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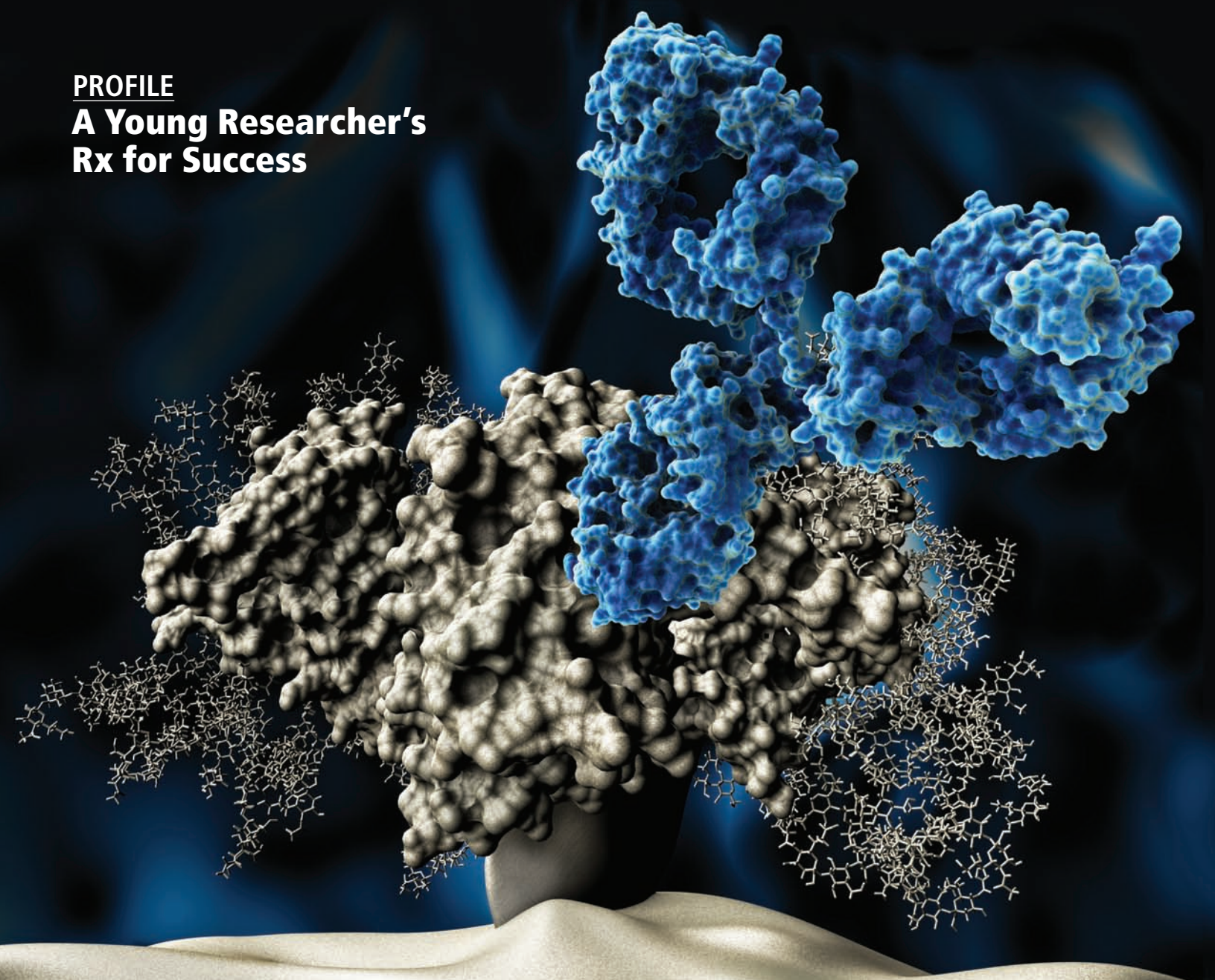
AIDS VACCINE 2010

ADVANCES ABOUND

PROFILE

A Young Researcher's Rx for Success



EDITOR'S LETTER

In September, more than 1,000 researchers gathered in Atlanta for AIDS Vaccine 2010. The feeling of optimism that has been palpable in the field over the past year persisted as a plethora of new, incremental scientific advances were reported over the course of the four-day meeting. And as usual, we devote a substantial amount of this issue to reporting on these advances (see *A Change of Tune*, page 4).

