turnover there is in these genital populations,” says Hunter. “It may be that the virus that establishes infection in the genital mucosa and then becomes systemic may have to have specific properties that enrichment in the genital fluid doesn’t provide.”

## Multiple founders

Given the mounting evidence that suggests HIV passes through a genetic bottleneck during sexual transmission, the frequency with which two or more viruses establish infection actually occurs more often than would be expected—in 24% of cases in Shaw’s study. “That’s much higher than you’d expect by chance,” he says, “something else must be going on.” Hunter and colleagues found that in individuals who became HIV infected by somebody other than their spouse—termed epidemiologically unlinked transmissions—the frequency with which more than one virus established infection increased dramatically. They also found a statistically significant association between the presence of either a chronic ulcerative disease or an inflammatory genital infection and multiple genetic variants establishing infection in the recipient. “It seems that the genetic bottleneck can be modulated by infections or inflammatory reactions, either by compromising the mucosal barrier or providing an enriched population of target cells for new infection,” says Hunter.

## Vaccine implications

These findings of a single founder virus and limited viral evolution may have important implications for vaccine design. “The good news is that in the majority of cases, very early on a single defined virus is initiating infection,” says Hunter. “That suggests that the frequency with which a virus that has the capacity to breach the mucosa and become systemic is quite low. It gives us hope

that if you can contain the newly infecting virus for long enough, with neutralizing or even binding antibody, to allow the CTL response to be triggered, you may be able to confer some protection with a vaccine and stop virus from becoming systemic.”

Shaw believes his data corroborates what the clinical data shows—HIV transmission is an uncommon event, perhaps as low as 1:1000 sexual acts under some circumstances. So it fits that infection is probably going to be due to only one or two viruses. “A vaccine that has breadth and potency will probably be effective because these are uncommon events,” says Shaw.

But John Moore, a professor of microbiology and immunology at Weill Cornell Medical College, advises caution. He thinks the perception that “because there’s only one virus expanding in the new host, you only have to block the transmission of one virus” is dangerous. “That’s like saying that to stop pregnancy a contraceptive only needs to stop one sperm from among the millions present,” adds Moore. “The analogy is not exact, but I’m concerned that some people in the vaccine and microbicide fields might take home the wrong message and misunderstand what’s really going on.”

## Monkey transmission

Both Hunter and Shaw are now using the simian immunodeficiency virus (SIV)/rhesus macaque model to complement their clinical studies and gain further insight into acute infection. The general aim of these SIV studies is to elucidate the early replication events in the eclipse phase, the period between virus transmission and the broader dissemination of the virus, when a vaccine might have its best chance of containing or eliminating an infection. “If we can understand the kinetics and the events that occur in this eclipse phase and then look at the effect of candidate vaccines on that eclipse phase, it could be a powerful tool,” says Shaw. That applies equally to clinical studies of vaccine candidates; characterizing founder viruses that establish infection after breakthrough infec-

**That’s like saying that to stop a pregnancy a contraceptive only needs to stop one sperm from among the millions present.**

– John Moore

tion in vaccinees (or SIV challenge in macaques) allows researchers to study routes of immune escape. “If we vaccinate with an immunogen that raises responses to certain T-cell epitopes, we can look very, very early at the virus that leads to breakthrough infection in those vaccinees and determine whether or not there is strong early selection pressure at the relevant epitopes,” says Shaw.

The research groups of Norman Letvin, professor of medicine at Harvard Medical School, and Gary Nabel, director of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases, have developed a low-dose, atraumatic (non-abrasive) mucosal challenge macaque model that uses heterogeneous inoculums. Shaw’s group has studied such animals and finds that “the virus that is transmitted is literally the same virus, nucleotide for nucleotide throughout the entire *env* gene, to a virus that is present in the inoculum,” says Shaw. They have also seen the same phenomenon in a clinical transmission pair—in an acutely infected individual with very low virus diversity and in a second individual who contracted HIV infection from that person, the transmitted virus is identical to a virus identified in the donor—not a single nucleotide has changed between the moment of transmission and about three to four weeks later at peak viremia.

Hunter is working in collaboration with David Evans, a researcher at the New England Primate Center at Harvard University, to set up a similar multiple low-dose rectal challenge in macaques. So far he finds a similar genetic bottleneck to that seen in humans. In four of six macaques challenged with SIV, a single genetic variant from the pool of challenge viruses established infection; the other two animals’ infection arose from two viruses.

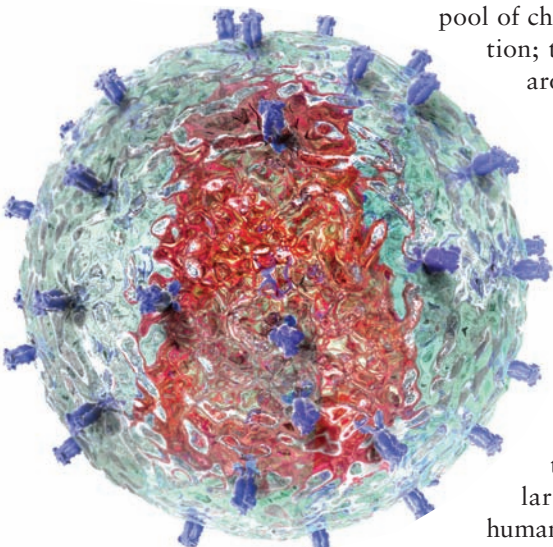
Moore thinks there is an important message to the field in these and other SIV studies of the transmitted virus, and hopes it will remove some of the prejudice against animal models, at least from the perspective of the viral dose. “It appears that it doesn’t really matter which non-human primate model is used, low- or high-dose challenge, rectal or vaginal, the data is very similar to naturally, mucosally infected humans,” he says. “That undermines the argument that animal models are misleading

due to the dose of virus being too high, or that the model is too stringent. The data coming out supports the contention that animal models are reasonable mimics of the human infection, at least from the perspective of the challenge dose.”

### Benefits of viral diversity?

Some studies indicate that the genetic diversity of HIV within an infected individual may actually be good news for vaccine researchers. In chronic infection, HIV is continually being selected by the host immune response, both neutralizing antibody and cytotoxic T-lymphocyte responses, and this immunological pressure forces the virus to mutate and generate escape mutants. The virus that gets transmitted, then, has been selected for survival in one immunogenetic environment. Hunter and his colleagues investigated how that viral quasispecies copes when it enters a new environment, how rapidly it escapes, and what happens to those escape mutations that were selected for in the chronically infected partner to see whether these escape mutations confer a fitness defect on the transmitted virus before it has chance to revert. Also, on a population basis, they looked at whether escape mutations in particular genes affect subsequent viral load.

In a study of 114 discordant couple transmission pairs, he and his colleagues found that multiple mutations in the *nef* gene didn’t seem to impair the transmitted virus. In contrast there was a progressive effect of escape mutations in *gag*, particularly in p24, such that when five or more escape mutations were present, the viral load in the newly infected partner was significantly lower (*J. Exp. Med.* 205, 1009, 2008). This suggests that the virus is placed at a disadvantage by having had to escape the immune response in the infecting partner. Those partners whose immune systems are most effective at targeting Gag may actually transfer viruses that are least fit, and this may have a positive long-term effect for the newly infected partner because peak viral loads and the attendant destruction of mucosal tissue might be reduced in those individuals. “It might also be telling us that if we can use a vaccine to target cellular immune responses to as many epitopes as possible in Gag,” says Hunter, “then the virus is really fighting an uphill battle because it’s trying to escape the immune response, but at the same time it’s decreasing its replicative capacity and committing harakiri.” ■





# PrEP Work

If effective, pre-exposure prophylaxis for HIV will offer many opportunities, but also numerous challenges

By Regina McEnergy

MORE THAN A DECADE AGO, highly active antiretroviral therapy (HAART) began rescuing HIV-infected individuals from the brink of death by aggressively suppressing viral replication. Yet this is only part of the critical role antiretrovirals (ARVs) have played in the battle against HIV. These drugs, alone or in combination, have also been effective soldiers in HIV prevention.

