

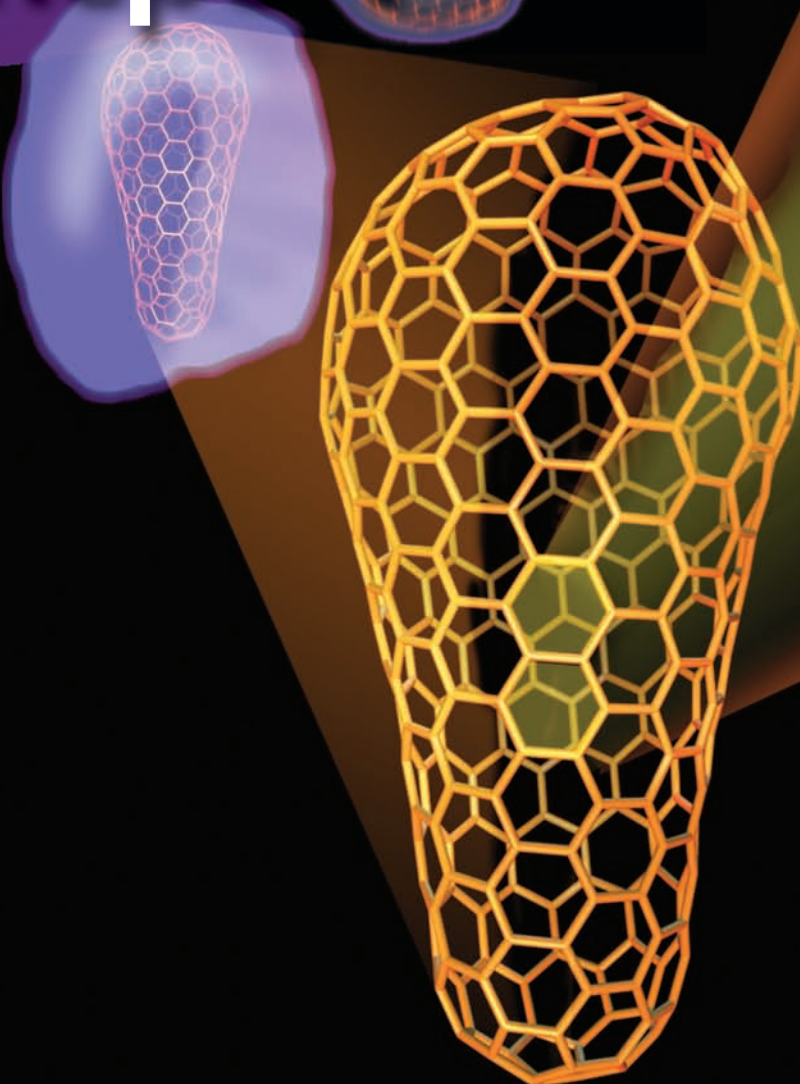
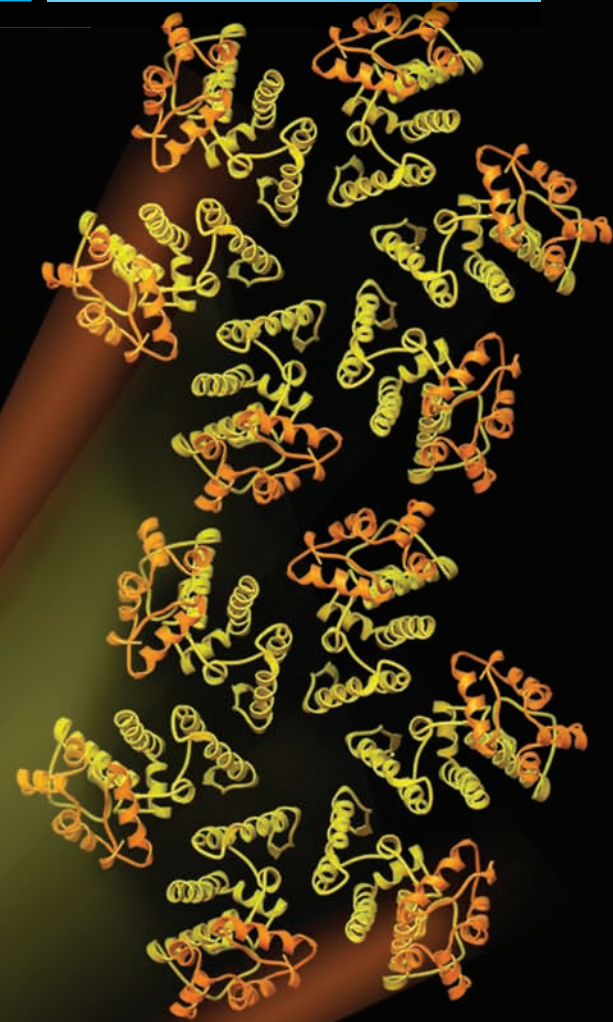
July-August 2009

IAVIR Report

The Publication on AIDS Vaccine Research

WWW.IAVIREPORT.ORG | VOLUME 13, NUMBER 4

IAS 2009: Conference Report



Challenge Models

Characterizing and optimizing
viral challenge models
for vaccine research

EDITOR'S LETTER

W

WHAT IF THERE WERE magic pills that could effectively treat HIV infection, prevent HIV-infected individuals from transmitting the virus to others, reduce prevalence of tuberculosis, and perhaps even protect uninfected individuals from acquiring HIV? Oh wait, maybe there are. They're called antiretrovirals (ARVs), and they have revolutionized the treatment of HIV infection.

So far, the road to developing biomedical interventions to *prevent* HIV infection has been a bit rockier. In his talk at the 5th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention, held recently in Cape Town, South Africa (see *Everything from Cause to Cure* for our report on the conference), Ronald Gray of Johns Hopkins University noted that of 29 trials evaluating the efficacy of different biomedical HIV prevention strategies, only four have shown significant success, and five have shown possible harm.

Until an effective vaccine or other HIV prevention strategy is developed, ARVs are being billed as one of the greatest hopes for controlling the global spread of HIV. One ARV-based approach to prevention is getting more HIV infected individuals on therapy. Evidence is accumulating that starting ARV treatment earlier in the course of HIV infection is beneficial. For prevention, the idea is that therapy, which efficiently and rapidly reduces viral load, could prevent those people already infected from transmitting HIV to others. This serves as the basis for the so-called test and treat strategy, which is explored in this issue (see *Test and Treat on Trial*).

But Stefano Bertozzi, who recently joined the Bill & Melinda Gates Foundation as HIV director, said at the IAS conference that twice as many people become HIV infected every day than are placed on treatment. In light of this, and the fact that there are still six million people who aren't getting treatment that will die without it, Bertozzi said, "It's really hard to imagine how [earlier treatment] could be a reality in the near future."

Considering this, the development of other HIV prevention strategies remains a priority. ARVs will likely play a role in preventing the spread of the virus, but in the eyes of many, the development of a vaccine is an imperative. One component of vaccine development analyzed in this issue is the plethora of viral challenge models used to evaluate different vaccine approaches in non-human primates (see *Looking for the Perfect Challenge*). José Esparza, senior adviser on HIV vaccines at the Bill & Melinda Gates Foundation, said a vaccine is "the best hope to stop the epidemic in the poorest countries and populations in the world."



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.

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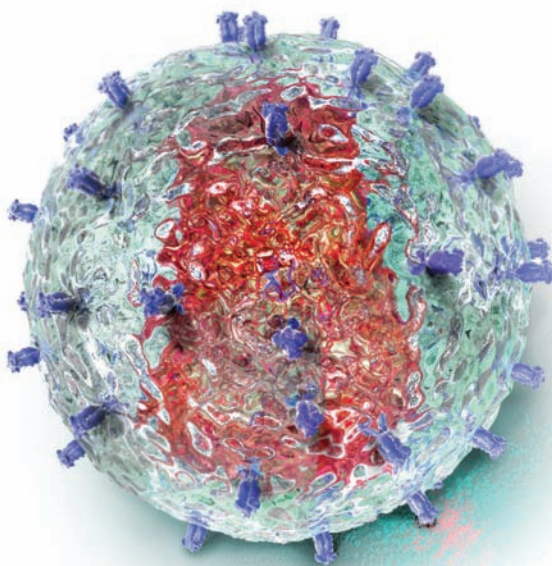
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[ON THE COVER]

Upon release from infected cells, HIV-1 undergoes a process of maturation that is triggered by protease cleavage of the spherically arranged Gag polyprotein. The resulting dramatic morphological changes culminate in particles that are infectious. During maturation, about 1,500 copies of the viral capsid (CA) protein assemble on a quasi-hexagonal lattice to form the characteristic conical capsid that encloses the RNA genome. Guided by previous results based on electron cryomicroscopy [Barbie Ganser-Pornillos et al., *Cell* **131**:70-79 (2007)], CA was engineered to form stable hexamers that were amenable to crystallization. The recently published X-ray structure at atomic resolution [Owen Pornillos et al., *Cell* **137**:1282-1292 (2009)] not only provides insight into the basis for assembly of the conical capsid but also reveals intermolecular interactions between CA subunits that may guide structure-based drug discovery efforts to inhibit capsid assembly.

Cover art by Michael E. Pique (*The Scripps Research Institute, TSRI*) and Mark Yeager (*TSRI and the University of Virginia*) using AVS software.

Everything from **CAUSE TO CURE**

Research presented at the biannual International AIDS Society Conference ran the gamut from studying early HIV infection to the search for a cure

By Kristen Jill Kresge

WITH THE THEME “From Cause to Cure,” the 5th International AIDS Society (IAS) Conference on HIV Pathogenesis, Treatment and Prevention, which was held from July 19-22 in Cape Town, South Africa, brought together more than 7,500 delegates to discuss a range of questions regarding everything from the earliest events of HIV infection to viral eradication. While the meeting covered the soup to nuts of HIV infection, Françoise Barré-Sinoussi, co-recipient of the Nobel Prize for the discovery of HIV, cited two main challenges plaguing researchers in her talk at the opening ceremony. “One challenge we have is to develop a vaccine, another is to have a cure for AIDS,” she said.

By no means are these small challenges. Research on viral eradication seems to have picked up steam recently, due in part to new funding from the US National Institute of Allergy and Infectious Diseases (NIAID), but it remains a formidable task. Barré-Sinoussi and the IAS continue to shed light on this effort. Next year, Barré-Sinoussi is organizing a two-day workshop in advance of the biannual International AIDS Conference in Vienna called “Towards a cure, HIV reservoirs and strategies to control them.”

In vaccine research, some of the emphasis has shifted to understanding the basic immunology

of HIV and using this to design improved vaccine candidates. Efforts continue to be focused on understanding the earliest events of HIV infection, as well as identifying properties that allow some individuals to control HIV immunologically. “This is a virus that can actually be controlled by the immune system,” said Bruce Walker, director of the Ragon Institute, in his plenary talk at the conference, adding that “a vaccine for HIV is possible, but still a long way off.” Anthony Fauci, director of NIAID, said that although the field is moving more toward basic research, “It’s not going to slow things down. I think it’s [actually] going to speed things up.”

Meanwhile, researchers continue to focus on implementing existing HIV prevention strategies, such as adult male circumcision. There is also a growing chorus of support for sustaining and increasing the availability of antiretroviral therapy (ART) and initiating treatment earlier in the course of HIV infection, both to save lives and prevent new infections from occurring.

In the midst of a global recession that threatens the sustainability of HIV/AIDS funding, the need to maximize the benefit of existing HIV treatment and prevention funding was a pervasive message at the conference. Stefano Bertozzi, who recently

joined the Bill & Melinda Gates Foundation as HIV director, suggested that the economic crisis might, paradoxically, help improve program efficiency and he called for better management and integration of services. “For the last 25 years we’ve had an emergency response to this epidemic,” said Bertozzi. “We have to switch that thinking.”

When to start

The approach to treating HIV has changed dramatically over the past 25 years. There are now more than 30 licensed antiretrovirals (ARVs), and combination regimens of these drugs. Effective therapy can reduce viral load to below detectable levels by standard assays and often even completely halt ongoing viral replication. Fauci said that now a newly HIV-infected 20-year-old who receives appropriate treatment has a life expectancy of at least 69 years. “The results are striking, historic, and to some degree unprecedented,” he said.