The antibody frenzy that kicked off a year ago with the first report of new HIV-specific broadly neutralizing antibodies continued in Atlanta. Several groups have fished out dozens of additional broadly neutralizing antibodies from HIV-infected individuals. Despite the ease with which researchers now seem to be able to isolate these antibodies, they are indeed rare. Only 1%-2% of HIV-infected individuals make very broad neutralizing antibodies. While it isn't clear what allows certain people to generate such antibodies, researchers are now exploring genetic factors as a possible explanation. They are also using genomic sequencing techniques to analyze how antibodies develop a series of mutations from their germline form, allowing them to better bind to and neutralize HIV.

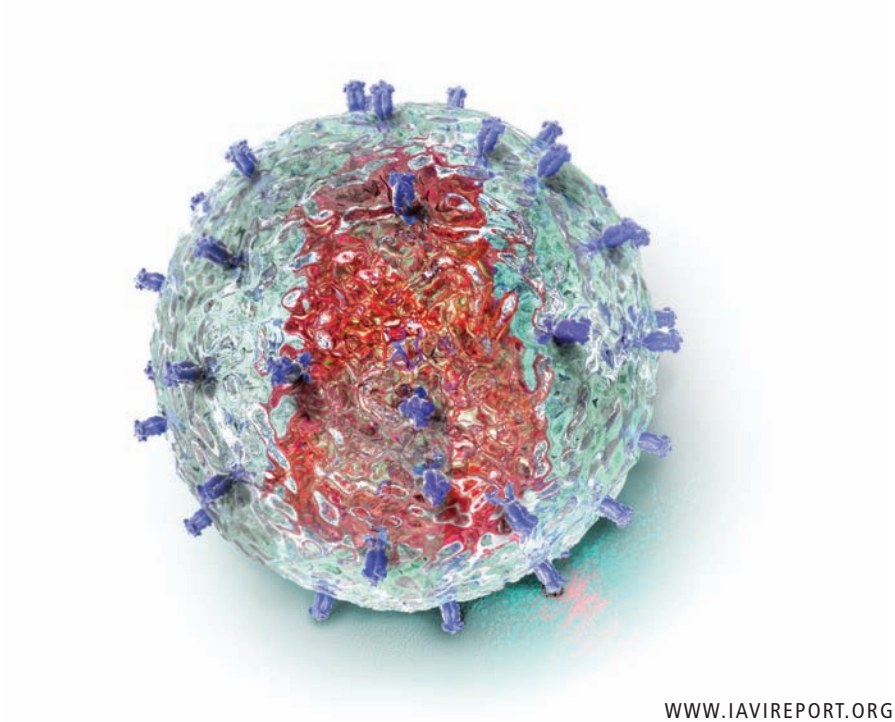
Another focus is on the structural details of these antibodies and how these insights can be used to engineer immunogens based on the HIV epitopes they target. Immunogen design is a burgeoning area of research, and though it poses many challenges, researchers are combining the principles of structural and computational biology to design immunogens capable of eliciting antibodies in animal models. Given the importance of immunogen design, it will be the focus of the next iteration of the Center for HIV/AIDS Vaccine Immunology, details for which were outlined recently (see *Vaccine Briefs*, page 19).

While developments in the antibody arena are likely to continue, more clinical trial activity is also expected. One new trial began recently (see *Vaccine Briefs*, page 18), and the plans for several others were outlined in Atlanta.

To round out this issue, there is a profile of Brandon Keele, a young researcher who recently established a laboratory at the National Cancer Institute after a successful six-year postdoctoral stint at the University of Alabama in Birmingham (see *Luck Favors the Prepared*, page 13). Keele's work led to publication of several seminal papers, and in this article he discusses some of the factors that contributed to his success.

KRISTEN JILL KRESGE

IN THIS ISSUE



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[ON THE COVER]

Structural model of HIV gp120 trimer (in silver) and immunoglobulin G (in blue) based on the work of Sriram Subramaniam at the National Cancer Institute, Peter Kwong at the Vaccine Research Center, Bill Schief at the University of Washington, and Erica Ollmann Saphire at The Scripps Research Institute (TSRI) in La Jolla, California.