In 1994, a landmark study showed that administration of AZT to pregnant women and newborns could reduce the risk of mother-to-child HIV transmission from 25% to 8% (*N. Engl. J. Med.* 331, 1173, 1994). Since then routine and timely delivery of ARV therapy to pregnant women and their babies has nearly eliminated perinatal transmission in developed countries. While curbing mother-to-child HIV transmission in developing nations continues to be a major battle, use of antiviral prophylaxis in low- and middle-income countries has grown from just 9% in 2004 to 33% in 2007, according to the latest figures from the Joint United Nations Programme on HIV/AIDS (UNAIDS).

ARVs are also thought to possibly block infection following known exposure to HIV in adults, a concept called post-exposure prophylaxis (PEP). Current recommendations call for a short course, typically one month, of ARVs to be given to health care workers within 72 hours following exposure to HIV from contaminated blood, medical supplies, or equipment, as a way to prevent the establishment of an HIV infection. PEP is also sometimes administered following known sexual exposure to the virus. While clinical evidence linking PEP with reduced rates of HIV transmission is sparse, one

study found that a month-long course of AZT reduced the risk of HIV infection by approximately 81% (*N. Engl. J. Med.* 337, 1485, 1997).

Considering all this, it's not surprising that researchers are now investigating whether delivering ARVs prior to HIV exposure, an idea known as pre-exposure prophylaxis (PrEP), can also be turned into an effective prevention tool. The first evidence that this strategy might work came from nonhuman primate studies conducted in 1995. Now, after some delay, there is a sudden surge of attention and money directed toward studying PrEP. Several large clinical trials are testing whether the ARV drug tenofovir disoproxil fumarate (Viread), or a combination pill of two ARVs—tenofovir and emtricitabine—known as Truvada, will be effective at preventing HIV transmission in high-risk adults.

If these trials yield promising results, there could finally be another biomedical weapon added to the stockpile of existing HIV prevention strategies that despite years of research still largely revolve around condom use, sexual abstinence, and syringe exchange (*Lancet* 370, 89, 2007). Male circumcision, the latest biomedical intervention against HIV, was found to reduce HIV acquisition by as much as 65% in heterosexual men in randomized controlled clinical trials, but because of logistical, cultural, and religious considerations, only a handful of countries so far have adopted policies recommending this surgical procedure for HIV prevention. "The challenge is really at the local level and getting national governments to understand the potential of large-scale prevention," says Robert Bailey, an epidemiologist at the University of Illinois, who has been studying

circumcision for more than a decade. “That is what PrEP is going to go through. You have to do lots of consultations, get people engaged, and set up committees. You have to get the community behind it.”

To ensure that PrEP, if found effective, doesn't face a similar fate as circumcision, HIV prevention researchers and advocates are now starting to consider some of the weighty challenges, both logistical and medical, that will need to be overcome to successfully introduce this new prevention tool. Governments and public health agencies, like the World Health Organization (WHO), will have to tackle numerous questions should PrEP work, including identifying who should be the recipients, determining the best systems for distributing the drugs, and monitoring any long-term effects they may cause. Massive public education campaigns will also be required to explain PrEP and counter any behavior change that might occur as a result of its use. Countries will also need a mechanism to track the development of HIV resistance in individuals who become infected despite taking PrEP. Other considerations include the cost of

*Intern. Med.* 146, 591, 2007). Pioneering studies published more than a decade ago first documented that injections of tenofovir, then still an experimental ARV, prevented SIV infection when administered either two days before, four hours after, or 24 hours following SIV challenge, with treatment continuing for four weeks (*Science* 270, 1197, 1995).

In 2001, California-based pharmaceutical company Gilead Sciences obtained regulatory approval and licensure from the US Food and Drug Administration (FDA) for tenofovir. Truvada, also developed and licensed by Gilead, received FDA approval in 2004. Both are considered attractive ARVs for PrEP because they are potent, require only a single daily dose, and cause low rates of adverse effects. These criteria opened the door to PrEP research (*Lancet* 370, 89, 2007).

Since then, some of the most significant data has emerged from the US Centers for Disease Control and Prevention (CDC), where researchers have focused on testing PrEP in a low-dose mucosal challenge model against a hybrid SIV/HIV known as SHIV. Their earliest studies showed that tenofovir, given orally, delayed the establishment of SHIV infection after repeated exposure and even prevented infection altogether in one of four rhesus macaques. It took a median of six challenges to infect animals treated daily with tenofovir and seven for those treated weekly, compared to a median of 1.5 challenges for the control animals to become infected (*J. Infec. Dis.* 194, 904, 2006).

At the 13th Conference on Retroviruses and Opportunistic Infections in 2006, the CDC reported that daily subcutaneous dosing of emtricitabine and tenofovir for nine days—with a higher dose of tenofovir than is approved for treatment—fully protected all six rhesus macaques following repeat rectal exposure to SHIV. And in a study published this year comparing daily and intermittent doses of this same regimen, the CDC found that all treated animals were completely protected against repeat mucosal SHIV challenge over a 14-week period, whether emtricitabine/tenofovir was given daily or intermittently—two hours before and 24 hours after challenge (*PLoS Med.* 2, e28, 2008).

Findings published this year also found Truvada effective in preventing vaginal transmission of HIV in a humanized mouse model developed by J. Victor Garcia-Martinez, an immunologist at the University of Texas Southwestern Medical Center (see *Mighty mice*, *IAVI Report*, Sept.-Oct. 2008). None of the humanized mice that received Truvada became infected after receiving inoculations

You need to be intellectually prepared to deal with the different scenarios based on how the trials come out.

— Kenneth Mayer

this intervention and its impact on the design and conduct of future HIV prevention trials.

All of this could add considerably to the already staggering costs of HIV/AIDS prevention, treatment, and care in many of the hardest-hit countries. But if it works, PrEP will also bring unprecedented opportunities. Despite achievements in treating HIV/AIDS, last year alone 2.7 million people were newly infected with the virus.

“In the absence of data, it is way too premature to frame the response dramatically because we don't know how good PrEP will be or in what settings it will work,” says Kenneth Mayer, a professor of medicine at Brown University, who has surveyed PrEP awareness. “But you still need to be intellectually prepared to deal with the different scenarios based on how the trials come out.”

### Animal evidence

Studies in nonhuman primates already offer strong evidence that ARVs administered systemically prior to exposure to simian immunodeficiency virus (SIV) can prevent infection, although the success of the intervention seems to vary with the challenge model and the ARVs used (*Ann.*

of HIV, while 88% of the untreated control mice were infected (*PloS Med.* 5, e13, 2008).

Although Truvada has become the drug of choice for PrEP studies—five of seven clinical trials are using this two-drug combination pill—other ARVs might also be effective at preventing HIV infection. The Tulane National Primate Research Center in Louisiana found that oral delivery of an experimental CCR5 inhibitor known as CMPD167 in rhesus macaques on the day of SHIV challenge and for 10 days following provided considerable protection (*Nat. Med.* 11, 1293, 2005).

### Seeking human data

There are now seven planned or ongoing clinical trials of PrEP that will enroll upwards of 18,000 individuals. These trials involve men who have sex

with men (MSM) and injection drug users (IDUs) in Asia, the US, Latin America, and Africa, and heterosexual men and women from Africa (see Figure 1). The first round of data—a safety study being conducted in 400 HIV-uninfected MSM in the US—is expected to be released next year and results of the first efficacy trial involving 2,400 IDUs in Thailand will closely follow.

There is anecdotal evidence suggesting that some high-risk individuals may already be using PrEP as a way to stay HIV free, but although there have been isolated reports of MSM taking antiviral drugs in advance of unsafe sex, the notion that there is widespread use of PrEP in this population has so far not been born out by the data. A survey of 1,819 MSM in San Francisco found that PrEP use and awareness was rare (*J. Acquir. Immune Defic.*

FIGURE 1

## The Status of PrEP Research

As of August 2008, seven large-scale PrEP trials are either underway or in the planning stages in high-risk populations around the world. If completed successfully, these trials will provide information that public health officials can use in determining if PrEP is effective, and how it should be used. Information in this table comes from *Anticipating the Results of PrEP Trials*, published by the AIDS Vaccine Advocacy Coalition.