Over time, therapeutic strategies have also changed, with researchers see-sawing between early and delayed initiation of ART. Early on, the approach was to hit hard as early as possible to try to stop the virus from wrecking the immune system. However, clinicians eventually became concerned about the toxicity of ARVs and tended to delay initiation of therapy until a person’s health began to decline. Also, because the availability of ARVs was severely limited in developing countries, guidelines were devised so that therapy was not administered until a person’s CD4+ T-cell count dropped below 200 in a microliter of blood (the clinical definition of AIDS).

Now, evidence is accumulating that suggests starting therapy earlier in the course of HIV infection is beneficial. Wafaa El-Sadr, a professor in the department of epidemiology at Columbia University, presented data from the SMART study, which was conducted at more than 300 clinical research centers in 33 countries and compared the clinical outcomes of nearly 5,500 HIV-infected individuals who were randomized to either start ART early in the course of their infections, or later, when their CD4+ T-cell levels fell below 250. Rates of serious AIDS- and non-AIDS-related events were 2.4 per 100 person years among those who started therapy early, versus 4.4 for those who delayed treatment.

Earlier initiation of therapy may be beneficial because of its ability to suppress ongoing rounds of viral replication that lead to chronic activation of the immune system, which can be detrimental over the long term. Researchers previously thought that dur-

ing the eight to 10-year period following infection, the virus was clinically latent, but more recent evidence suggests clinical latency is a “misconception,” according to El-Sadr. Ongoing viral replication during the period of asymptomatic HIV infection induces “inflammatory changes that are associated with an increased risk of mortality,” she said. Inflammation is an important cause of organ damage, disease progression, and death, added El-Sadr. “HIV is much more toxic than any drug you can throw at it,” said Julio Montaner, president of IAS.

Another study provided additional evidence that starting therapy earlier can improve clinical outcomes. The study, known as CIPRA HT 001, involved 816 HIV-infected adults in Haiti with CD4+ T-cell levels between 200 and 350. Half of the participants were randomly selected to start treatment within two weeks of enrollment, while the remaining volunteers did not receive ART until their CD4+ T-cell counts dropped below 200, in accordance with the current treatment guidelines set by the World Health Organization (WHO). At an interim review, the study’s data safety monitoring board (DSMB) found that starting treatment at CD4 counts between 200-350 improved survival rates compared with deferred treatment. The mortality rate was four times higher among volunteers who deferred treatment until their CD4 count dropped below 200. Twice as many people in the group that received deferred therapy also developed tuberculosis (TB) during the study. Based on these findings, the DSMB recommended the trial be stopped and that all volunteers be offered ART.

Additional data indicates that ART may be an effective strategy in controlling TB. Keren Middelkoop, a principal investigator at the Desmond Tutu HIV Center, presented data from a follow-up HIV/TB survey conducted in a township community in Cape Town, South Africa, in 2008. The first survey of this community was conducted in 2005 and included 762 participants. At that time approximately 12% of those HIV infected were receiving ART. In 2008, after the South African government instituted a large-scale ARV program, a second survey of 1,251 people was conducted and 31% of those HIV infected who qualified for treatment were receiving it.

Middelkoop reported that HIV prevalence rose from 23% in 2005 to 25% in 2008. But after adjusting for age, sex, and HIV infection status, the population-level TB prevalence in 2008 was significantly lower—it dropped from 3% in 2005 to 1.8% in 2008. This decline was driven primarily by a

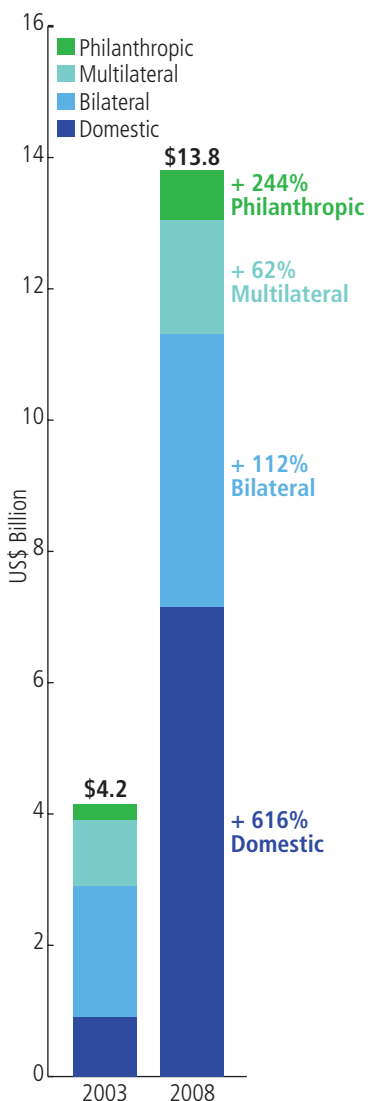
Current Antiretroviral Treatment Guidelines

Organization	CD4+ T-cell Count Threshold
US Department of Health and Human Services (2008)	< 350 (recommendations stronger if < 200)
International AIDS Society (2008)	< 350 (consider if > 350 in some situations)
European AIDS Clinical Society (2008)	< 350 (consider if > 350 in some situations)
British HIV Association (2008)	< 350 (consider if > 350 in some situations)
World Health Organization Europe (2007/2008)	between 200-350
World Health Organization (2006)	< 200 (consider between 200-350)

[AIDS FUNDING]

A snapshot of the increase in AIDS funding from different sources over the past five years.

Source: Stefano Bertozzi



reduction in prevalence of previously undiagnosed TB in HIV-infected individuals from 9.2% to 3.6%. During this time there were no changes in any of the TB programs or immigration levels that could have led to the decline in prevalence. Middlekoop concluded that the strong temporal association between reduction in TB prevalence and the initiation of an ART program suggests it is possible that ART reduces TB transmission.

“Everything seems to point toward earlier therapy,” said Fauci. This suggests that perhaps the current treatment guidelines may need to be revised. The IAS has already altered their recommendation, suggesting therapy be commenced at <350 CD4+ T cells, and many think the WHO may soon modify their guidelines as well. Most developing countries follow the WHO’s recommendation, so if they adopt earlier treatment, many more people would qualify for ART, dramatically increasing global treatment costs. But Fauci said earlier therapy is still a cost-effective strategy. “It doesn’t actually cost more money [because] it’s twice as expensive to care for people who don’t start early,” he said.

However, the optimism about early initiation of treatment was tempered by warnings about potential drug shortages in some countries that could jeopardize access to therapy, even for individuals already on treatment. Bertozzi said that there is still so much to be done to get treatment to people who will die without it that, “It’s really hard to imagine how [earlier treatment] could be a reality in the near future.”

Treatment as prevention

As researchers reconsider the optimal time to begin therapy, there is also an ongoing push to expand availability of ARVs to help stem the spread of HIV (see *Test and Treat on Trial*, page 14). “Highly active antiretroviral therapy is an essential tool to curb the growth of the pandemic,” said Julio Montaner, president of IAS and a pioneer of early initiation of treatment, who called therapy a “cost-averting intervention even in a fiscally challenging environment.” Bernard Hirschel, head of the HIV/AIDS unit at the University Hospital of Geneva, even declared that, “Treatment as prevention is the topic of the year.” He pointed to HIV transmission data from a recently published study of circumcision as a reason to explore ARVs for prevention (*Lancet* 374, 229, 2009). In this study, despite free condoms and HIV counseling, the likes of which he said would never be achieved outside of the setting of a clinical study, the HIV transmission

rates in both the control and intervention groups were above 10%. “Is treatment the answer?” Hirschel asked. “I don’t know, but we better find out.”

In a study presented by lead author Patrick Sullivan of Emory University, researchers looked at the rates of HIV transmission in 2,993 serodiscordant couples from Rwanda and Zambia over almost a year and a half. During this time, 172 linked infections between couples occurred when the infected partner was not on ART, while only four occurred if the infected partner was on treatment (initiated at a CD4+ T-cell count less than 200). Based on this data, there was a 79% reduction in HIV transmission associated with the infected partner taking ART.

All four of the HIV transmissions that occurred when the infected partner was taking ART were from women to men, but Sullivan concluded that there were too few transmissions to reach a conclusion on whether relative rates of HIV transmission among serodiscordant couples on ART differ by sex. Sullivan also noted several caveats to this study, including the fact that viral load measurements were not routinely taken so they could not link transmission to viral suppression. He said that although these data can’t be generalized outside of these specific cohorts, they do “support the idea that treatment of patients with clinical indication may reduce HIV transmission.”

Others were more circumspect about the possibility of treatment as prevention. Sarah Fidler, senior lecturer at Imperial College in London, said directing treatment to the most infectious individuals would have the greatest effect on reducing transmission rates, but this requires identifying infected individuals within a few months after seroconversion. She noted that three main factors determine infectiousness: the viral load of the infected individual, whether or not the individual has other sexually transmitted infections (STIs), and the stage of their HIV disease. Fidler said that it is during primary or acute infection that an HIV-infected individual is the most infectious. A study involving serodiscordant couples in Rakai, Uganda, found that during primary infection, an individual is estimated to be 26 times more infectious than during the long duration of asymptomatic infection that follows (*J. Infect. Dis.* 198, 687, 2008). Infectiousness increases again during the late stage of infection (10-19 months before death), but to a lesser degree than during the acute phase. During acute infection, HIV-infected people are often not aware that they are infected, are likely to

also have other STIs, and have “extremely high” plasma and genital tract viral loads, according to Fidler. Also, recently transmitted HIV variants are often biologically more transmissible.

The period of “high infectiousness” during primary infection was estimated in the Rakai study to last approximately three months after initial seroconversion. Fidler said that if the “high viral load is short-lived, the increased risk of transmission is also short-lived.” As no treatment guidelines call for initiation of therapy during acute infection, Fidler casted doubt on whether earlier treatment could substantially lower HIV transmission rates on a population level.

Early control of HIV

There is also an ongoing effort to better understand the earliest events of HIV infection. “We know an awful lot about the pathogenesis of HIV but there are still questions we can’t answer,” said Fauci.

Some researchers are focusing on the early stage of HIV infection in controllers—individuals who maintain viral loads of less than 2,000 HIV RNA copies/ml. HIV controllers are the subject of much research, which is of particular interest to vaccine researchers, but little is known about how these individuals control virus during acute infection. Toshiyuki Miura of the Institute of Medical Science at the University of Tokyo presented an analysis of host genetics and virus characteristics in a group of 18 controllers during acute HIV infection, so-called acute controllers. Based on human leukocyte antigen (HLA) typing, Miura reported that host genetics were not responsible for effective control of HIV—the frequency of acute controllers expressing any of the HLA class I alleles that are considered protective against HIV (B*13, B*27, B*51, and B*57) was actually significantly lower than among HIV controllers during chronic infection. Only two acute controllers expressed the B*57 allele.

Miura and colleagues then constructed chimeric viruses derived from the Gag-Protease proteins isolated from virus in acute controllers. Researchers observed that these chimeric viruses had reduced replicative capacity, attributed in part to cytotoxic T lymphocyte (CTL) escape mutations. Viral sequencing indicated that viruses in acute controllers had a higher frequency of drug resistant mutations than the viruses in progressors during acute infection. Two of the acute controllers who possessed the B*57 allele had a T242N viral escape mutation that affects viral fitness. Miura concluded from this small study that individuals who control viremia following acute

infection are infected with viruses that have reduced replicative capacity that is attributable to the transmission of drug resistant strains or fitness costs that are the result of CTL escape mutations.