Illustration by Christina Corbaci at TSRI; provided courtesy of Ann Hessell at TSRI.

A Change OF TUNE

Following the first trial showing efficacy and continuing progress in other areas of research, a new chord of optimism was struck at AIDS Vaccine 2010

By Kristen Jill Kresge and Regina McEnery

A few years ago it was not uncommon to hear Anthony Fauci, the veteran director of the National Institute of Allergy and Infectious Diseases (NIAID) at the US National Institutes of Health (NIH), publicly question whether it would be possible to develop an AIDS vaccine. And although the prime-boost vaccine regimen tested in the controversial RV144 trial in Thailand provided only modest efficacy (31.2%), it was enough to make Fauci a convert.

"It is feasible to block acquisition of HIV infection. We know from the Thai trial that it can be done," Fauci said at AIDS Vaccine 2010, held in Atlanta, Georgia, from Sep. 28 to Oct. 1. "Before, I was not so sure it was feasible. The proof of concept here is huge."

During his signature overview talk at the annual conference, Fauci highlighted recent progress in the isolation of HIV-specific broadly neutralizing antibodies and their role in structure-based vaccine design, as well as the insights from and plans to build upon the results of RV144. Advances in these areas, as well as many others, including new plans for designing clinical trials and additional data from viral vectors, were presented in the jam-packed sessions that occurred throughout the four-day meeting.

"I believe we are seeing a real reason for optimism," said Alan Bernstein, executive director of the Global HIV Vaccine Enterprise, which co-hosted AIDS Vaccine 2010 with Emory University's Center for AIDS Research. Fauci was also enthusiastic about the progress being made. "Our task now is to use the science to get us closer to a much more effective vaccine," said Fauci. "I don't think there's any question we're going to get there. We have a light at the end of the tunnel. All we need to do is follow the light, follow the science."

RV144: The search continues

Without a doubt, the results of the RV144 trial helped galvanize AIDS vaccine research. Now, researchers are mining the samples from the trial in the search for possible immune correlates of protection that could potentially allow researchers to rationally improve upon what Nelson Michael, director of the US Military HIV Research Program (MHRP), called the "early but nondurable efficacy" seen in RV144 with the ALVAC canarypox-based prime and AIDSVAX gp120 boost. One year into the RV144 trial the efficacy was as high as 60% (based on the modified intent-to-treat analysis), but it slowly waned over the course of the three-and-a-half-year trial.

Michael reported that MHRP and the 35 investigators at 20 different institutions who are collaborating on the RV144 correlates analysis are still in the initial phase of the process that

involves evaluating a broad range of assays and selecting those that will be used, come January 2011, to evaluate the precious RV144 samples in a case-control design. Although the case-controlled studies have not yet begun, researchers at MHRP have already made some intriguing observations, which Michael summarized.

In an exploratory analysis, researchers found that a small group of 60 vaccinated volunteers from RV144 who remained HIV uninfected and who had a positive response by interferon (IFN)-γ ELISPOT (greater than or equal to 20 million spot-forming cells per million cells and at least four times background) had a high frequency of $CD4^+$ T-cell responses that targeted two distinct epitopes (peptides 44 and 49) in the V2 loop of gp120. T-cell responses directed toward these epitopes were not seen in 68 volunteers with breakthrough infections (who are being followed in a separate trial, RV152), and are very rare in HIV-infected Thais (only one person from natural history studies has been found to have these responses).

"That may be on the pathway to a correlate," said Michael. But he cautioned that this finding was far from conclusive because these immune responses may have occurred in all vaccinated volunteers but then been eradicated when an individual became HIV infected.