*BMGF—Bill & Melinda Gates Foundation; CDC—US Centers for Disease Control and Prevention; FHI—Family Health International; MTN—Microbicide Trials Network; NIH—US National Institutes of Health; USAID—United States Agency for International Development; TDF—tenofovir; TDF/FTC—Truvada*

Location	Sponsor/ Funder	Population (mode of exposure)	PrEP strategies being tested	Status/ Expected completion
United States	CDC	400 men who have sex with men (penile and rectal)	TDF	Fully enrolled – ongoing / 2009
Thailand	CDC	2,400 injection drug users (parenteral)	TDF	Enrolling / 2009
Botswana	CDC	1,200 heterosexual men and women (penile and vaginal)	TDF/FTC (switched from TDF in 2007)	Enrolling / 2010
Brazil, Ecuador, Peru, South Africa, Thailand, US (iPrEX Study)	NIH, BMGF	3,000 men who have sex with men (penile and rectal)	TDF/FTC	Enrolling / 2010
Kenya, Uganda (Partners PrEP Study)	BMGF	3,900 serodiscordant heterosexual couples (penile and vaginal)	TDF; TDF/FTC	Enrolling / 2012
Kenya, Malawi, South Africa, Tanzania (FEMPrEP)	FHI, USAID	3,900 high-risk women (vaginal)	TDF/FTC	Planning – anticipated start 2009 / 2012
Southern Africa – specific locations TBD (VOICE Study)	MTN, NIH	4,200 sexually active women (vaginal)	TDF; TDF/FTC; TDF gel	Planning – anticipated start 2009 / 2012

*Syndr.* 47, 241, 2008). “I wasn’t totally surprised that not that many people had heard of it,” says Albert Liu, director of HIV prevention and intervention studies for the San Francisco Department of Public Health, who conducted the survey and is also involved in an ongoing PrEP clinical trial. “It’s still a relatively new concept and the PrEP trials are still getting underway.”

Mayer conducted a survey on PrEP among 250 MSM in Boston and found that fewer than 20% had ever heard of it. “When we explained what PrEP was, we found a lot of enthusiasm,” he says, but people also raised some concerns, including potential side effects and cost.

### The challenges

The rapid escalation in the number and breadth of large-scale clinical trials that has occurred recently has generated excitement about PrEP—it was one of the hot topics at the XVII International AIDS Conference in Mexico City in August. This has motivated advocates, governments, and public health organizations to start considering the process of implementing PrEP should it prove safe and

**If a study shows it is highly effective then recommendations will be made on how best to use it and in what populations.**

— James Rooney

effective. Researchers most closely involved in the study of PrEP agree that it is productive to start conversations now, particularly with key stakeholders in the PrEP debate, including organizations such as the WHO that often set policies that developing countries adopt. But some researchers also stress caution on moving too quickly before the studies are completed.

“Countries hardest hit by the epidemic have a lot of other things going on,” says Lynn Paxton, the coordinator of PrEP studies with the CDC. “They don’t have much money and to ask them to start intensive preparation for something that might not have been shown to work yet is difficult.”

The AIDS Vaccine Advocacy Coalition (AVAC) has spearheaded many of the discussions about PrEP so far, even though its central mission has historically centered around AIDS vaccines. AVAC’s executive director Mitchell Warren says the group diversified its message for two reasons. “First, we are many years from vaccine efficacy,” says Warren.

“We will also begin to get answers about PrEP over the next two years and there has been pitifully little said about what we will do if it works.” Warren says response plans that are adequately funded and which correctly identify the high-risk uninfected individuals most likely to benefit from this intervention should be developed sooner rather than later.

### Adherence and access

As conversations about PrEP implementation get underway, researchers and advocates are just beginning to address the challenges associated with this potential HIV prevention measure. One key challenge will be identifying possible strategies for implementing PrEP, if effective. It is likely that PrEP programs will, at least initially, target high-risk individuals in communities in which the HIV infection rates are highest. But many stakeholders say it is premature to determine now who should receive PrEP or what the obligations of government and industries should be in providing it.

“If a study shows it is highly effective then recommendations will be made on how best to use it and in what populations,” says James Rooney, vice president of medical affairs for Gilead. “In conjunction, there will also be discussions on whether the current infrastructure would allow for PrEP to be provided or whether there needs to be further discussions on how the drugs could be made available.”

With the exception of AZT, which is used to prevent mother-to-child HIV transmission, there are no indications for ARVs around prevention, and even if PrEP performs well in clinical trials Gilead does not plan to seek regulatory approval to market tenofovir or Truvada as preventive drugs against HIV. “Gilead does not view prevention of HIV as a commercial opportunity that they would actively promote,” says Rooney. “We do view PrEP as a potentially important public health intervention and we would want to provide appropriate education to physicians about PrEP use and the fact that it is not a substitute for other well demonstrated means of preventing HIV infection.”

Another key obstacle will be adherence. There are studies from both developed and developing countries that have documented adherence to ARVs in HIV-infected individuals, but the data are variable. At the 15th Conference on Retroviruses and Opportunistic Infections in Boston earlier this year, researchers from the CDC estimated adherence to HAART among a cohort of 926 men and women from rural Uganda to be around 95% during three years of treatment.

There are clear-cut medical reasons for HIV-infected individuals to stick to treatment. Failure to do so could make them more susceptible to drug resistance and accelerate progression to AIDS. In contrast, motivating high-risk but uninfected individuals to take a daily dose of ARVs could prove difficult, much like convincing men and women to use a condom every time they have sex or IDUs to use clean needles every time they inject drugs. Though PrEP is hardly as intrusive to sexual spontaneity as stopping intercourse to apply a condom, some advocates see adherence as potentially the biggest barrier to PrEP effectiveness.

To avoid issues with daily adherence, some researchers are eyeing the possibility of testing intermittent PrEP use—such as before and after high-risk activity. “It will be important to understand if intermittent PrEP is feasible and effective,” says Timothy Mastro, senior director of research at Family Health International. “Taking the drug intermittently around the time one might be exposed is probably more feasible for many people in the world.” IAVI is considering utilizing excess clinical trial capacity to evaluate the feasibility of intermittent PrEP use. Such a study could also provide insight into immunological questions that may be important for AIDS vaccine research.

Another concern among researchers and advocates is that even though PrEP will unlikely be 100% effective at protecting against HIV, PrEP users may feel protected and therefore increase their risk behaviors, a phenomenon social scientists refer to as behavioral disinhibition. Ume Abbas, formerly an assistant professor in the Department of Infectious Diseases at the University of Pittsburgh School of Medicine and now with the infectious disease division at the Cleveland Clinic, used mathematical modeling to measure the potential impact of PrEP in sub-Saharan Africa, home to about 66% of the 33 million people living with HIV. The model determined that about 2.7 million to 3.2 million new HIV infections in southern Africa could be prevented over 10 years, factoring into the model a 90% effectiveness of the PrEP regimen targeting individuals at high risk for HIV with no behavioral disinhibition (*PLoS ONE* 2, e875, 2007).

Even with a 100% increase in high-risk behavior, a PrEP regimen carrying 90% efficacy predicted a reduction of HIV infections between 23% and 63%. It is only when the efficacy of PrEP hovers around 50% that the model shows that sexual disinhibition could actually increase the number

of new HIV infections. “We found that effectiveness of PrEP is key to determining the impact on HIV prevention. If PrEP has a high level of efficacy and adherence, it will be able to absorb a greater degree of sexual disinhibition,” says Abbas.

While the mathematical models are used as just that, models to predict “real life” outcomes, their forecasts can be instructive. In this case, they underscore the need for counseling and education. “If the public feels that they can take a pill and now have more sex, the effect of PrEP will go way down,” says John Mellors, a professor of medicine at the University of Pittsburgh who directs clinical research activities in HIV/AIDS.