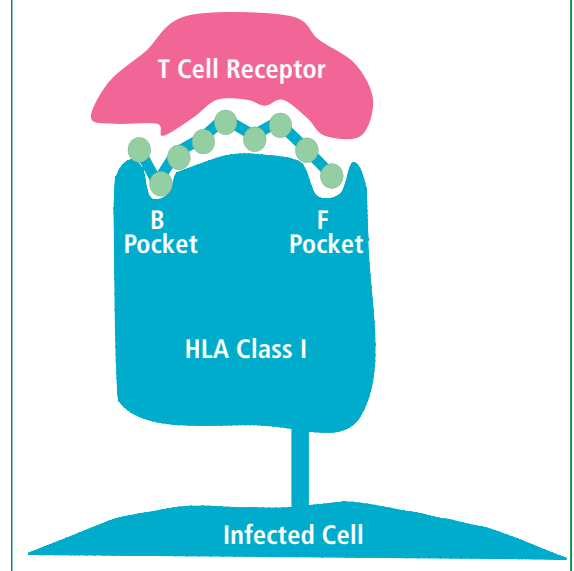
Host genetics and immune control

Walker and colleagues are one of the main groups studying HIV controllers to mine for clues that may inform vaccine development. Their research is also uncovering important characteristics about the role of host genetics in immune control of HIV. According to Walker, HLA B alleles are “really important in this disease,” as they greatly influence viral load. HLA class I alleles display viral peptides on the surface of infected cells that are recognized by T-cell receptors. There are 817 HLA class I B alleles, some of which have previously been associated with control of HIV. In a study conducted in KwaZulu-Natal, South Africa, Walker and colleagues did high resolution HLA typing of approximately 700 individuals. They found that some alleles, such as B*57, B*8101, and B*5801, are associated with protection against HIV and result in lower viral loads, while others such as B*5802 are associated with disease progression and higher viral loads. HLA distribution within the population largely reflects ethnic ancestry—both B*5801 and B*5802 are common in African populations. In this study, individuals with the B*5801 allele had a median viral load of 14,650 HIV RNA copies/ml, while individuals possessing a B*5802 allele had a median viral load of 75,200. Individuals with neither of these alleles had a median viral load of 33,000 copies/ml.

In another study, Walker and colleagues confirmed the observation that broader Gag-specific CD8⁺ T-cell responses are associated with lower levels of viremia, while conversely, broader Env-specific CD8⁺ T-cell responses were associated with higher viral load. The B*5801 allele primarily presents Gag epitopes, while the B*5802 allele pre-

T-Cell Immunity and HLA Molecules

Human leukocyte antigen (HLA) class I alleles, which are determined by host genetics, display HIV peptides or epitopes that enable immune recognition and killing of HIV-infected cells by CD8⁺ T cells. There are 817 HLA class I B alleles, some of which have previously been associated with control of HIV.



[CIRCUMCISION PROGRESS]

Multiple sessions at the 5th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention focused on progress in implementing adult male circumcision (AMC) for HIV prevention. Kawango Agot, of the Universities of Nairobi, Illinois, and Manitoba Project, gave an overview of progress on this front in the Kisumu district of Kenya. Since the Kenya National Voluntary Male Circumcision Program was initiated in November 2008, around 30,000 males have been circumcised through the public system, largely with support from donor agencies. One obstacle to implementing circumcision programs was the lack of trained medical professionals. In June, Kenya revised its health regulations to allow nurses to conduct the surgical procedure, and Agot's group has found that after training, adverse events following AMC did not differ if the procedure was performed by nurses.

It is still unknown whether AMC reduces rates of HIV infection in men who have sex with men (MSM), but Tim Lane of the University of California in San Francisco, presented data from a study in Soweto, South Africa, showing that uncircumcised MSM had nearly five times higher HIV infection rates than their circumcised counterparts. Lane said that about 40% of men in the Soweto cohort also had sexual relations with women, therefore, "reducing the risk of HIV among MSM could benefit entire communities." He called for further research to assess the acceptability of an AMC trial for HIV prevention among MSM.

—Daisy Ouya, Program Manager, Information, Education and Communication for IAVI in Nairobi, Kenya

dominantly presents HIV Env epitopes, explaining at least in part, why one allele is associated with protection and the other with progression.

Using a viral suppression assay, Walker and colleagues then compared the ability of CD8⁺ T cells freshly isolated from chronically HIV-infected individuals with high Gag-specific CD8⁺ T-cell responses, to those from individuals with similar viral loads yet low Gag-specific CD8⁺ T-cell responses. They found that the CD8⁺ T cells from high Gag responders were better able to neutralize HIV and inhibit viral replication. "There's a qualitative difference depending on what part of the virus is being targeted," said Walker. Further analysis showed that this is, in part, because Gag-specific CD8⁺ T-cell responses select for viral fitness mutations that cripple HIV.

To evaluate the population-level impact of epitope-specific immune pressure on HIV, Walker described efforts led by Philip Goulder, an immunologist at Oxford University, to conduct HLA typing and virus sequencing of Gag from 2,800 chronically HIV-infected individuals from nine cohorts, spanning five continents. Goulder and colleagues then analyzed the HLA prevalence and detection of escape mutations in HIV. This work, recently published in *Nature*, suggests that the more prevalent an HLA allele, the more immune escape viruses that are transmitted (*Nature* 458, 641, 2009). "HIV is being shaped by the immune response [against it] on a global level," said Walker, "and some protective epitopes are being lost." He and the other study authors suggest that keeping up with the changing immunological landscape of HIV will be a challenge for vaccine researchers.

Host genetics can also impact HIV transmission. In research, which will soon be published in the *Journal of Virology*, Walker and colleagues found that among mothers who express protective HLA alleles, transmission of HIV to their infants is less common. Additionally, infants born to mothers with protective HLA alleles, or who themselves had protective alleles, were more likely to be slow progressors.

The status of HIV prevention

Ronald Gray, professor in population and family planning at Johns Hopkins University, pointed out in a plenary talk on the state of HIV prevention that out of 29 completed trials evaluating the efficacy of different biomedical interventions to date, only four had shown significant success (three evaluating adult male circumcision and the other evaluating treatment of sexually transmitted infections to reduce HIV risk). Five showed pos-

sible harm. "HIV prevention trials are extremely difficult and expensive," said Gray. As such, he challenged researchers to urgently improve the design of trials, to do a better job of pre-clinical screening and assessment, and to collect better pre-trial HIV incidence estimates.

Gray suggested that treating sexually transmitted infections such as herpes simplex virus (HSV)-2 is still a priority, but its effect on the HIV epidemic may be limited. However, Jairam Lingappa, medical director of the Partners in Prevention HSV/HIV study, presented data showing that treatment of HSV infection with acyclovir delays HIV disease progression by 17-19%. Lingappa and colleagues analyzed 3,381 of the HSV/HIV-infected partners in the study with median CD4⁺ T-cell counts of about 400. The primary analysis was conducted based on a composite endpoint of reaching a CD4⁺ T-cell count of less than 200, starting ART, or dying during the study. Of those receiving acyclovir, 284 individuals reached this composite endpoint compared to 324 individuals who received placebo—a statistically significant difference. Lingappa said there are multiple interpretations for this observation. He presumed that the effect is mediated by the observed 0.25 log reduction in HIV RNA copies/ml of plasma observed in the acyclovir group, though he did also refer to recent studies that have shown that acyclovir has direct antiretroviral activity *in vitro*. He concluded that this presents a new concept for delaying HIV disease progression without ART. However, a question was raised about a possible downside of this observation. If ART can reduce HIV transmission, delaying initiation with acyclovir could only prolong the period of time that an HIV-infected person is able to transmit HIV to others.

Another possible strategy for reducing HIV infection risk may be vaccination against human papilloma virus (HPV). Jennifer Smith, a researcher at the University of North Carolina in Chapel Hill, reported that men in a circumcision trial in Kenya who were HPV infected had an 80% higher risk of HIV infection through 42 months, even after controlling for circumcision status and HSV-2 infection. Smith said there were many possible explanations, including that HPV could potentially induce specific local cytokines, such as macrophage inflammatory protein-3 or interleukin-8, which may increase HIV susceptibility, and added that HPV prevention efforts may help reduce HIV acquisition. Two vaccines against HPV are now licensed for use in many countries throughout the world. ■

Looking for the **PERFECT CHALLENGE**

Researchers are characterizing existing challenge models and tweaking them to better mimic HIV transmission and pathogenesis

By Andreas von Bubnoff

ANIMAL MODELS ARE THE MAJOR way to gain insights into human diseases and how to prevent them. In the field of AIDS vaccine research, non-human primates (NHPs), especially rhesus macaques, have emerged as the most relevant animal model. While these animals cannot be infected with HIV, they can be infected with simian immunodeficiency virus (SIV), the monkey equivalent of HIV, and SIV-derived challenge stocks have become the major challenge virus used in AIDS vaccine research.

But the quantity of challenge virus stocks is limited, so researchers have to propagate them, often by passaging the existing viruses in cultured cells. This can alter the challenge virus stock, leading to genetic and biological differences. Some challenge stocks are more infectious than others, while some can be neutralized more easily than others.

This has raised concern that even challenge stocks that initially came from the same source—such as SIVmac251—might become different in their biological properties once they have been propagated in different labs. Such differences can make it difficult to compare the outcomes of different NHP studies of vaccine candidates, even if they use the same challenge stock.

The host animals used in challenge studies can also differ from each other. Some respond to viruses with higher viral loads than others, and even animals within the same host species can have genetic differences, including major histo-

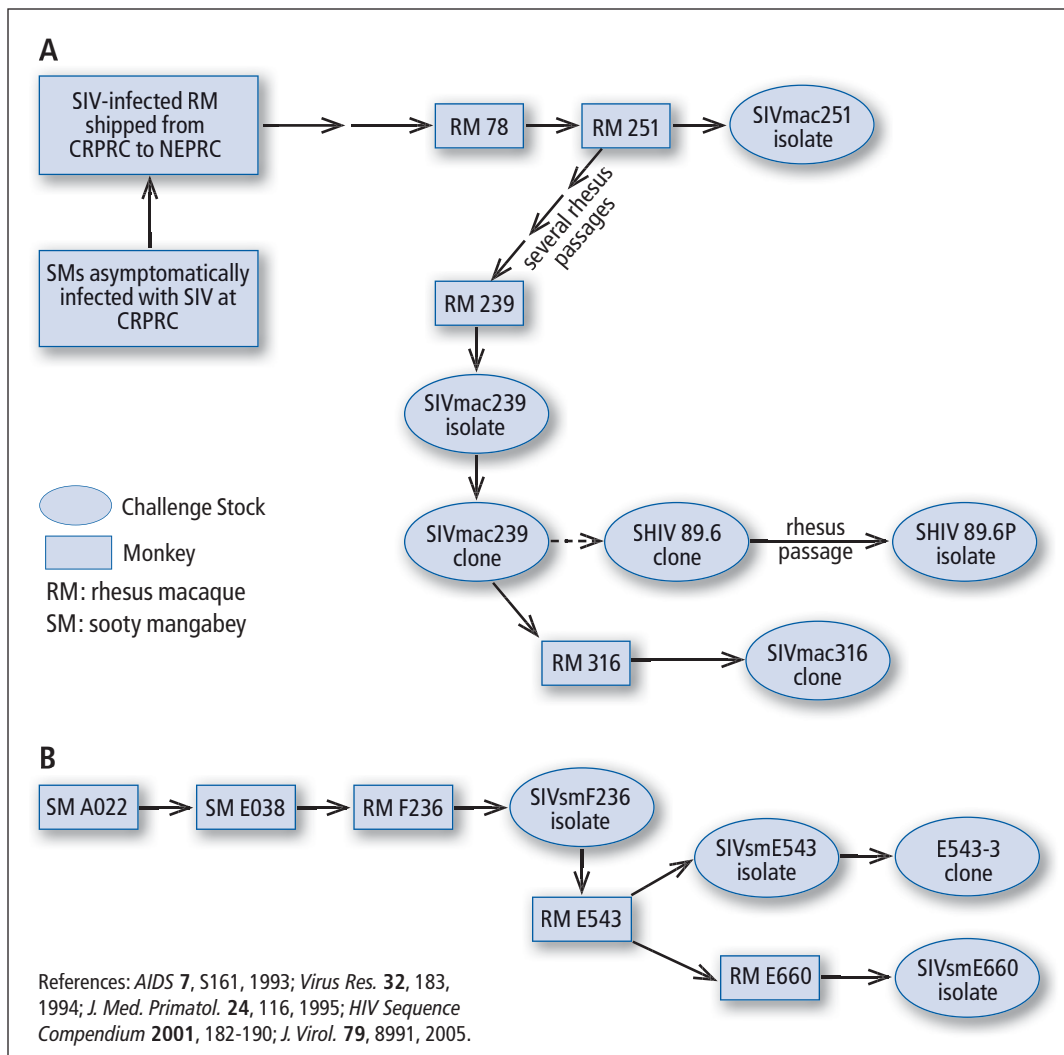
compatibility complex (MHC) type I alleles that protect against the challenge virus.