What is unique about peptide 44 on the V2 loop is that it includes the binding motif for the integrin α4β7, which was shown by James Arthos and colleagues in Fauci's lab to be an additional receptor on $CD4^+$ T cells that HIV gp120 binds to and signals through (*Nat. Immunol.* **9**, 301, 2008). "This receptor defines a subset of T cells in the cervix and rectum that is highly susceptible to HIV infection," said Fauci. The α4β7 integrin mediates the migration of activated T cells to gut-associated lymphoid tissue, where HIV wreaks the most substantial, and often irreversible, damage to the immune system very soon after infection. Arthos and colleagues found that the efficiency of gp120 binding to α4β7 varied considerably. According to Fauci, Env from newly transmitted HIV has been shown to bind better to α4β7 than chronically replicating virus that is more heavily glycosylated. He concluded that an Env confirmation that is defined by easy binding to α4β7 "should seriously be considered as a vaccine target."

Linear epitope mapping of the B-cell responses in RV144 volunteers conducted by the Vaccine Research Center (VRC) at NIAID also indicate that antibody responses to the V2 loop are more frequent in RV144 volunteers than in volunteers in the HIV Vaccine Trials Network (HVTN) 204 study, which tested the DNA/adenovirus (Ad) serotype 5 prime-boost regimen developed at the VRC that is now being tested in the large Phase II trial, HVTN 505.

Taken together, these observations have piqued the interests of investigators and Michael said that assays looking at vaccine-induced immune responses to the V2 loop, and to the peptides that contain the α4β7 binding site specifically, "will make the cut for case-control studies."

Meanwhile, there are also plans in place for additional clinical trials to build on the results of RV144. One of these follow-up studies is focused on determining the correlates of protection and is being planned with optimal specimen collection. Another study will test the effect of administering an additional gp120 protein boost to already vaccinated individuals from RV144.

Some of these studies will get underway as early as next year, but it will take until 2013 or 2014 for the next efficacy trial to begin with the same regimen that was tested in RV144. "The field wasn't in the position to capitalize on success," said Michael, who reviewed plans for two RV144 follow-up efficacy trials. The first is a Phase IIb trial in Thailand that Michael called a "top priority" because it has the potential to lead to licensure of the vaccine candidate in this region. This trial, which will be funded by the US Army, the Thai Government, the NIH, and Sanofi Pasteur, will test the same ALVAC/AIDSVAX regimen used in RV144 with an additional gp120 protein boost administered six months after the fourth vaccination (12 months after the first vaccination). This trial will enroll men who have sex with men (MSM) at high risk of HIV infection, a much different population than was enrolled in RV144. Immediately after the RV144 results were reported, some researchers speculated that the almost exclusively heterosexual and low- to moderate-risk trial population may have been an important factor in the vaccine's modest success. Several studies have shown that despite substantial viral diversity in the infected partner, the majority of heterosexual infections can be traced back to a single transmitted founder virus—only about 20% of cases are the result of more than one founder virus. However, more than one transmitted founder virus is evident in about 40% of the infections studied among MSM, suggesting this mode of transmission may widen the range of viruses a vaccine would have to block to be protective.

Another Phase IIb efficacy trial, which would also start in 2014, is being planned in southern Africa. This trial will involve high-risk heterosexual volunteers and is being funded by the Bill &

Melinda Gates Foundation, the NIH, the HVTN, Sanofi Pasteur, and Novartis RSA, among others, according to Michael. He said the objective of this trial is to see if the efficacy seen in RV144 can be extended to other geographic regions where there is greater viral diversity. Investigators collaborating on this trial are still deciding which gp120 protein will be used as the boost in this trial.

At the NIH's AIDS Vaccine Research Subcommittee meeting, which took place the week before the Atlanta conference in Rockville, Maryland, there was extensive discussion about whether to use two clade C HIV gp120s or one clade A and one clade C. Although clade C is predominant in southern Africa, there is evidence from the Phambili trial, the Phase IIb trial of Merck's adenovirus-based candidate that was halted early, that clade A virus may be emerging in South Africa. Additionally, researchers think that including two gp120 boosts of different clades may increase the breadth of the immune response induced by the prime-boost regimen.