Mastro agrees and says that communicating accurate information about PrEP will be essential to achieving its goal of reducing the number of new HIV infections. “Once you give someone a biomedical intervention, if they think they have been given a get-out-of-jail-free card they may think, why be safe?” says Mastro, who is also involved in a large clinical trial of PrEP in multiple African countries. “To me that is one of the major challenges of PrEP. They are only partially effective interventions. You will still need to be careful.”

### The threat of resistance

Another big concern is the possibility that people will become unknowingly HIV infected despite taking PrEP, either because it is partially effective or due to poor adherence, and continue to take the drugs. This could spur the development of drug-resistant strains of HIV and could compromise an individual’s treatment options over the long term.

For now, researchers can mostly only speculate about the likelihood that HIV drug resistance will develop when a person taking PrEP becomes HIV infected. The only clinical data available comes

**If the public feels that they can take a pill and now have more sex, the effect of PrEP will go way down.**

— John Mellors

from a randomized controlled PrEP trial with tenofovir conducted in Ghana, Nigeria, and Cameroon that was never fully completed. Two seroconversions occurred in individuals receiving tenofovir and standard genotypic analysis found no evidence of drug-resistant mutations (*PLoS Clin. Trials* 5, e27, 2007). Aside from that study, the only other evidence comes from a recent case report of a man

who had been taking Truvada intermittently to prevent HIV during high-risk sexual encounters with other men and became HIV infected (*J. Acquir. Immune Defic. Syndr.* 49, 117, 2008). In this case there was also no evidence of HIV resistance.

The development of HIV resistance, along with safety and efficacy, is being investigated in ongoing PrEP trials. Researchers conducting one trial involving 3,900 serodiscordant couples in Uganda and Kenya will look for evidence of drug-resistant HIV being transmitted or acquired among the seroconverters in the study.

Mellors said HIV drug resistance and its impact on ARV efficacy is the question he is asked about most often. But there are no clear-cut answers. “It is reasonable to assume that individuals will become infected on PrEP and will likely develop resistance unless PrEP is stopped,” he said during a talk at the

XVII International AIDS Conference. Mellors used a computer model to predict how drug resistance might evolve in these large-scale PrEP prevention trials and how severe the impact will be if it isn’t properly addressed. Mellors said if only a handful of people enrolled in the trial are unknowingly infected with HIV, the impact in the general population will likely be minimal. But if the number of HIV-infected people taking PrEP is large, according to Mellors, the potential benefits of PrEP could be nullified by the amount of drug-resistant virus circulating throughout the population.

Mellors says this shows routine testing will be required. “Any rollout program needs to identify who is infected and who is not and not make the mistake of giving PrEP to people who are already infected,” he says. While monitoring thousands of people in a three-year clinical trial is a manageable exercise, repeatedly testing PrEP users in the general population will be more challenging. And HIV resistance testing, which is used extensively in developed countries to optimize ARV treatment, is still an expensive luxury in many poor countries where second-line treatment regimens are often limited and unaffordable.

In addition to tracking HIV drug resistance, PrEP programs will also need to monitor individuals for adverse effects from the drugs. Though studies have found the drugs to be well tolerated, tenofovir and emtricitabine have been known to cause nausea and diarrhea, and tenofovir has also been associated with renal toxicity, says Rooney. There is also some evidence in animal models that tenofovir causes reduction in bone density, although long-term follow up in clinical trials found no increased incidence of fractures in HIV-infected individuals (*HIV Clin. Trials* 8, 164, 2007).

Another concern will be planning for and conducting other HIV prevention trials. If PrEP’s efficacy is established in more than one randomized controlled clinical trial and government policies endorse the strategy, organizations conducting AIDS vaccine trials would likely be asked to provide PrEP or refer volunteers to a clinic where it is available. Including enough volunteers in a trial to determine a vaccine’s benefit, in addition to PrEP and circumcision, would add significantly to the complexity and cost of conducting AIDS vaccine trials. There are also possible safety and biological complications that would need to be evaluated during clinical trials in which vaccine candidates were tested in combination with PrEP, according to Fran Priddy, director of medical affairs at IAVI. ■

## Weighing the Costs

Just as pricing is one of the biggest points of contention for HIV treatment, it is also one of the key questions surrounding possible implementation of PrEP. The cost of HIV treatment has grown astronomically since the advent of HAART. To meet the goal of universal access, UNAIDS estimates it will cost approximately US\$54 billion each year to provide ARVs to those in need in low- and middle-income countries by 2015.

The price tag for tenofovir or Truvada used for PrEP will be the same as for treatment, says James Rooney, vice president of medical affairs at Gilead. The company now charges developing countries about \$17 and \$26 a month respectively for tenofovir and Truvada. As treatment costs soar, it will be difficult for cash-strapped countries with the highest HIV/AIDS burdens to take on the additional cost of providing drugs for HIV prevention.

“I think many of these questions will be worked out once the trials are completed and if the trials demonstrate PrEP is safe and effective,” says Rooney. “There are a variety of discussions already surrounding regulatory and commercial issues, and of course reimbursement. Those discussions are in the preliminary stages at this point in time.”

The US government is now the biggest financial backer of AIDS treatment in developing countries—it will be funneling nearly \$50 billion in aid over the next five years through its US President’s Emergency Plan for AIDS Relief (PEPFAR) to expand existing HIV/AIDS prevention, treatment, and care efforts worldwide (see *Vaccine Briefs, IAVI Report*, July-August 2008).

In its report, “Anticipating the Results of PrEP Trials,” the AIDS Vaccine Advocacy Coalition (AVAC) noted that PEPFAR, the Global Fund, and other major funders of AIDS services should have plans in place to make PrEP available rapidly should it work. “Almost all prevention and treatment services in developing countries are dependent on development assistance—providing male and female condoms, male circumcisions, ARVs, voluntary counseling and testing etc.,” says Mitchell Warren, AVAC’s executive director.

In the developed world, where public and private insurance programs pay for the bulk of ARV treatment, cost could also be an issue. It remains to be seen whether public or private insurance programs will be willing to pay the \$6,000 to \$9,000 a year it now costs for tenofovir or Truvada in order to fend off HIV.

# *Mucosal Vaccines:* Insights from different fields

Researchers are joining forces to understand mucosal immunity and develop mucosal vaccines

By Andreas von Bubnoff

EVEN THOUGH HIV TRANSMISSION most often occurs at mucosal surfaces, there is still much that is unknown about the role of mucosal immunity in blocking the virus (see *The great barrier, IAVI Report*, March-April 2008). But a recent meeting on Modern Mucosal Vaccines, Adjuvants & Microbicides showed that research is now underway that may help fill this knowledge gap. “I was hoping that we were able to rejuvenate this whole area,” said Pearay Ogra, a professor of pediatrics at the State University of New York, who was one of the organizers of the meeting. He said the meeting succeeded in bringing together people from varied backgrounds and accordingly, the research presented covered diverse topics, including the application of antiretrovirals and antibodies as microbicides, live vaginal bacteria microbicides, using plants to manufacture proteins for vaccines, and utilizing microparticles to enhance the induction of immune responses. The researchers also discussed broader topics like which type of immune response is the most relevant to measure in mucosal tissues and novel routes of administering mucosal vaccines.

## Joining forces

Several speakers emphasized the importance of microbicide and vaccine researchers working together to develop approaches to prevent mucosal

acquisition of HIV. The potential for synergy became evident in a presentation by Martin Cranage, a professor and chair of molecular vaccinology at St. George’s, University of London, who described research where an intra-rectally applied gel containing 1% of the antiretroviral tenofovir protected six out of nine Indian rhesus macaques against intra-rectal simian immunodeficiency virus (SIV) challenge (*PLoS Med.* 5, e157, 2008). He also mentioned an ongoing project with Robin Shattock, also at St. George’s, in which the researchers are testing a gel-based version of gp140 protein that is applied vaginally in rabbits, nonhuman primates (NHPs), and humans.