Researchers have started to characterize both host animals and challenge stocks to more fully understand these differences. “There is an increasing realization within the field that we have to be more exact where we talk about the viruses and the animals that we are using,” says David O’Connor, assistant professor of pathology and laboratory medicine at the University of Wisconsin-Madison, one of the researchers involved in the characterization effort. Eventually, better characterization may help researchers to better compare and interpret the results of vaccine studies.

But just understanding the differences may not be enough. Some researchers are calling for the development of a standardized challenge virus stock for evaluating AIDS vaccine candidates in NHPs. “What would really benefit the field would be to have a standard challenge stock of SIV that had desirable infectivity and pathogenic properties and that also had a neutralization phenotype that resembled primary isolates of HIV,” says David Montefiori, who directs the laboratory for AIDS vaccine research and development at Duke University Medical Center.

The origin of challenge stocks

The origin of most virus challenge stocks that are currently used in AIDS vaccine research is sooty mangabey SIV (see Fig. 1), which is thought to have



▲ FIGURE 1

Origins of Virus Stocks

(A) The challenge stocks SIVmac251, SIVmac239, and SIVmac316 originated from SIV-infected RM number 78 at the New England Primate Research Center (NEPRC). This SIV is thought to initially be derived from SIV-infected SMs at the California Regional Primate Research Center (CRPRC). At CRPRC, the infected SMs are thought to have infected rhesus macaques, some of which were shipped to the NEPRC in 1970. There, they are thought to have infected other RMs and eventually RM 78.

(B) The challenge stocks SIVsmE660 and E543-3 also originated from an SIV-infected SM that infected RM F236.

given rise to HIV-2 in humans. While sooty mangabey SIV typically doesn't cause disease in its natural hosts, it can infect and cause disease in rhesus macaques. In contrast, chimpanzee SIV, which is thought to have given rise to the HIV-1 epidemic, is unlikely to infect rhesus macaques because it is very similar to HIV-1, which does not replicate in rhesus macaques, says Brandon Keele, a senior scientist at the National Cancer Institute at Frederick.

Two of the most commonly used rhesus macaque SIV challenge stocks, SIVmac239 and 251, are derived from rhesus macaques that are thought to have been infected with SIV from sooty mangabeys (see Fig. 1A). About 20 years ago, Ronald Desrosiers' lab at the New England Primate Research Center isolated SIVmac251 from an infected Indian rhesus macaque (number 251) that had lymphoma. SIVmac251 is a biological isolate or a swarm, which means that it contains many dif-

ferent virus variants or quasispecies, similar to HIV that naturally infects humans. Desrosiers passaged this SIVmac251 from one macaque to another up to animal number 239, from which he derived a stock, and then cloned SIVmac239 from that. As a clone, SIVmac239 consists of genetically identical copies of the same SIV strain or variant, and is therefore much more defined than 251.

A third stock currently in use is SIVsmE660, a swarm virus that also originated from a sooty mangabey SIV that infected a rhesus macaque (see Fig. 1B). It was then passaged through an additional Indian rhesus macaque before it was eventually isolated from macaque number E660, according to Vanessa Hirsch, a senior investigator at the National Institute of Allergy and Infectious Diseases, who isolated this stock along with Philip Johnson in the late 1980s (*AIDS* 7, S161, 1993; *Virus Res.* 32, 183, 1994).

The fact that most challenge stocks are derived from the SIVmac239/251 and the SIVsmE660 lines (see Fig. 1) is a problem, says Cristian Apetrei, an associate professor at the University of

Pittsburgh, because it doesn't reflect the genetic diversity of HIV-1. "When we have only two strains in order to develop vaccine studies, [we] will be handicapped by the lack of virus divergence in the animal model," he says. To add genetic diversity to the available challenge stocks, he has been deriving new SIV stocks from rhesus macaques infected with plasma from SIV-infected sooty mangabeys (*J. Virol.* 79, 8991, 2005; *Virology* 362, 257, 2007). Another advantage of the new strains is that they have not been changed by passage in animals or *in vitro*, he says. "[These are] unadapted strains directly derived from sooty mangabeys."

In the mid-1990s, researchers also started to develop hybrid viruses called SHIVs that are clones of SIVmac239 combined with HIV Env protein. SHIVs are useful for studies of HIV-specific antibody responses. More recently, researchers developed SHIVs that contain the reverse transcriptase

(RT) from HIV to test drugs that inhibit this enzyme. Some SHIVs, for example 89.6, have been made more pathogenic by passaging them through animals, says Adrian McDermott, director of immunology and vaccine discovery at IAVI. Passaging between animals can make a stock “hotter” or more virulent, perhaps because it selects for virus variants that replicate more quickly, adds Alan Schultz, a project director of the NHP program at IAVI.

Researchers also create “boutique” challenge viruses to answer specific research questions, O’Connor says.

“To culture is to disturb”

The different ways labs maintain and propagate challenge stocks has some researchers concerned that it could be difficult to compare the results of different studies. There is less concern with stocks that are clones like SIVmac239, than for stocks like 251 that are swarms composed of many different virus variants or quasispecies, O’Connor says, adding that there are at least three or four different 251 stocks, and different virus variants within these stocks could predominate depending on who prepared them. “Multiple people have virus stocks that they refer to as SIVmac251, but in fact these viruses, while closely related, are distinct,” adds R. Paul Johnson of the New England Primate Research Center.

Differences between virus stocks can arise when labs propagate them by passaging them in animals or in tissue culture, R. Paul Johnson says. “To culture is to disturb,” he says, quoting the French virologist Simon Wain-Hobson. “Even very seemingly minor changes in how the virus is propagated can perturb its characteristics,” R. Paul Johnson adds. Passaging the 251 stock in tissue culture can increase its susceptibility to neutralizing antibodies. Preston Marx, a professor of tropical medicine and microbiology at Tulane University, says his 251 stock has been passaged in tissue culture, which made it a little less pathogenic and a little more susceptible to antibody than the Desrosiers stock.

But despite the concern that challenge stocks might be different from each other, little is known about the extent of these differences. “Really the only way to understand those differences is to sequence the entire genome of the infecting viruses,” adds O’Connor, who is one of several researchers who have started to characterize different challenge stocks. In a collaboration initiated by R. Paul Johnson, O’Connor and colleagues are

examining possible differences in the sequences of the 251 stocks from Desrosiers and Chris Miller at the University of California in Davis.

Keele and colleagues have found differences when they recently used single genome amplification (SGA) to characterize *env* gene sequences from different 251 stocks. SGA allows researchers to determine the proportion of virus strains with a certain sequence of the *env* gene within a challenge stock. The analysis found differences between the 251 stock from the lab of Norm Letvin, a professor of medicine at Harvard Medical School, and the Desrosiers stock, Keele says. While both stocks are still very similar, they have accumulated some unique changes due to independent propagation in different labs over time. “There aren’t any viruses in [Letvin’s] stock that are identical to the viruses in [Desrosiers’] stock,” Keele says. “It’s like taking a monkey on an island and then leaving it for thousands of years.”

Keele says it is unclear if such differences can result in biological differences that could affect the evaluation of candidate vaccines in animals challenged with the two strains. He says experiments are underway to see if there are differences in how the two stocks are transmitted in macaques.

O’Connor predicts there could be a wake-up call for the field when studies with different 251 stocks show different outcomes. “I think the field needs to have a clear-cut example of a case where viruses that were called the same thing had different outcomes,” he says. “I think that we will probably pick up such an example with SIVmac251 if we look hard enough. That will create a moment of action that will motivate the field to establish uniform standards for applying these viruses.”

But not everyone is convinced that differences between stocks matter that much. Letvin acknowledges differences between 251 stocks, but says that they should not matter once a vaccine candidate clearly works. “My working assumption is that these differences won’t obscure our ability to see a dramatically better vaccine approach than what we currently have available,” Letvin says.

Escaping variability

One way to improve the comparability of results would be to have a standardized challenge stock everyone in the field uses, says Montefiori. Ideally, such a standardized challenge stock should be a clone, Montefiori adds, to better allow researchers to find the immune correlate of protection. This is because in *in vitro* neutralization assays, they can

[CLONES OR SWARMS?]

In addition to developing challenge stocks that better mimic the biological properties of HIV, researchers are also trying to better mimic HIV transmission in animal models by using swarm viruses because humans are exposed to swarms, not clones. A recent study by Brandon Keele, of the National Cancer Institute at Frederick, and colleagues found that the swarm challenge stocks 251 and E660 actually mimic HIV transmission quite well in rhesus macaques. They used single genome amplification (SGA) to characterize *env* gene sequences of an SIVmac251 stock and an SIVsmE660 stock from a different lab (*J. Exp. Med.* 206, 1273, 2009). The study found that within each stock, the virus strains differed from each other by about 1%-2% at most, similar to the average HIV diversity in the blood of an HIV-infected person during the first few years of infection.

The study also determined a dose where rectal infection of rhesus macaques with these stocks leads to infection by just one or a few transmitted founder viruses, which is similar to what has been observed in HIV infection. The virus strains from the challenge stock that established infection were different in each infected animal, suggesting that just like in humans, the initial selection of the virus strains that established infection appears to be random, Keele says. Recently, David Watkins of the University of Wisconsin-Madison collaborated with Keele and George Shaw of the University of Alabama, to use SGA to determine a dose at which only a few transmitted founder viruses of SIVsmE660 establish infection in a repeat rectal challenge study (see *Capsules from Keystone, IAVI Report* March-April, 2009; *J. Virol.* 83, 6508, 2009). This study is also an example of efforts to make challenge models more realistic by using heterologous challenge strains like SIVmac239, which some believe better simulate the huge variation in HIV strains that infect humans.

then use the exact same virus strain that was used for the challenge to test if the animal's serum has antibodies that can neutralize the challenge strain.

If, however, researchers use a swarm like uncloned SIVmac251 for a challenge, a different virus variant establishes infection in different animals, Montefiori says. As a result, the virus used in an *in vitro* neutralization assay of the animal's serum after challenge will likely be different than the virus that established infection in the challenged animal. In many cases, researchers are measuring neutralizing antibodies as an immune correlate of protection with a different quasispecies or a different clone than what is transmitted, Montefiori says. He suggests that this complexity could be eliminated by using a molecularly cloned virus for the challenge and the assays.

Better reproducibility is also the reason why an IAVI project that compares different vectors for candidate vaccines uses SIVmac239, a clone, as a challenge virus. "Because it's a clone, we will get the same virus loads in those animals every single time," McDermott says. In addition, the type and time of escape mutations from the host's T-cell response are very well characterized with 239, which means that any deviation from that pattern will give researchers clues as to whether there is a vector-specific effect.

The drawback of using a clone, however, is that it's not very realistic (see left margin). Humans are not usually exposed to a single virus variant but rather a swarm. To combine the more realistic features of a swarm with the reproducibility of a clone, Desrosiers is trying to create a challenge stock that is a mixture of cloned viruses. It consists of SIVmac239 as a backbone with different versions of the envelope sequences present in the original 251 stock he generated in his lab. "I think to provide greater standardization and better control I would definitely like to see the field move toward the use of cloned viruses or even mixtures of cloned viruses," Desrosiers says. "I just think it's much better defined. You can have total control of what you are looking at."

With all these different choices, getting the field to agree on one challenge stock as a standard could be difficult, Montefiori says. "You just cannot get the people at the primate facilities to agree—they all have their favorite challenge stocks, and since many of them have been using those challenge viruses for many years, it's hard to get them to change." Others see advantages to having an array of challenge virus stocks available. "I don't think there is going to be one model

that fits all," Letvin says. "It depends on exactly what experiment one is doing."