In the tenofovir study, the macaques that remained uninfected and showed no systemic antibody response to SIV after being protected by the tenofovir gel nevertheless showed an SIV-specific T-cell response both locally in the gut, as well as systemically. This suggests that rectal exposure to the virus in the presence of tenofovir might have a similar effect to vaccination, Cranage said. “This is the potential bridge between microbicides and vaccines,” he said.

Laurel Lagenaur, a senior scientist at California-based Osel Inc. described the development of a live vaginal protein-based microbicide by introducing genes into *Lactobacilli*, bacteria that nor-

## [ ABOUT THE CONFERENCE ]

The Modern Mucosal Vaccines, Adjuvants & Microbicides meeting, which was held from October 22-24 in Porto, Portugal, attracted about 100 researchers from diverse backgrounds, including microbicide research, immunology, and vaccinology. The meeting was quite unique in that sense, said Nicholas Mantis, a research scientist at the New York State Department of Health.

[ IgA: NOT THE WHOLE STORY ]

One important question is which mucosal antibody responses are the most critical. Antibody measurements in mucosal tissues typically focus on secretory Immunoglobulin A (IgA), but several speakers at the meeting cautioned that a potential role for other antibody types in protection should not be neglected. Lou Bourgeois, a scientific officer at the Program for Appropriate Technology in Health (PATH), said that secretory IgA is not always essential for mucosal immunity—for example, most people with IgA deficiency do not have an increased susceptibility to infections. He said researchers should also look at the protective effect of IgG at mucosal surfaces. Jan Holmgren of the Vaccine Research Institute in Sweden said that in IgA-deficient people, IgG and especially IgM responses might compensate and that at some mucosal surfaces, such as the lungs and the vagina, systemic antibodies may actually leak through—or get transported—to the mucosal surface.

Another issue is how to correctly measure mucosal immune responses. Some researchers use absorbent sponges called Weck-Cel to obtain vaginal and rectal secretions to measure antibody concentrations. But Jiri Mestecky of the University of Alabama at Birmingham pointed out that such measurements might be misleading because the sponges could damage the mucosal epithelium and therefore take up systemic antibodies that leak into mucosal surfaces. In mucosal tissues like the female genital tract it could then be hard to distinguish circulating IgG from IgG that has been produced locally, he added.

mally live in the vaginal mucosa. Deficiency in these bacteria is associated with increased acquisition of sexually transmitted infections, including HIV. She showed that it is possible to colonize NHP vaginas with a transgenic *Lactobacillus* strain expressing the protein cyanovirin, which binds HIV surface glycoproteins. In addition, the protein was actually expressed by the bacteria in the macaques. Next, the company plans to conduct an *in vivo* challenge study in NHPs.

Larry Zeitlin, president of San Diego-based Mapp Biopharmaceutical, also emphasized a synergy between vaccinology and microbicides. He described the use of a combination of monoclonal antibodies to herpes simplex virus (HSV) and CCR5, a cellular chemokine receptor used by HIV to enter its target cells, for a vaginal microbicide called mapp66. He said that mapp66 can neutralize both HSV and HIV *in vitro*. The company is planning Phase I trials with this microbicide later this year or in early 2009. “As we are developing a microbicide like this and learn what the protective dose is,” Zeitlin said, “that would provide some guidance for vaccinologists in terms of what neutralizing titers we need to target in the vagina.”

**A serving of transgenic potatoes**

Zeitlin’s talk also illustrated another theme at the conference: the potential of using plants in vaccine production. To produce the monoclonal antibodies for the mapp66 microbicide, his company uses tobacco plants. Zeitlin said the antibodies can be made in accordance with good manufacturing practice (GMP) standards, much faster and cheaper than when using mammalian cell culture. GMP is a set of standards required by regulatory agencies like the US Food and Drug Administration (FDA) for products that are tested in humans (see *Cooking up candidates*, IAVI Report, Jan.-Feb. 2008).

Yasmin Thanavala, a professor of immunology and oncology at the Roswell Park Cancer Institute in New York, presented research in which people who had previously been vaccinated with hepatitis B vaccine were given transgenic raw potatoes expressing hepatitis B surface antigen. At least half showed increased hepatitis B antibody titer after eating two or three doses of the transgenic potatoes. This suggests that oral delivery of antigens in minimally processed plant materials can provoke immune responses, Thanavala said. It also is an advantageous way to deliver vaccine in developing countries, since it eliminates injection and the cold chain (*Proc. Natl. Acad. Sci.* 102, 3378, 2005).

“Apparently it works, [although] there are some limitations because these are raw potatoes,” said Jiri Mestecky, a professor of microbiology and medicine at the University of Alabama at Birmingham, adding that other researchers are working with other plants like tomatoes, for example, which might be more appealing for consumption.

**Considering tolerance**

One concern with delivering antigens in food is that the immune system may develop tolerance to them. Oral vaccines therefore need to contain additional “danger signals” that can alert the immune system. These warning signals often come from adjuvants, according to Jan Holmgren, director of the Vaccine Research Institute at the University of Gothenburg in Sweden. In developing countries, oral vaccines—especially live attenuated viral and bacterial vaccines—are often less effective than in developed countries, Holmgren said, adding that the reasons are not well understood. Possible explanations include nutritional deficiencies, competing microflora for live vaccines, and perhaps also that people there tend to be exposed to so many antigens that oral vaccination can be like “spitting in the sea,” said Holmgren.

Mestecky pointed out that for a novel antigen, the induction of tolerance depends on whether the first vaccination is mucosal or systemic. His group found that oral or nasal immunizations of humans with a novel antigen called keyhole limpet hemocyanin resulted in diminished T-cell responses following subsequent systemic immunizations with that same antigen. This only occurred when the first immunization was delivered mucosally, not when systemic preceded oral immunization. He said this could be a concern for HIV vaccines, in that vaccinating mucosally first with a novel antigen might induce tolerance that could dampen the cellular immune response to HIV.

**The route can make a difference**

There are many different routes to deliver mucosal vaccines. Typically vaccines are delivered directly to mucosal surfaces to induce a mucosal immune response. More traditional routes include oral or nasal administration, but novel routes like transdermal or sublingual administration also show some promise. Typically, the strongest response is at the vaccinated mucosa, with the next best at adjacent mucosae, although the nasal and perhaps sublingual routes can also stimulate a genital mucosal immune response.



Increasingly, researchers are finding that the choice of route can make a difference in the immune response. For example, Charani Ranasinghe, a research fellow at the Australian National University, and her colleagues showed that in mice, nasal priming followed by intramuscular or nasal boosting elicits a higher avidity CD8<sup>+</sup> cytotoxic T Lymphocyte (CTL) response than a systemic (intramuscular) prime-boost with pox vectors expressing HIV genes (*J. Immunol.* **178**, 2370, 2007). In addition, the researchers found that the high avidity of CTLs generated by the mucosal immunizations correlates with lower expression of interleukin-4 (IL-4) and IL-13 cytokines by CD8<sup>+</sup> CTL, with IL-13 being especially important, according to knockout studies in mice. This is the first study which demonstrates the importance of IL-13 for CTL avidity *in vitro* or *in vivo*, Ranasinghe said.

Susan Barnett, a senior director for viral vaccine research at Novartis Vaccines and Diagnostics, presented data from a Phase I trial that showed that intranasal priming using gp140 Env protein combined with systemic boosting can elicit HIV-specific Immunoglobulin A (IgA) and IgG in cervicovaginal secretions. In the trial, led by David Lewis of St. George's, University of London, women were primed several times intranasally with the Env protein with or without the adjuvant LTK63, a nontoxic mutant of *Escherichia coli* enterotoxin, and then boosted twice intramuscularly with the Env protein and a different adjuvant called MF59. "I don't think anyone has gone into women and elicited a vaginal IgA response with an envelope based vaccine," said Barnett, who was also part of a recent study that showed that intramuscular immunization alone or combined with intranasal immunization can protect rhesus macaques against a vaginal SIV/HIV hybrid virus, known as SHIV, challenge (*AIDS* **22**, 339, 2008). "We can elicit vaginal mucosal responses with intranasal priming, that's the take home [message]."