Looking to better mimic HIV

Challenge stocks also differ in how closely they mimic the biological properties of HIV. SIVmac239, for example, is harder to neutralize than HIV, which means that testing candidate vaccines with 239 could underestimate their ability to induce protective neutralizing antibody responses, Montefiori says. Also, SHIV89.6P infects target cells via the CXCR4 coreceptor in primates, unlike most mucosally transmitted strains of HIV, which infect target cells with the CCR5 coreceptor (R5), says Ruth Ruprecht, a professor of medicine at Harvard Medical School. As a result, 89.6P eliminates naive CD4⁺ T cells, unlike recently transmitted HIV which targets CD4⁺CCR5⁺ memory cells. Most monkeys infected with 89.6P lose their entire CD4⁺ T-cell population irreversibly within two weeks after virus challenge. This is not what happens to HIV-infected humans during acute infection. "People don't die of AIDS in a year," Ruprecht says. "So too pathogenic is not a good thing."

Researchers are therefore looking for new challenge strains with more similar biological properties to HIV. Hirsch is experimenting with a clone called E543-3 that can be neutralized more easily than 239. It was derived from the same animal which was the source of the virus stock that gave rise, after passage in an additional macaque, to SIVsmE660 (*Virus Res.* 32, 183, 1994). Another alternative is SIVmac316, which was cloned from macaques infected with 239. SIVmac316 is macrophage tropic like most HIV strains and not T-cell tropic like SIVmac239, according to Schultz. It is also more neutralization sensitive and not as pathogenic as 239.

Researchers have also been developing R5-tropic SHIV challenge viruses. Ruprecht has developed neutralization-sensitive, R5-tropic SHIV strains that contain an HIV clade C envelope glycoprotein from a recently transmitted HIV strain isolated from an infected child, who was part of a mother-infant cohort in Lusaka, Zambia (*AIDS* 21, 1841, 2007). These SHIV strains cause disease much more slowly than other SHIVs and pathogenic SIVs, making them more similar to HIV, Ruprecht says. "AIDS vaccines should focus on the transmitted forms of HIV-1," she adds, "not on the highly aggressive, highly pathogenic end stage forms that are not typically transmitted among humans."

Paul Bieniasz and Theodora Hatzioannou, of the Aaron Diamond AIDS Research Center in New

York City, and colleagues recently developed another cloned challenge virus that is an engineered version of HIV called simian tropic HIV-1 (stHIV-1). It differs from HIV-1 only in that it contains an SIV-mac239 version of its *vif* gene. This SIV Vif can destroy the APOBEC3 defense proteins of pigtail macaques, and can therefore infect them, although it doesn't make them sick (*Proc. Natl. Acad. Sci.* 106, 4425, 2009). The acute virus levels in the stHIV-1 infected pigtail macaques are almost as high as in acutely infected humans, but taper off later toward low or undetectable levels, similar to what is observed in human long-term nonprogressors.

However, the current stHIV-1 virus has an Env protein that is primarily X4 tropic, which means that it tends to infect different target cells than most HIV-1 strains currently circulating in humans. Bieniasz is currently developing versions of stHIV-1 with an R5 tropic Env protein, and hopes that future versions of stHIV-1 will eventually even cause disease in the pigtail macaques.

What about the host?

Another factor that differs in challenge models is the animal host. The vast majority of researchers in the field use rhesus macaques of Indian origin, in part because they were used in the polio vaccine effort, says O'Connor. "That's what people had access to," he adds. Other host animals being used for HIV vaccine research are rhesus macaques of Chinese or Burmese origin and cynomolgus macaques.

These host animals differ in many ways, including their susceptibility to infection. Indian rhesus macaques tend to be highly susceptible to the commonly used SIV challenge strains SIV-mac239 and 251 because these strains were initially adapted to Indian rhesus macaques. "If you want to work with a highly pathogenic infection, then Indian rhesus would be the best," Marx says. McDermott agrees, noting that SIVmac239 or 251 will cause higher viral load in these animals than HIV in humans. In contrast, Chinese rhesus macaques infected with these same strains die more slowly and show similar peak viral loads to HIV-infected humans, Schultz says.

However, the genetics of the immune system in Chinese rhesus macaques are largely undefined, whereas for Indian rhesus macaques, MHC alleles like Mamu-B*08 and -B*17 are known to be associated with protection. This enables researchers to exclude them from studies for a more rigorous evaluation of candidate vaccines. "When we do vaccine challenge studies in our laboratory, we always

MHC class I type the animals so that we don't include some animals that have those protective class I genes and others that don't," Letvin says.

Such genetic differences may be the reason that even if the same host species is used, the old adage that mice lie, and monkeys don't always tell the truth may be correct. O'Connor's group found that genetic differences in the MHC could explain why in a study of an adenovirus-based vaccine, both the vaccinated and the control group controlled the challenge virus (*Vaccine* 26, 3312, 2008).

In light of such findings, O'Connor says, it is worth revisiting past studies because perhaps what was considered an effective vaccine candidate was really more attributable to the monkeys in the study. "If you look back through the literature," he says, "there are lots of animals that have been spontaneous controllers of virus in vaccine studies or animals that fared particularly poorly that often get written off in the process of writing a paper or get aggregated with the other animals in the study. If the fact that they are outliers has nothing to do with their treatment and has everything to do with their genetics, then that changes the way we may have to interpret a lot of those studies."

O'Connor uses Mauritian cynomolgus macaques for his studies of the role of cellular immune responses in protection. They are genetically very similar to each other because they come from a small isolated population on the island Mauritius. "[They are] almost like a monkey mouse," says McDermott, referring to inbred mouse strains. This may allow researchers to better study the effect of T cells on protection. "You are trying to keep the animal constant, keep the virus constant, and see what's involved in protection," says McDermott. O'Connor is characterizing cynomolgus macaques to better understand their protective MHC alleles and other factors that could affect the outcome of vaccine studies.

Biological differences are not the only factor that determines which animals researchers use for their studies. Sometimes, the reasons are more practical such as cost and availability. Because females are often used as breeder animals, fewer of them tend to be available for actual experiments, according to Letvin. As a result, mucosal challenge experiments in monkeys more often involve rectal than vaginal challenges. "There is no perfect way to model heterosexual transmission of the virus in monkeys because of simple animal availability," Letvin says. Cost is a factor as well, says Ruprecht. "If someone tells me you can have a free Chinese origin rhesus monkey, would I say no? Of course not. I'll take it." ■



Andreas von Bubnoff

▲ Indian rhesus macaque on Cayo Santiago, an island off the coast of Puerto Rico that houses a colony of almost 1,000 Indian rhesus macaques. The island, also known as "monkey island," is a facility of the Caribbean Primate Research Center and one of the largest providers of Indian rhesus macaques for AIDS vaccine research in the US. The animal in the picture is an adult eight-year-old male, a resident of the smallest of the six social groups on the island. He likes walks on the beach and bananas.

Test and Treat ON TRIAL

A strategy to implement universal HIV testing and immediate treatment is receiving increased attention and scrutiny from prevention researchers

By Regina McEnery

HIGHLY ACTIVE ANTIRETROVIRAL therapy (HAART) is remarkably effective at controlling viral replication and reducing viral load to below the limits of detection by standard assays. And because viral load is considered to be the principal predictor of heterosexual HIV transmission, scientists have postulated that widespread distribution of antiretrovirals (ARVs) among HIV-infected individuals, in addition to decreasing morbidity and mortality, might also reduce HIV incidence (*Lancet* 370, 1923, 2007 and *N. Engl. J. Med.* 342, 921, 2000).

Despite years of research, new biomedical HIV prevention strategies—apart from adult male circumcision—have not materialized. “With no vaccine or microbicide on the horizon, we need to look at other approaches,” said Sarah Fidler of Imperial College, in a talk at the 5th International AIDS Society (IAS) Conference on HIV Pathogenesis, Treatment and Prevention, held from July 19-22 in Cape Town, South Africa. One approach that is receiving increased attention and scrutiny from researchers is test and treat, which calls for universal testing and immediate treatment for anyone found to be HIV infected.

The move toward earlier treatment of HIV-infected individuals was a major theme at the recent IAS meeting, and test and treat also received con-

siderable attention (see *Everything from Cause to Cure*, page 4). IAS President Julio Montaner says researchers have been confident for several years that ARVs, if appropriately employed, provide a dual benefit. “Number one, they decrease morbidity and mortality rates among HIV-infected [individuals],” he says. “In addition, adequately treated people become, at the very least, less likely to transmit HIV. So if you treat a larger number of people, the impact on the epidemic could be significant.”

But despite endorsements from some researchers and mathematical models showing that this strategy could possibly even eliminate HIV in 50 years, there is little evidence to suggest test and treat is feasible. Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases (NIAID), cautions that this approach “may not work.” Speaking at the IAS conference, Fauci said, “We need to address that before we can promote it.” NIAID and others are now studying test and treat, including how much of an effect ARVs will have on reducing HIV transmission and the feasibility of implementing universal HIV testing.

“We need to determine these things factually or we’ll be in trouble,” says Myron Cohen, director of the Institute for Global Health and Infectious Diseases at the University of North Carolina, who is also studying test and treat.

Even if shown to work, there are numerous logistical and financial obstacles to implementing this strategy. Achieving universal testing is a considerable challenge, not to mention the cost of providing treatment to all infected individuals despite their disease status. Current guidelines for initiating treatment vary by country and circumstance. The World Health Organization (WHO) recommends that treatment be initiated when a person develops AIDS (as defined by having fewer than 200 CD4⁺ T cells in a microliter of blood) or an AIDS-related illness. Many developing countries adopt WHO guidelines in determining when an HIV-infected individual should begin therapy, but the IAS as well as leading government health agencies in the US and Europe have adopted 350 CD4⁺ T cells as the threshold for treatment based on accumulating evidence that suggests that earlier treatment is beneficial. An analysis of 18 HIV cohort studies concluded that the benchmark of 350 CD4⁺ T cells should be the minimum threshold for initiating therapy because deferring treatment was associated with higher rates of AIDS and death (*Lancet* 373, 1352, 2009). And a recent study from the US and Canada found mortality rates declined even further if HIV-infected individuals started therapy when their CD4⁺ T-cell counts were between 350 and 500 (*N. Eng. J. Med.* 360, 1815, 2009).

Based on the current treatment guidelines, only about a third of people who need therapy are receiving it. “Why are you even thinking of test and treat if you can’t get all infected people [who need treatment now] on antiretroviral therapy?” Fauci asked at the IAS conference. He then answered his own question by saying these are complementary issues that need to be addressed at the same time.

The model

The mathematical model that sparked much of the current discussion regarding the viability of test and treat was developed by a quintet of researchers from the WHO (*Lancet* 373, 48, 2009). The authors of this study used South Africa to model a generalized HIV epidemic and predicted that by testing all adolescents and adults at least 15 years of age annually and providing ARVs immediately to those who are found to be infected, HIV incidence would drop from slightly more than 1% a year to .05% a year, effectively ending the epidemic within 50 years. The authors suggested that the epidemic would end because no new infections

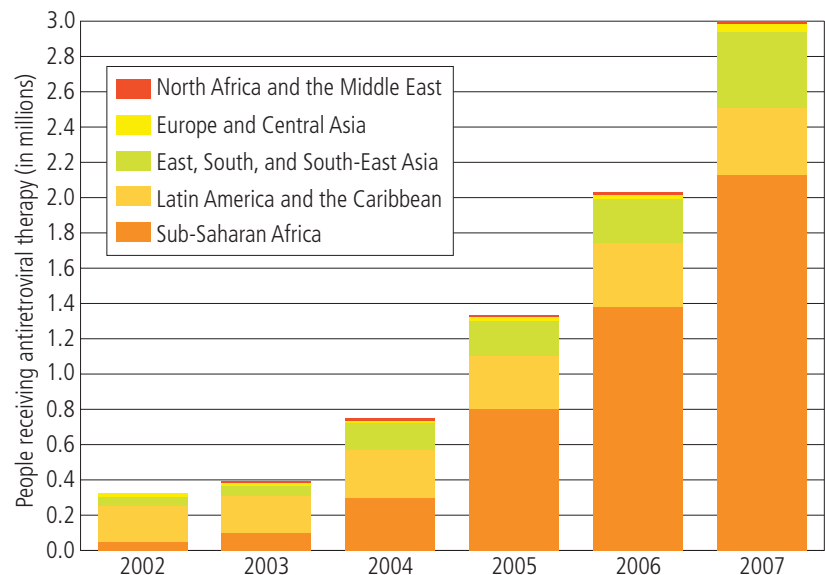
would occur and those already HIV infected would eventually die. The WHO researchers also plugged actual figures from Malawi into the South Africa model because the data from Malawi’s ARV treatment program is considered to be the most reliable, according to Brian Williams, a member of the WHO team who developed this model.

The WHO model is based on the assumptions that all HIV transmission is heterosexual, that 95% of the population receiving ARVs is compliant to therapy, and that access to second-line HAART regimens would be available. The modelers also assumed that the period of acute or primary infection, when viral load is highest and the risk of HIV transmission considered greatest, lasts only about two months, and contributes to only 10% of HIV transmissions.

“Their assumptions about the effect of this strategy are highly optimistic,” wrote David Wil-

Number of People Receiving Antiretroviral Therapy in Low- and Middle-Income Countries

Substantial progress has been made in expanding the availability of antiretroviral (ARV) treatment in low- and middle-income countries. An estimated 3.8 million to 4.3 million people were receiving ARVs by the end of 2008, according to data presented at the 2009 International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention. Sub-Saharan Africa, which has the highest number of HIV-infected people, accounts for about 75% of those on treatment and represents the most dramatic increase in ARV access over the past six years, which has been largely funded by foreign aid such as the US-funded President’s Emergency Plan for AIDS Relief and the Global Fund to Fight AIDS, Tuberculosis and Malaria. *Source: Reuben Granich*



[ASSESSING COMPLIANCE]

Adherence to therapy is one factor essential for the test and treat strategy to work, and researchers will need to study whether starting antiretroviral therapy sooner has any impact on long-term compliance rates.

David Bangsberg, a Harvard Medical School researcher, and his colleague Norma Ware helped develop a model for adherence to antiretrovirals (ARVs) in Nigeria, Tanzania, and Uganda that incorporated more than 400 one-on-one interviews and field observations to explain high adherence rates in areas of extreme poverty (*PLoS Med.* 6, e1000011, 2009).

“The reason people adhere so well, we believe, is because they start their ARVs during late-stage disease when they are bed-bound,” says Bangsberg. “On ARVs, they become strong and healthy again and become an economic resource rather than an economic drain. Doctors call it the Lazarus effect.” It remains to be seen, says Bangsberg, whether adherence would remain high if treatment was initiated before an individual becomes ill.

Researchers will also need to look more closely at patterns of drug resistance and transmission of drug-resistant viruses among HIV-infected individuals who aren't compliant to therapy. —RM

son, an AIDS researcher at the University of New South Wales in Sydney, in a commentary three months after the model was published (*Lancet* 373, 1077, 2009). “They assume that ARV therapy reduces infectiousness by 99%,” Wilson wrote. “This level of reduction is unlikely.”

Williams, who is now with the South African Centre for Epidemiological Modeling and Analysis, says the volume of emails and press coverage that the *Lancet* study generated exceeded anything he had experienced before. Many researchers raised questions about the assumptions made in the model. The WHO researchers acknowledge that better data on test and treat is needed, and they hope studies are done to determine if the approach is feasible. “The real obstacles are political, not scientific,” says Williams. “If you don't have the political will it won't work.”

Other researchers who have modeled test and treat have reached less rosy predictions, yet their analyses still suggest the strategy could help reduce HIV incidence. Based on a model developed by Montaner and colleagues from the British Columbia Centre for Excellence in HIV/AIDS, expanding access to HAART in British Columbia—where only about 50% of those medically eligible are receiving HAART—would decrease the number of new HIV infections by up to 60% over the next 25 years (*J. Infect. Dis.* 198, 59, 2008).

Rochelle Walensky, an associate professor of medicine at Harvard Medical School, used mathematical modeling to measure the impact test and treat could have in Washington, D.C. The US capital has the highest HIV prevalence in the country—an estimated 3% of the half-million residents are HIV infected. In a poster presented at the IAS conference, Walensky concluded that a test and treat strategy could potentially decrease the number of new HIV infections there by as much as 30% over the next five years.

Walensky's model, which used data from a hospital-based treatment center in Washington, D.C., assumed adherence to both testing and treatment would be lower than in the WHO model. “Not everyone will be offered a test, not everybody will accept it, not everybody will be linked to care, not everyone will start ARVs, and not everyone, once they are on ARVs, will remain adherent,” says Walensky.

Walensky says the WHO model was intended to shake things up and push researchers to “think outside the box” at a time when effective preven-

tion strategies are not being developed as fast as the AIDS community would like. “What I worry about is people who read the results and think we are going to easily clear the epidemic, when the model is only theoretical,” she says. “It's not that this strategy does not hold great promise, but we need to keep expectations realistic.”

Determining feasibility

Several researchers are now trying to determine the feasibility of a test and treat strategy. NIAID, along with the US Centers for Disease Control and Prevention (CDC), is developing and sponsoring pilot studies that address the feasibility of universal HIV testing and explore which voluntary counseling and testing strategies work best in specific high-risk populations. HIV transmission risk is highest during the acute stage of HIV infection, so testing strategies would need to target individuals at greatest risk.

But getting high-risk individuals to undergo routine testing has proven difficult, even in countries where treatment is accessible and routine testing is recommended. A recent CDC analysis of 34 US states found 38% of individuals progressed to AIDS within a year after their HIV diagnosis, underscoring the failure to identify HIV-infected individuals soon after they became infected. And the WHO estimates that nearly 80% of HIV-infected adults in sub-Saharan Africa do not know whether they are infected.

Researchers say the reasons people fail to get tested regularly, if at all, varies based on the risk group and geographical region. “One of the critical epidemics [in the US] is among gay men,” says Steven Deeks, who co-directs the population and clinical science core at the GIVI Center for AIDS Research at the University of California in San Francisco. “There is easy access to testing and effective therapies yet they are not getting tested. The typical patient we see, with newly diagnosed HIV, has very advanced disease.”

The transition from testing to treatment would also occur more smoothly if HIV-infected individuals could access ARVs at the same facility where they are tested. However, this is often not the case. Fauci noted in a recent commentary that individuals frequently are diagnosed in settings apart from those where they ultimately receive treatment, and significant barriers impede the efficient movement from diagnosis to care, including lack of health insurance and denial of HIV status (*JAMA* 301, 2380, 2009).

Complexities of transmission

Scientists assessing the feasibility of test and treat will first have to measure how effective ARVs are in actually preventing transmission. Research from the Rakai Health Science Program (formerly the Rakai Project) in Uganda established nearly a decade ago that viral load is the chief predictor of the risk of heterosexual transmission of HIV and that transmission is rare among persons with less than 1,500 copies of HIV RNA per milliliter of blood (*N. Eng. J. Med.* 342, 921, 2000). However, viral load in blood may not always correlate with viral load in semen, and HIV transmission can still occur even if an individual is on suppressive therapy (see *Canvassing CROI, IAVI Report*, Jan.-Feb. 2009).

“I love mathematical modeling,” says Cohen, “but the [WHO] model in question is driven by assumptions that if we test everybody, treat everybody, and the therapy works well, it will prevent transmission. It’s a far cry from having absolute veracity.”

Cohen is leading a trial in Africa, Asia, and Latin America that is looking at whether earlier initiation of ARVs can actually reduce the risk of heterosexual HIV transmission. The study will compare HIV transmission rates among serodiscordant couples based on whether the HIV-infected partner starts ARV therapy early or only when their CD4⁺ T-cell count drops to between 200 and 250. Plans are to enroll 1,750 couples in the seven-year study.

More research will also be necessary to study the relationship between the stage of HIV infection and the likelihood of transmission, as this has implications for the effect ARVs will have on transmission rates. There is scant clinical data showing what percentage of HIV infections actually get transmitted during the acute stage of infection, though it is thought to be high. A study of 235 heterosexual serodiscordant couples conducted by the Rakai Health Science Program found that the rate of transmission per coital act was highest during early-stage infection. The researchers found that among 23 heterosexual couples where the index partner became infected following enrollment in the study, 43% or 10 of the 23 HIV-infected individuals transmitted the virus to their monogamous partners approximately 2.5 months after their seroconversion. The rate of transmission decreased to 15% for the period of six to 15 months following seroconversion of the index partner, and declined even further to 3% between 31 and 40 months later. Rates of transmission increased again during late-stage infection,

with 37% or 19 of 51 transmitted infections occurring during the six to 35 months before death (*J. Infect. Dis.* 191, 1403, 2005).

The financial burden

Even if the science holds up, test and treat would still be extremely expensive to implement. At the IAS conference, much attention was focused on the sustainability of global HIV treatment programs given the current economic crisis. The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Bank reported in July that some international treatment programs are facing drug shortages, resulting in interruptions in treatment after the global recession prompted some public and private donors to reduce their funding. And, in many countries, availability of second-line drug regimens is limited and often prohibitively expensive. This has some researchers wondering where additional money for implementing test and treat would come from.

What I worry about is people who read the results and think we are going to easily clear the epidemic when the model is only theoretical.

— Rochelle Walensky

UNAIDS estimates that US\$25 billion will be required to achieve universal access to HIV treatment, prevention, care, and support in low- and middle-income countries by 2010, including \$7 billion for provision of treatment based on the current treatment guidelines.

While the WHO researchers who developed the test and treat model suggest that implementing the strategy would require substantially more money in the short term—about \$3.5 billion a year in South Africa and \$85 billion total—the financial burden would be alleviated as the number of new infections started to decline. “I think test and treat is absolutely doable,” says Williams. But others are not as confident. “The proponents of this [test and treat] strategy are not being realistic and could do a lot of harm to treatment programs if there is a competition for resources,” says Ron Gray, a professor of population and family planning at the Johns Hopkins Center for Global Health. ■

Kristen Jill Kresge contributed reporting to this article.

Vaccine BRIEFS

Phase II Prime-Boost Trial Begins in the US

A PHASE II TRIAL TESTING the safety and efficacy of a DNA/Ad5 prime-boost regimen of two vaccine candidates developed at the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID) recently began enrolling volunteers. The trial, which is sponsored by NIAID and is being conducted by the HIV Vaccine Trials Network (HVTN), is referred to as HVTN 505. Researchers aim to enroll 1,350 men who have sex with men (MSM) at 15 sites in 12 US cities.

The VRC prime-boost regimen involves three vaccinations with DNA encoding HIV clade B Gag, Pol, and Nef, and Env from HIV clades A, B, and C, followed by a vaccination with an adenovirus serotype 5 (Ad5) vector encoding clade B Gag and Pol, and Env from clades A, B, and C. The HVTN 505 trial will evaluate the safety of the prime-boost regimen, as well as its ability to decrease set point viral load in volunteers who become HIV infected despite vaccination.

The study is not powered to detect whether the prime-boost regimen can prevent HIV infection altogether. The VRC candidates being evaluated in HVTN 505 were originally slated to be tested in a much larger Phase IIb test-of-concept trial known as PAVE 100, involving 8,500 HIV-uninfected men and women from North and South America and Africa. But in 2007, after another Ad5-based candidate developed by Merck—known as MRKAd5—failed to prevent transmission of HIV or slow disease progression in vaccinated volunteers, the start of PAVE 100 was postponed. The protocol for this trial then went through a number of revisions before NIAID settled on the current version.

Results of the STEP trial indicated that male volunteers who received the vaccine candidate had a higher risk of acquiring HIV if they were uncircumcised and had pre-existing antibodies against the Ad5 vector as compared to placebo recipients with the same characteristics. Based on these results, only circumcised MSM with no pre-existing antibodies against Ad5 will be eligible to enroll in HVTN

505. “We are deeply committed to the population that we are hoping to recruit for this trial,” says Scott Hammer, study chair of the HVTN 505 trial. “We know we are asking a major commitment of them and we will do our part to provide them with counseling and education about vaccines and make sure that they are fully aware of the STEP trial results.”