### Open up and say aaaaah...

Mucosal immune responses can also be induced by using a relatively novel route: sublingual immunization, in which liquid drops are applied under the tongue, according to Cecil Czerkinsky, deputy director of the International Vaccine Institute in Seoul. He showed that in mice sublingual immunization with ovalbumin plus cholera toxin adjuvant induced ovalbumin-specific mucosal antibody and CTL responses in the lung (*Vaccine* **25**, 8598, 2007) and in the female reproductive tract. Sublingual immunization with live or inactivated influenza

vaccine induced systemic and mucosal IgA and IgG antibodies and CTL responses and protected against lethal influenza challenge in mice (*Proc. Natl. Acad. Sci.* **105**, 1644, 2008). He said the sublingual area is good for immunizations because it is not keratinized, which makes it more permeable. It also contains dendritic cells that are similar to the skin's Langerhans cells, and in mice, sublingually administered antigen does not go into the olfactory bulb epithelium, suggesting it is not neurotoxic. He also showed preliminary results of a human study that suggests that sublingual administration of recombinant cholera toxin B subunit is safe.

### From potatoes to particles

Not only the route of administration, but also the formulation of a mucosal vaccine can make a difference in the immune response it induces. One novel formulation currently under investigation uses particles coated with antigenic proteins. Maarten van Roosmalen, a senior scientist at the Dutch company Mucosis, described a particle called GEM that is made by hot acid treatment of *Lactococcus lactis*, a type of bacteria used to produce some types of cheese. This removes the proteins, lipids, and DNA, and the particles are then used to carry antigens. The antigen-covered particles are much more efficiently taken up by antigen-presenting cells than antigen alone, and peptidoglycans in the particle stimulate the innate immune system, resulting in a more robust and stronger immune response, according to van Roosmalen. In mice, intranasal vaccination with GEM-based streptococcus and influenza vaccines induced IgA secretion in the nasopharynx, lung, and vagina, and protected from lethal challenge with these pathogens.

In general, particle-based vaccines may be more immunogenic than soluble antigens, said Ed Lavelle, a lecturer in the school of biochemistry and immunology at Trinity College. He used a mimetic of a lectin called UEA-1 that binds to intestinal microfold (M) cells in mice. M cells overlie Peyer's patches in the intestine, sample proteins and antigens from the luminal side of the gastrointestinal tract, and present them to underlying immune cells. Lavelle found that in mice, association of ovalbumin and UEA-1 to polystyrene microparticles enhanced cellular immune responses to intranasal and oral delivery, compared with delivering these two substances in soluble form. "Just the fact that you put an antigen on a bead is enough to stimulate a decent immune response," said Nicholas Mantis, a research scientist at the New York State Department of Health. ■

### Find out more:

Mucosal immunity and vaccines. J. Holmgren and C. Czerkinsky in *Nat. Med.* **11**, 545, 2005.

Perspectives on mucosal vaccines: is mucosal tolerance a barrier? J. Mestecky, M.W. Russell, and C.O. Elson in *J. Immunol.* **179**, 5633, 2007.

New generation of mucosal adjuvants for the induction of protective immunity. Y. Yuki and H. Kiyono in *Rev. Med. Virol.* **13**, 293, 2003.

Needle-free vaccine delivery. G. Kersten and H. Hirschberg in *Expert Opin. Drug Deliv.* **4**, 459, 2007.

# *An Interview with* ALAN BERNSTEIN

## At the Helm of the Global HIV Vaccine Enterprise

By Kristen Jill Kresge

**How did you make the decision to join the Enterprise as its first executive director?**

My decision to join the Enterprise was motivated by several factors. One was obviously the size of the problem. HIV/AIDS is the number one health challenge facing the world today and so it's hard to say no to the opportunity to participate. Secondly, the scientific challenges are so great that I was intrigued by the opportunity to contribute whatever I could, as an outsider to this field, to solving the scientific issues that are involved. Also, the uniqueness of the Enterprise model really interests me. I think the opportunity to be involved with an organization that represents a partnership between all the major funders in HIV research around the world, and to convene a conversation on their behalf that hopefully will articulate the fastest way forward to a vaccine, was intriguing, especially given my background at the Canadian Institutes of Health Research (CIHR). I had to deal with some similar issues at CIHR and so it also appealed to me to apply that experience at a new organization with a different, but overlapping, vision.

When I put all that together, and chatted with my wife, it became a no-brainer that I would say yes. Actually, after leaving CIHR, I would have been quite happy to sleep for a year.

**Not to mention that you joined the AIDS vaccine field at a rather interesting time—your acceptance was officially announced not long after the results of the STEP trial were released. What was that like?**

My appointment was announced about two weeks after the STEP trial results were released and it was indeed an interesting time. The scientific community reacted so negatively to those results; there was so much disappointment. It went way beyond what I would have anticipated and I realized that I was missing something. Everyone kept saying to me, “But Alan, you don't understand, the STEP trial failed,” and my reaction was yes, trials fail all the time. And I realized in a sense, that my reaction was the right one. I think the expectations in this field have been so high and the pressure to deliver a vaccine as soon as possible has been so great, that every scientist and every funder, whether they were directly involved or not, felt pain over the STEP trial.

I think that speaks to one of the great strengths of this field, which is that everybody wants a vaccine, whether they're the ones who develop it or not, because they understand the humanitarian cost of not having one. At the end of the day, that's what really matters and is what



## ALAN BERNSTEIN

Alan Bernstein, PhD, is a renowned researcher whose wide-ranging career has spanned the study of embryonic development, stem cells, hematopoiesis, and cancer. In each of these areas he's contributed extensively, authoring more than 200 peer-reviewed scientific publications. As the founding president of the Canadian Institutes of Health Research (CIHR), Bernstein helped develop CIHR into a leading research agency that supports more than 11,000 scientists with an annual budget of US\$1 billion. Prior to that, he was director of research at Mount Sinai Hospital.

It was during his postdoctoral work in London that Bernstein first began studying retroviruses, foreshadowing his future work with one of the most infamous retroviruses. In January 2008, Bernstein started the next phase of his career, taking up the helm at the Global HIV Vaccine Enterprise as its inaugural executive director. His appointment came just months after the results of the Phase IIb test-of-concept trial known as STEP showed that Merck's vaccine candidate failed to provide any protection against HIV. This set off a recalibration of research efforts in the field and Bernstein, as a newcomer, set out to bring his fresh perspective and expertise from other areas of research to bear on the development of an AIDS vaccine.

makes this field different. In areas that I know best, like cancer research, most trials don't work. When a cancer trial makes the front page of a newspaper, it is when it works, not when it doesn't. And it's not that cancer is not a serious problem, because of course it is, there's just an expectation that most trials won't work. That's what I was used to.

Joining the HIV vaccine effort was and is a fascinating learning curve for me. It's been a very interesting time for me to understand the science, the psychology in the field, what led to the STEP trial, and how the science should be framed going forward.

### What are some of the other differences between cancer research and the AIDS vaccine field that you've observed so far?

I think the image of the HIV vaccine field is that it is simply about product development as opposed to the need for doing great science, which is the case in cancer research. That's one reason why I think young people don't necessarily see a role for themselves in the AIDS vaccine field. I'm generalizing because there are obviously a lot of young people in the field, but there aren't the numbers that I'm used to in cancer research or in other areas. I think if we're going to be in this for the long haul, and it does look like it is going to be a long haul, we need to make sure we renew the current generation of very distinguished scientists, many of whom came into the field back in the mid-1980s when the virus was first discovered.

There's also been a whole slew of new technologies that have been developed due to advances in the field of genomics, which again, we need to make sure are fully incorporated into the search for developing an HIV vaccine, as they are in cancer research.

### The need to recruit a new generation of researchers has become a focus of the Enterprise, but what is being done to ensure it actually happens?