Alan Fix, chief of the Vaccine Clinical Research Branch at the US National Institutes of Health, says there are a number of differences between the regimen being assessed in HVTN 505 and MRKAd5. In the STEP trial, volunteers

received three doses of the Ad5-based vaccine candidate, whereas in HVTN 505 they will receive only one dose of the Ad5 candidate as a boost, following three immunizations with the DNA candidate. And MRKAd5 did not contain HIV Env, whereas the VRC’s DNA and Ad5 candidates both encode HIV Env. There are also differences between the Ad5 vectors used in the STEP and HVTN 505 trials, not just the immunogens. “We don’t know if this vaccine, with its similarities and differences from the Merck vaccine, will behave the same way,” says Fix, which is why researchers have decided to restrict the study population for HVTN 505. The prime-boost regimen is not being evaluated for future commercial licensure, according to Fix.

The Fenway Community Health Center in Boston is one of the research centers now screening volunteers for the HVTN 505 trial. Ken Mayer, principal investigator at this site, says the informed consent documents include information about the STEP trial results and community information forums are being held to help potential participants understand the trial and put the results in their proper context. “While we hope to be vaccinating people very soon, it’s been an intense screening process,” says Mayer. Other sites are also holding town hall meetings in the coming months to clarify any questions regarding participation in HVTN 505. —Regina McEnergy

We don’t know if this vaccine, with its similarities and differences from the Merck vaccine, will behave the same way.

— Alan Fix

South African AIDS Vaccine Initiative Launches Phase I Trial

THE SOUTH AFRICAN AIDS VACCINE INITIATIVE (SAAVI) commemorated the launch of the South African arm of a Phase I AIDS vaccine trial at the Emavundleni Prevention Centre in Cape Town on July 20. The purpose of the trial is to evaluate the safety and immunogenicity of a prime-boost regimen, comprised of a DNA plasmid vaccine candidate followed by a modified vaccinia Ankara (MVA) vector-based candidate, developed by researchers in South Africa.

The trial, known as SAAVI102/HVTN 073, is being conducted in collaboration with the HIV Vaccine Trials Network (HVTN) and the National Institute of Allergy and Infectious Diseases (NIAID) at the US National Institutes of Health (NIH).

Researchers plan to enroll 36 volunteers at two clinical research centers in South Africa—the Emavundleni Prevention Centre and Chris Hani Baragwanath Hospital in Soweto. Twelve volunteers have already been successfully enrolled and vaccinated in the US arm of the study, which is being conducted at the Fenway Community Health Center in Boston.

The DNA vaccine candidate was constructed in South Africa using a plasmid provided by the Vaccine Research Center at NIAID and the MVA candidate was developed by researchers at the University of Cape Town with funding from SAAVI and the NIH. Both candidates were manufactured in the US and carry HIV clade C antigens. “We wanted to have a vaccine based on the subtype of the virus that circulates in South Africa,” said Anna-Lise Williamson, of the University of Cape Town, who led the development of the

vaccine candidates and remarked on the significance of this. “We’re seen as the place to test vaccines, now we’ve developed one,” she said. “It usually happens the other way around.”

SAAVI is the lead program of the South African Medical Research Council (MRC) and was established by the South African government and the energy supply company Eskom in 1999 to coordinate the development of an HIV vaccine for southern Africa. Anthony Mbewu, president of the MRC, called the launch of this trial a “scientific milestone,” which he said “ensures that South Africa will be better able to design and develop vaccines against infectious agents in the future.”

Anthony Fauci, director of NIAID, said that scientists in South Africa received more NIAID funding last year than any other country outside the US. “You have the intellectual capital and people who are passionate about health, especially in the arena of HIV/AIDS,” he said. Fauci also mentioned the “extensive challenges” facing AIDS vaccine researchers.

Despite these challenges, many speakers noted the importance of continuing AIDS vaccine research. “The cost of [HIV] treatment is very high,” said Naledi Pandor, the South African Minister of Science and Technology. “I therefore cannot overstate the importance of the development of a vaccine for the South African population.”

“While we have the biggest antiretroviral roll out program in the world, vaccines are the best way to control infectious diseases,” said Williamson. —*Kristen Jill Kresge*

Tracking Spending on Vaccine, Microbicide, and PrEP Research and Development

FUNDING LEVELS FOR HIV vaccine research declined last year for the first time since 2000, most notably within the pharmaceutical industry, according to the HIV Vaccine and Microbicide Resource Tracking Working Group, which released a report at the 5th International AIDS Society (IAS) Conference on HIV Pathogenesis, Treatment and Prevention, held July 19-22 in Cape Town. The resource tracking group says, however, that over the past nine years investment in all experimental biomedical prevention strategies, including vaccines, has increased. The AIDS Vaccine Advocacy Coalition (AVAC), the Alliance for Microbicide Development, the Joint United Nations Programme on HIV/AIDS, and IAVI helped collect the figures and compile the report, which is available online at www.hivresourcetracking.org.

According to the report, US\$1.2 billion was spent in 2008 on HIV prevention research—\$868 million for AIDS vaccine research and development, \$244 million for microbicides, and

close to \$44 million for the study of pre-exposure prophylaxis (PrEP)—administration of antiretrovirals in an effort to block HIV infection. Spending on AIDS vaccine research fell 10% from 2007, while research for microbicides and PrEP rose by 8% and 13% respectively. The report cites shifts in scientific priorities and the likely influence of the global financial downturn as contributing factors to the decline in vaccine research and development funding. The cessation of immunizations in late 2007 in the STEP and Phambili trials, which were testing a candidate vaccine developed by Merck, ended one of the pharmaceutical company partnerships for HIV vaccine research and development.

Kevin Fisher, policy director at AVAC, says the estimates were drawn from a range of sources, including one-on-one interviews and publicly available information on clinical trials and spending by public agencies like the National Institute of Allergy and Infectious Diseases that support research and trials. —*Regina McEneaney*

Research BRIEFS

Early Damage to the Gut Environment B Cells Need to Mature

WHILE T-CELL DEPLETION in the gut-associated lymphoid tissue and in blood has been well documented, not much is known about the effect of early HIV infection on B cells. A study has now taken a closer look, and found a possible explanation as to why the body produces HIV-specific neutralizing antibodies much later after infection than in response to other pathogens (*PLoS Med.* 6, e1000107, 2009).

Researchers found that just a few weeks after HIV infection, more B cells have matured into antibody-producing memory B cells in blood and in the gut of HIV-infected individuals than in uninfected people. These B cells are often not HIV specific, and instead include influenza and autoreactive antibodies. As a result, HIV-infected people have fewer naive B cells available to mature into B cells that produce HIV-specific antibodies. To make matters worse, the study also found that just weeks after HIV infection, the environment in the gut that could induce the maturation of naive B cells into cells that produce HIV-specific antibodies is damaged.

It's a bit like looking at a car wreck and saying, I know a wreck has occurred, but exactly how did it happen? —Anthony Moody

“People have identified damage to the T-cell arm of the immune system,” says Anthony Moody, an assistant professor of pediatrics at Duke University and one of the lead authors of the study. “I think our paper added that damage is also occurring to the environment that supports the B cells. It adds another face onto the picture of acute HIV infection.”

The researchers analyzed B cells and their environment from biopsies of the terminal ileum section of the gut as early as 47 days after HIV infection. The terminal ileum is the last part of the small intestine and contains a large amount of lymphoid tissue. They found that HIV-infected people had damage to the gut follicles where B cells collect and mature to become antibody-producing cells. This damage included cell death and disruption of the arrangement of dendritic cells, which present antigen to the B cells and support the different cell types in the follicle.

In addition, fewer B cells in the gut follicles were dividing in HIV-infected people, although there did not seem to be fewer B cells overall in the gut than in uninfected people. “This data is suggesting

that the environment of the B cells to mature into antibody secreting cells is being damaged,” says Moody. “That may be part of the reason why patients infected with HIV don’t make a good antibody response to HIV.” However, what leads to the damage is still an open question. “It’s a bit like looking at a car wreck and saying, I know that a wreck has occurred, but exactly how did it happen?”

Researchers also found that the terminal ileum of HIV-infected people also contained fewer naive B cells, and more memory and plasma B cells as a percentage of all B cells. The same was observed in blood samples taken as early as 17 days after infection. However, many of these memory and plasma B cells were not HIV specific, and instead included antibodies to the body’s own antibodies usually seen in patients with rheumatoid arthritis. “They are just making antibodies to everything,” Moody says. “It’s not a response the body generally would want to make. Any specific HIV response is probably being drowned out by all of the non-specific responses that are being driven through.”

One possible cause for this early unspecific B cell maturation, Moody says, might be an early production of cytokines in HIV-infected people such as interleukin 15 and interferon- α . This might be the earliest sign of the chronic inflammation associated with HIV infection that eventually leads to AIDS.

Taken together, these observations could explain why HIV-infected people only show neutralizing antibodies to HIV 40 or more days after infection, much longer than the week it often takes for the body to mount such a specific response to other pathogens, Moody says.

“While B cell dysfunction is known to be a feature of chronic HIV infection, this is the first study to demonstrate B cell pathology in gut tissues and peripheral blood in acute and early HIV infection,” says Savita Pahwa, a professor at the University of Miami School of Medicine who was not involved in the study. “These findings highlight the need for a vaccine that at the very least has to act early enough so that it can prevent the virus from causing the damage to the immune system that ensues rapidly after HIV infection.”

Next, Moody plans to study the bone marrow of HIV-infected people to see if there is any damage to the generative environment of B cells. “The bone marrow is where [B] cells originate, and if there is damage, that would be another reason why people may have damage to their immune systems by HIV,” he says. —*Andreas von Bubnoff*

Lower Antibody Levels Can Protect from Low-Dose Challenge

PREVIOUS STUDIES HAVE FOUND that high serum concentrations of broadly neutralizing antibodies are required to completely protect rhesus macaques from a high-dose intravenous challenge. But according to a recent study, much lower concentrations of the broadly neutralizing antibody b12 can significantly lower the risk of infection in rhesus macaques exposed to a repeat low-dose vaginal challenge regimen with a simian immunodeficiency virus (SIV)/HIV hybrid virus (*Nat. Med.* 15, 951, 2009). Such a regimen is considered more similar to HIV transmission than a single high-dose challenge.

This is promising news for vaccine researchers as it suggests that an HIV vaccine would have to induce lower levels of antibodies than previously thought to significantly decrease the risk of infection.

Researchers infused rhesus macaques once a week with a dose of 1 mg/kg of the broadly neutralizing antibody b12, and then challenged them vaginally twice a week with a low dose of SHIV162P3. This dose of the challenge virus was at least 30-times lower than what is usually used in high-dose intravenous challenge studies in macaques. At this dose, it took 10 challenges to infect four macaques that were untreated or treated with a mock antibody, while it took 108 challenges to infect the same number of b12-treated macaques.

Before the new study, it was perceived that the levels of elicited neutralizing antibodies needed to protect against HIV challenge may be unachievable, says Ann Hessell, a senior research assistant at The Scripps Research Institute and first author of the study. For example, one previous study used a 25-fold higher dose of b12 antibody than what was used in the new study to

protect all macaques from an intravenous high-dose challenge with a different challenge virus (*J. Virol.* 75, 8340, 2001). The fact that the two studies used different challenge viruses makes it difficult to compare the antibody doses, Hessell says. Still, the new study suggests that a much lower dose of antibody can decrease the risk of infection from a repeat low-dose challenge than the antibody dose needed to protect from a high-dose challenge.

It has often been said that it will take supraphysiologic levels of neutralizing antibodies to protect... these data support a more encouraging model. —John Mascola

“It has often been said that it will take supraphysiologic levels of neutralizing antibodies to protect against HIV-1 infection,” says John Mascola of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases, who was not involved in the study. “These data support a more encouraging model suggesting that modest levels of vaccine-elicited neutralizing antibodies could have a major impact on acquisition of HIV-1 infection.”

“If you had this amount of antibody on board [in humans], then you could decrease the risk of becoming infected, and that’s a benefit,” says Dennis Burton, a professor at The Scripps Research Institute, who led the study. “[But] this is a model and it’s one antibody and it’s one virus and so it should be taken very carefully.” —*Andreas von Bubnoff*

2G12 Revisited

AMONG THE HANDFUL of broadly neutralizing antibodies to HIV that have so far been isolated, 2G12 is unique because it recognizes the sugar coat of HIV, as opposed to the viral proteins. A study in 2000 showed 2G12 is also exceptional because even though it neutralizes virus relatively poorly *in vitro* compared with other broadly neutralizing antibodies, it protects animals similarly well (*Nat. Med.* 6, 207, 2000).

In a recent study, Ann Hessell, a senior research assistant at The Scripps Research Institute, and colleagues confirmed these earlier findings that 2G12 could protect rhesus macaques from infection better than what would be expected from its poor neutralization ability *in vitro* (*PLoS Pathog.* 5, e1000433, 2009). 2G12 completely protected three out of five rhesus macaques from high-dose vaginal challenge with the hybrid simian immunodeficiency virus (SIV)/HIV challenge strain SHIV162P3.

2G12 protected at about twice the dose of the b12 antibody dose that had previously been shown to protect all animals from

a similar high-dose challenge (*J. Virol.* 75, 8340, 2001). While it is difficult to compare the antibody doses in the two studies because they used a different challenge virus, this suggests that 2G12 can protect from high dose challenge at a similar dose as what was previously shown for the b12 antibody. However, the new study also showed that 2G12 fared about 100-times worse than b12 at neutralizing the challenge virus *in vitro*.

“Generally we tend to think that neutralization correlates with protection,” says Dennis Burton, a professor at The Scripps Research Institute, who led the study. “[But 2G12] boxes above its weight in the sense that it protects [animals] at relatively low neutralization titers.”

John Mascola, of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases, who led the 2000 *Nature Medicine* study, says the new 2G12 study is “more definitive and clearly shows that relatively modest levels of 2G12 have a major *in vivo* impact.”

However, just why 2G12 does so much better *in vivo* than

what would have been expected from its *in vitro* neutralization ability is still unclear. The new research ruled out the possibility that 2G12 may be better transported from blood to mucosal tissues, such as those in the vagina, than other antibodies like b12.

Another explanation could be that there are many biological components that are present *in vivo* but not *in vitro*, Hessell says. “*In vitro* you don’t have all of the mechanisms of the human immune system. You don’t have other cells and things like complement that can be brought in,” she says. “*In vivo* you bring in these other aspects that possibly influence the mechanism by which the antibody is able to protect.”

Whatever the case, there might be something advantageous

to antibodies like 2G12 that are directed against the sugar coat of HIV as opposed to protein targets, Burton says. Perhaps 2G12 acts especially strongly on HIV transmission in that it inhibits interactions between the sugars on the HIV surface and proteins called lectins on host cells that pick up the virus during transmission, according to Burton.

Hessell is also the first author of another single high-dose challenge study submitted by Burton’s lab that came to similar, although less dramatic, conclusions for the broadly neutralizing antibodies 2F5 and 4E10, suggesting that these two antibodies are also better at *in vivo* protection than what would be expected from their *in vitro* neutralization ability. —*Andreas von Bubnoff*

Two Studies Add to Understanding of HIV Assembly

THE EXACT WAY HIV assembles during and after budding from an infected cell is still not completely understood, but two recent studies give more detailed insight into this process, which might eventually lead to the development of new drugs that inhibit HIV assembly.

One study shows, at a higher resolution than previously reported, how full-length Gag proteins assemble to form a honeycomb-shaped lattice of hexamers under the plasma membrane of immature HIV particles as they are released from the host cell (*Proc. Natl. Acad. Sci.* 106, 11090, 2009). The study used a method called cryoelectron tomography, where HIV samples are frozen extremely quickly and then analyzed by electron microscopy. This avoids the formation of ice crystals that could destroy biological structures.

John Briggs, a researcher at the European Molecular Biology Laboratory in Heidelberg, Germany, and lead author of the

study, says it was already known from previous studies that the spheres formed by the lattice are incomplete, with sometimes as much as one third missing, similar to an egg with its top cut off (*EMBO J.* 26, 2218, 2007; *Cell Host Microbe* 4, 592, 2008).

But it was unclear how the continuous part of the honeycomb-shaped Gag lattice could be curved because a lattice of hexamers should be flat. “We always knew that [the lattice] had to have something other than hexamers going in there because otherwise [it] couldn’t be a curved structure,” Briggs says. “The question was, what are those things?” One possibility, he says, was that it contained pentamers just like a soccer ball, although it was also considered possible that the lattice contains holes.

Researchers found evidence for holes and not pentamers (see Fig. 2). “This, I think, is the first time that it has been shown convincingly that those are irregular shaped defects rather than anything regular,” says Briggs.

Elizabeth Wright, an assistant professor at Emory University School of Medicine and the first author of the 2007 *EMBO Journal* study, says the new study complements previous studies in its demonstration that immature HIV virions and immature *in vitro* assembled particles are not composed of a completely enclosed protein shell. In addition, she says, it “unequivocally [illustrates] that the hexagonal lattice appears to be a continuous sheet into which irregular defects are included to generate the curvature necessary to make an enclosed particle.”

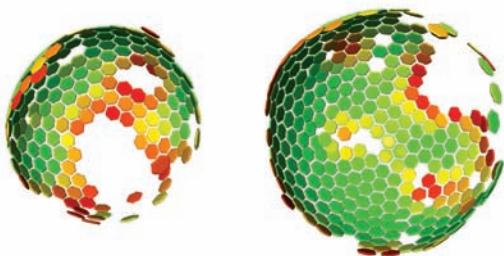
Another recent study took a detailed look at the cone-shaped capsid of mature HIV particles, which forms at a later stage in the HIV life cycle and encloses the genetic material of the virus (*Cell* 137, 1282, 2009). The study, led by Mark Yeager of The Scripps Research Institute and the University of Virginia, is the first X-ray crystallographic analysis of the capsid protein CA, which is generated when HIV protease cleaves Gag in the immature Gag protein lattice studied by Briggs. The CA proteins then form the cone-shaped capsid by assembling as about 250 hexamers and 12 pentamers.

The CA proteins arrange in a noncovalent way to form the hexamers and pentamers, but exactly how they are arranged

FIGURE 2

Lattice Maps for Immature HIV Particles

The 3D computer reconstruction shows the immature Gag lattice of HIV that matures to form the protein shell of the infectious virus. Maps are shown in perspective such that hexamers on the rear surface of the particle appear smaller. The side of the particle toward the viewer lacks ordered Gag. *Image courtesy of John Briggs/ European Molecular Biology Laboratory.*



has been unclear. This was in part because the capsid of each HIV particle is slightly different, making it impossible to grow crystals of intact capsids to determine the structure at high resolution using X-ray crystallography, Yeager says. In addition, the CA protein itself has two domains that are connected with a flexible linker that impedes growth of protein crystals.

So the researchers used a trick. Guided by a model of the hexameric CA lattice based on a lower resolution structure determined using electron cryomicroscopy, they engineered CA hexamers that were stable enough to grow crystals. This enabled the determination of the X-ray structure of the CA hexamer at an unprecedented atomic resolution of two Ångstrom (see cover image).

The study for the first time identifies the atomic structure

of the so-called NTD-CTD interface that holds the N-terminal and C-terminal domains, or opposite ends, of adjacent CA proteins in the hexamers together. It shows that water molecules make the surface of the interacting proteins somewhat slippery. This, Yeager says, could explain how the CA protein is flexible enough to both arrange in the form of hexamers as well as pentamers.

“We had little clue about the molecular details of the NTD-CTD interface,” says Ian Taylor of the National Institute of Medical Research in London, who was not involved in the study. “This paper now provides these details and furthermore confirms the idea that interdomain flexibility is the driver of shell curvature.” Knowing the NTD-CTD interface could help develop drugs that inhibit assembly and maturation of HIV. —*Andreas von Bubnoff*

Public Database of Viral Vectors Released

COMPUVAC, A CONSORTIUM OF 140 SCIENTISTS from 11 countries that was established in 2005, held a day-long symposium on June 29 at the Collège de France in Paris to commemorate the public release of an interactive database and bioinformatics tool that allows researchers to compare different viral vectors and virus-like particles (VLPs) to predict the best potential immunization strategy for the development of novel vaccines. This database was the culmination of an €8 million grant from the European Union under its Sixth Framework Programme.

CompuVac was created to help standardize the evaluation of viral vectors and aid the rational development of novel recombinant vaccines. “Many novel technologies for development of new vaccines exist but their design or improvement profoundly lacks a reliable evaluation system,” said David Klatzmann, CompuVac’s coordinator. This is because it is difficult to compare results from different studies because researchers often use different antigens or methods to evaluate vaccine vectors or VLPs.

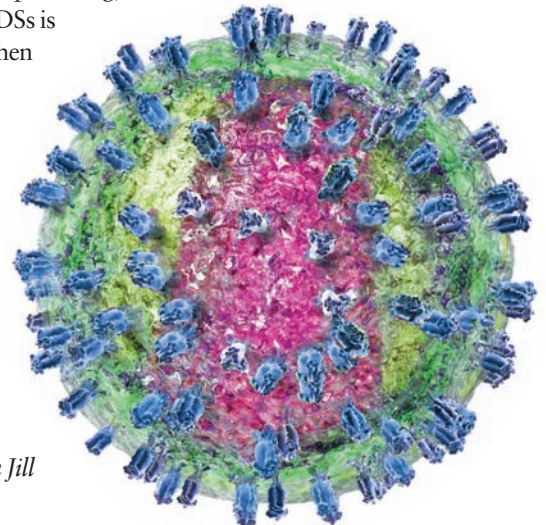
Cedrick Britten, a T-cell immunologist from Johannes Gutenberg University in Germany, spoke at the symposium about efforts to encourage standardization in cancer research. He is pioneering an effort that would require any publication of immunology data to provide an explanation of methods. Although this “reporting standard,” as he called it, falls short of requiring use of standardized assays, it is a first step. Britten joked that a legal body would be required to realistically impose standardization because researchers are so unwilling to accept another’s protocol, but then cited an example involving a Grand Challenges in Global Health grant from the Bill & Melinda Gates Foundation that successfully promoted standardization. This grant provided free reagents to researchers as a way to motivate them to use the same reagents.

Peter Piot, chairman of the board of the Global HIV Vaccine Enterprise, who also spoke at the symposium, said that the biggest resistance to standardization and sharing data comes from academic researchers, and not industry. He said that many of the “organizational challenges” in the AIDS vaccine field are “as big

as the scientific ones.”

In an effort to standardize evaluation, CompuVac set out to study several vaccine vectors, including adenovirus, herpes simplex virus-1, measles virus, modified vaccinia Ankara, Bacillus Calmette-Guérin, and DNA, as well as many VLPs, using standardized antigens—the GP33-41 epitope from lymphocytic choriomeningitis virus to evaluate T-cell responses and the envelope protein from vesicular stomatitis virus to evaluate B-cell responses. In all, CompuVac researchers fully assessed the immunogenicity of 51 different vectors with these so-called “gold-standard” antigens, based on their ability to induce T- and B-cell responses, as well as their ability to protect against an infectious challenge in mice. CompuVac researchers also assessed the gene-expression profiles induced by the different vectors and VLPs expressing the standard antigens. The results of these assessments are available in the Genetic Vaccine Decision Support system (GeVaDSs), the online database created by the consortium that stores this data and allows users to create associations and develop algorithms to compare new vaccine vectors or VLPs to those previously analyzed (available at www.compuvac.org).

The premise of GeVaDSs is that researchers can then predict, based on the data sets for each of these single vectors, the best homologous or heterologous prime-boost combinations to evaluate further. The GeVaDSs system is what Klatzmann refers to as a “systems vaccinology approach.” —*Kristen Jill Kresge*



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