The Enterprise is putting together a group of young researchers from around the world, chaired by Dan Barouch of Beth Israel Deaconess Medical Center and Thumbi N'dungu of the University of KwaZulu-Natal, and asking them that very question. I think young people have the right perspective about what they need and what's missing for them in the field. There are definitely issues we've identified regarding

long-term funding and mentorship and I think our responsibility now is to continue to explore them and then put forward our findings in a way that will be useful to funders.

We're also losing many talented young researchers who are trained in the developed world and then go back to developing countries and don't have the resources there to continue their research, so we need to address that as well.

### Another recent mantra in the field is the need for innovation. You mentioned genomics—what are some of the areas of science that you think should be more actively investigated in the AIDS vaccine field?

Well, I think systems biology is a big one. We need to better understand a person's immune response to HIV. We have a virus that does very powerful things to the immune system and yet we haven't completely documented the immune responses when someone becomes infected. For example, there are some people who have high levels of virus in their bloodstream, while other people, like elite controllers, have very low levels of virus, and we don't yet understand why. We need to understand the cellular and molecular mechanisms behind those differences and to do that, we need to apply a systems biology approach to understanding the complexity of the human immune response to HIV. People are starting to look at this now, but it's just begging for some great science.

Traditionally, immunologists have measured their favorite protein or gene and then the next week when a new protein or gene is discovered, they start measuring that. That's what we used to do in cancer research. But what people are doing now is looking at everything, because they can. And the big advantage of that is it makes no prior assumptions as to what's important and what isn't, because the truth is we don't know what's important. As soon as you know you are going to measure interferon- $\gamma$ , which is one of the assays almost everybody uses, you're assuming that this is actually an important parameter, but there's no convincing evidence, as far as I know, that it is. So I think we need to make sure that we are applying the very latest technologies to look at the totality of the immune response to HIV.

The Enterprise hosted a meeting in October, Systems Biology and HIV Vaccine Development, that brought together card-carrying

systems biologists and HIV vaccine researchers to have a conversation about the opportunities for applying these newer concepts and technologies to developing an HIV vaccine. This meeting, which was chaired by Arnold Levine, is just one example of an area where the technologies have changed so much that we can now do things you couldn't even think about doing five or 10 years ago. And we've got to make sure that all of those new ideas, where relevant, are being applied to developing a vaccine.

### What are some of the key roles you see for the Enterprise over the following months?

I have an open mind about where we can best add value to what other organizations are already doing. One of our top priorities over the next year will be to update the existing scientific strategic plan that was created in 2005. This scientific plan is designed to provide a broad framework for the field and it should reflect the profound changes in science that have taken place over the past five years. The new strategic plan will identify opportunities in the field, such

as systems biology, as well as what some of the obstacles are, along with concrete suggestions about how to address them. Then we can renew the scientific plan annually or every two years and see how we are doing. I think that's one way we can add value.

There are currently four areas of focus for the Enterprise: attracting and retaining young and early career investigators, ensuring that systems biology becomes part of the armamentarium of HIV vaccine research, closing the gap between preclinical and clinical HIV research, and actively encouraging a culture of knowledge and data sharing. The Enterprise has also formed a Science Committee that will hold its first meeting in January. The committee is made up of 18 of the top HIV and biomedical researchers in the world. Their task will be to identify those areas of HIV vaccine research that require greater attention and resources and those that should be dropped. They will also begin developing the new scientific plan and help guide the Enterprise's scientific agenda. I am especially pleased that Rafi Ahmed, director of the Emory Vaccine Center, will chair the committee.

## An Enterprising Strategy

The Global HIV Vaccine Enterprise is an international alliance of researchers, funders, and advocates committed to accelerating the development of an HIV vaccine. The idea for the Enterprise was originally proposed in a 2003 *Science* article authored by 24 leading AIDS vaccine researchers, who argued that the scale of research at the time was insufficient for solving the major scientific challenges impeding the development of an AIDS vaccine (see *An enterprising solution takes one step forward*, *IAVI Report*, Dec.-March 2005). The approach of the Enterprise, modeled in part on the Human Genome Project, was to attract additional funding to support large-scale, collaborative efforts across multiple organizations and institutions. In 2005, the Enterprise published its Scientific Strategic Plan in *PLoS Medicine*, laying out a shared vision of the research priorities for the field.

Following this, the Enterprise quickly succeeded in mobilizing significant levels of new funding to the AIDS vaccine effort. In July 2005, the National Institute of Allergy and Infectious Diseases (NIAID) at the US National Institutes of Health (NIH) awarded US\$300 million over seven years to establish the Center for HIV/AIDS Vaccine Immunology (CHAVI), a consortium of investigators from various medical and research institutions. Then, in August 2006, the Bill & Melinda Gates Foundation awarded \$287 million to the Collaboration for AIDS Vaccine Discovery (CAVD), another large-scale initiative that unites major players in the field through support of 16 AIDS vaccine development centers. Both of these projects, which fall under the auspices of the Enterprise, encourage data and sample sharing across research teams that are tackling some of the most pressing issues in AIDS vaccine research and development.

### What do you think about the idea of HIV pre-exposure prophylaxis?

I think there's great excitement about it now and I hope that it is warranted. Two years ago, there was great excitement about microbicides and since then there have been a number of trials in which the candidates have not worked. That's to be expected but I'm just worried that we're all setting our expectations too high first on vaccines, then on microbicides, and now on pre-exposure prophylaxis. We need to view all of these approaches as tough challenges that may not yield something immediately.

We've become spoiled in the AIDS field because treatment has worked so spectacularly well. But it is important to remember that these drugs have side effects, they're expensive, and they don't cure anybody of the disease, so we haven't really solved the treatment problem until we solve prevention.

### Do you think more funding for AIDS vaccine research would help lead to a scientific breakthrough?

It is hard to say in any area of science whether you need more money or not. What we don't

know, and would never know, is if you had more money invested in research, would you speed up the development of a vaccine. I think there are still a lot of good ideas to pursue that aren't being funded at the moment.

Following the STEP trial, there has also been a lot of discussion about the balance between spending on clinical trials and basic research, and obviously Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases, has taken this into serious consideration. I absolutely think we need to be doing more basic research, but I also think we need to do more research to understand the human immune response to HIV and to HIV immunogens.

I also think that nonhuman primate models are very important for vaccine development. They are the best preclinical model we have and we have not been making enough use of them in trying to understand the immune response to SIV [simian immunodeficiency virus], which is very similar in its biology to HIV. There is a lot of great science that needs to get done, some of it involves humans, some involves non-human primates, and some involves mice.

### Are there any parallel lessons from cancer research or other fields that are relevant to AIDS vaccine research?

Yes, I think there are parallels and overall, I think we need more cross contamination

between HIV vaccine research and research going on in other areas. The Agence Nationale de Recherche sur le Sida (ANRS) is funding a trial now that is built on that idea. They're trying to target dendritic cells with a vaccine candidate, which is the basis for more modern types of cancer vaccines. There is an important difference however: Cancer vaccines are really treatments and we're talking about preventing HIV infection. But I think scientifically, there are a lot of parallels. Again, it highlights the point that we have a lot to learn from other fields and hopefully, vice versa.

### What is your overall impression of the AIDS vaccine field after your first year with the Enterprise and what thoughts do you have about what should be done differently?

I have been very impressed with the quality of the individuals working in the field as well as the different teams and networks. The challenge for me is how to add value given the talent that's already out there. I know I made the right decision to come into this field because of how warmly I have been received by everybody in the scientific community, as well as by the funders.

What I do think we need to do differently is to urgently move away from the expectation that the next trial will be a home run because I think there are a number of unfortunate side effects to that type of thinking. One is psychological; we all go into a depression afterward. We shouldn't be thrown off because one or two trials have failed or are not going ahead, that's just not the way science advances.

The other consequence of designing trials on the hope that they will be a home run is probably more serious in the long run, which is, you don't learn anything from a trial if you don't get protection, so you don't know how to make the vaccine better. We haven't been placing enough emphasis on what we're measuring. We need to start focusing on this so that, in an iterative way, we can design better vaccine candidates that will elicit a more rapid, potent, and broader specificity of immune response. Understanding the global architecture of the response to HIV is, to me, the best way forward toward a vaccine. ■

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— Alan Bernstein



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# Vaccine BRIEFS

## IAVI Opens AIDS Vaccine Laboratory in New York City

ON NOVEMBER 12, IAVI CELEBRATED the opening of its AIDS Vaccine Design and Development Laboratory, the first research facility in the world dedicated exclusively to the research and development of an AIDS vaccine. The 36,000-square-foot lab is housed in an historic building in New York City known as the Brooklyn Army Terminal (BAT), at which the city and state governments, along with private entities, are developing a state-of-the-art bioscience center. New York City Mayor Michael Bloomberg, who spoke at the opening of the Design Lab, said investing in bioscience is a way to diversify the city's economy in troubling economic times. IAVI, the first research group to occupy the bioscience center at the BAT, received US\$12 million from the New York City Economic Development Corporation to renovate the laboratory space. "The potential to change the world is right here in this building," said Bloomberg. "New York City is very glad to partner with IAVI in hastening the day to the development of a vaccine."

Scientists at the new Design Lab will be working with a broad network of researchers affiliated with IAVI's established research consortia, as well as partners in both academia and industry. "This past year has been really

tumultuous for the AIDS vaccine field," said Seth Berkley, founder and president of IAVI, referring to the unexpected failure of Merck's vaccine candidate in the STEP trial. There are many scientific challenges facing AIDS vaccine researchers and the Design Lab is meant "to focus on these challenges and solve them as quickly as possible," he said.

One of the key challenges is identifying immunogens that are capable of inducing broadly neutralizing antibodies against HIV, and this is one of the main areas of focus at the Design Lab. "We know in principle that there are antibodies out there that do what we want them to, the problem is how to induce them," said Dennis Burton, a professor of immunology and molecular biology at the Scripps Research Institute and head of the Neutralizing Antibody Center (see *Vaccine Briefs, IAVI Report*, Sept-Oct. 2008). Burton, who spoke both at the opening ceremony and a science symposium held earlier that afternoon, said that all vaccines that are in use today have an antibody component and that a protective AIDS vaccine candidate will have to induce both broadly neutralizing antibodies and potent T-cell responses. While inducing antibodies against HIV has proven much more difficult than for other viruses, HIV does have weaknesses, Burton said, and "we're confident that in the end we will defeat this virus."

Researchers at the Design Lab will also focus on developing T-cell vaccine candidates, based on replicating viral vectors, which are capable of controlling HIV infection as well as the live-attenuated simian immunodeficiency virus (SIV) vaccine can control SIV infection in nonhuman primates. There is some evidence emerging from recent studies that suggests T-cell responses can effectively control virus in vaccinated animals (see *Research Briefs*, this issue, and *AIDS vaccine researchers STEP up to the challenge, IAVI Report*, Sept.-Oct. 2008). "T cells might be able to do a lot better than we initially thought," said David Watkins, director of the department of pathology at the University of Wisconsin-Madison.

Berkley said the Design Lab is well positioned to test and develop these new T-cell or antibody candidates. "We've got an engine to move things through quickly if there's promise."  
—Kristen Jill Kresge



Photo by Olga Shmaldenko

▲ **A LAB GROWS IN BROOKLYN** New York City Mayor Michael Bloomberg spoke about the city's commitment to HIV prevention, education, and treatment, but said, in the end "you have to have a vaccine."

# Research BRIEFS

## Prime-Boost Regimen with Ad26 Offers Renewed Hope for T-cell Vaccines

RHESUS MACAQUES THAT RECEIVED a heterologous prime-boost vaccine regimen comprised of an adenovirus serotype 26 (Ad26) vector expressing simian immunodeficiency virus (SIV)mac239 Gag, followed by an adenovirus serotype 5 (Ad5) vector expressing the same Gag, showed reduced viral loads and remained healthy for 500 days following intravenous SIVmac251 challenge (*Nature* doi:10.1038/nature07469). This heterologous prime-boost regimen reduced peak viral load in vaccinated monkeys by 1.4 logs and setpoint viral load by 2.4 logs, compared to unvaccinated control animals, and is the first to show this level of control in such a stringent challenge model, says Dan Barouch, an associate professor of medicine at Harvard Medical School, who led the study.

The overall message of the paper is that we are not at the end of the road when it comes to T-cell vaccines.

— Dan Barouch

The challenge model used in this study was stringent in that it used a lethal dose of SIVmac251 in animals lacking the MHC class I alleles Mamu-A\*01 and B\*17, which are associated with more efficient control of viral load. Three of the 22 macaques in the study had a protective allele called B\*08, but when an analysis was done excluding these animals, the statistical significance of the results still held, Barouch says.

In contrast, a homologous Ad5/Ad5 prime-boost regimen also evaluated in this study did not have any effect on setpoint viral load or protect macaques from dying following challenge. In previous studies, a homologous prime-boost Ad5/Ad5 regimen and a heterologous DNA/Ad5 prime-boost regimen also failed to provide any level of

protection against a similar challenge, adds Barouch. “I am really delighted that they are able to improve on the protection studies of the past,” says Gary Nabel, director of the Vaccine Research Center at the US National Institute of Allergy and Infectious Diseases, adding that the control of viral load for 500 days is encouraging compared with previous studies. “In many of the other studies the control of viral load is lost after a certain period of time,” Nabel says.

In this study the magnitude and breadth of Gag-specific T-cell responses correlated with control of setpoint viral load, according to Barouch. Only gag was used as an insert, suggesting that primarily T-cell responses and not antibody responses were responsible for protection, he adds. “[This] shows very clearly that the Gag-specific T

cells were doing the job.”

T cell-based approaches were called into question after the results of the STEP trial,

which showed that Merck’s Ad5 candidate vaccine regimen failed to protect vaccinees from HIV infection, and that pre-existing immunity to this relatively common adenovirus serotype appeared to be associated with an increased risk among vaccinees of acquiring HIV. To avoid concerns about pre-existing immunity, Barouch chose to develop a vector based on Ad26, a much rarer serotype. He says this study suggests that there is still hope for T-cell vaccine candidates that control viral load after infection by stimulating T-cell responses. “The overall message of the paper is that we are not at the end of the road when it comes to T-cell vaccines.”

In addition, optimizing the breadth of Gag-specific T-cell responses may be a desirable feature for next generation vaccines.

This study “begins to define the correlates that we need in terms of magnitude and breadth of responses,” says Bruce Walker, director of the Partners AIDS Research Center at Massachusetts General Hospital.

Barouch says the Ad26/Ad5 regimen won’t be tested as a candidate vaccine in humans because it contains Ad5, to which many people may have pre-existing immunity. “We think it’s important to avoid Ad5 altogether,” he says. However, Barouch is currently testing an Ad26 vector containing an Env clade A insert in a Phase I safety trial. This vector is a prototype to test safety and immunogenicity of this approach, Barouch says. He is also currently evaluating a variety of heterologous prime-boost regimens that use two rare serotype Ad vectors, which will be tested in nonhuman primates and then possibly in clinical trials.

The study is not the only finding that suggests that T-cell vaccines are still promising. Recently, Nancy Wilson, an associate scientist in the lab of David Watkins at the University of Wisconsin-Madison, reported that a DNA/Ad5 regimen encoding all SIVmac239 genes except *env* can control viral load in macaques following five low-dose mucosal challenges with the heterologous swarm virus SIVsmE660 (see *AIDS vaccine researchers STEP up to the challenge*, *IAVI Report*, Sep.-Oct. 2008). — Andreas von Bubnoff

### [ ON THE IAVI REPORT CALENDAR ]

26TH ANNUAL SYMPOSIUM FOR NON-HUMAN PRIMATE MODELS FOR AIDS:  
December 9-12, 2008, San Juan, Puerto Rico

16TH CONFERENCE ON RETROVIRUSES AND OPPORTUNISTIC INFECTIONS:  
February 8-11, 2009, Montreal, Canada

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