Longer-term follow up of STEP

The RV144 trial may have opened the long-awaited door to AIDS vaccine efficacy, but it is not the only late-stage clinical trial informing researchers these days. Clues continue to emerge from the Phase IIb test-of-concept trial known as STEP, which was stopped in 2007 after an interim analysis revealed that Merck's Ad5 vector-based vaccine candidate MRKAd5 appeared to be ineffective in both preventing acquisition of HIV and in reducing levels of the virus in individuals who became infected despite vaccination. And going even further back, scientists have also turned their attention to a 12-year-old efficacy trial, Vax004, which tested AIDSVAX, the same gp120 protein candidate tested in RV144, by itself.

Ann Duerr, HVTN associate director for Scientific Affairs and an investigator in the STEP trial that enrolled 3,000 volunteers at high risk of HIV infection in North and South America, the Caribbean, and Australia, reported on a long-term follow-up study (HVTN 504) of STEP participants. The most complete analysis conducted to date showed that among 1,836 male participants there were 99 HIV infections reported among vaccinated men and 73 infections among unvaccinated men. This analysis excluded women because at the time vaccinations were halted in the STEP trial in 2007 only one HIV infection had been reported among 1,134 female volunteers. An analysis of the combined STEP and HVTN 504 data showed that all vaccinated men were at an increased risk of HIV acquisition compared to placebo recipients, based on the modified intent to treat analysis. However, the increased risk of infection in men who were uncircumcised and/or who had preexisting Ad5 immunity waned over the course of the four-year study. Interpreting the results of the post-hoc analyses has been tricky, however, because retention rates for vaccine recipients were slightly higher than they were for placebo recipients, particularly when the trial was halted and unblinding occurred. Differences in behaviors between men who received vaccine and placebo could also have impacted the relative risk, according to Duerr.

Researchers still do not know why uncircumcised male vaccine recipients with high Ad5 antibody titers at baseline had a higher rate of HIV acquisition. Some researchers speculated it might have been because the vaccine induced a long-lasting increase in HIV target cells that homed to the gut mucosa. But, Nicole Frahm, associate laboratory director of the HVTN, presented data from a study of blood and mucosal tissue from the lower GI tract of 23 HIV uninfected STEP trial participants—half of whom received MRKAd5—which showed that 30 months after the final injection there were no significant differences in expression of CCR5, CCR9 (a chemokine receptor that is usually present at higher levels on activated Ad5-specific $CD4^+$ T cells), and CD103 (an integrin expressed on activated T cells) in either $CD4^+$ or $CD8^+$ T cells in the gut mucosa of vaccine or placebo recipients, even when stratified by baseline Ad5 titer, therefore failing to explain the increased risk of HIV infection among vaccinated volunteers who were uncircumcised and had higher pre-existing Ad5 immunity. "Even though we don't have a mechanism, we're pretty sure this is a real biologic effect," said Larry Corey, principal investigator of the HVTN.

Another look at AIDSVAX

It's certainly not news that AIDSVAX, which was tested in the first two Phase III AIDS vaccine trials, failed to protect against HIV infection. Still, the 5,400-person Vax004 trial that enrolled mostly MSM at high risk of HIV infection in North America and the Netherlands continues to engage and inform researchers, most recently in Atlanta, where it was highlighted by theoretical biologist Bette Korber, who heads the HIV Database and Analysis Project at Los Alamos National Laboratory (see *Tracking HIV Evolution*, *IAVI Report*, May-June 2010).

[FIRST TRIAL IN INFANTS]

Results from the first AIDS vaccine trial in Africa in breast-fed infants born to HIV-infected mothers were presented at AIDS Vaccine 2010 in Atlanta. The Phase I, double-blind, randomized, placebo-controlled HPTN 027 trial evaluated the ALVAC-HIV vCP1521 canarypox vector-based vaccine candidate—the same one used in the RV144 trial as part of a prime-boost regimen—in a cohort of 60 infants.

The four-year study found the four-dose regimen, given at birth, and then at one, two, and three months of age, was safe, but not very immunogenic. HIV-specific T-cell responses were not observed by interferon (IFN)-γ ELISPOT assay, but were observed in vaccine recipients using two other assays, cytokine staining and carboxyfluorescein succinimidyl ester CFSE, which measures T-cell proliferation using flow cytometry. However, the difference in immunogenicity between the vaccine and placebo groups was not statistically different during any time point in the trial. Results of antibody assays should be presented early next year, said Huyen Cao, director of cellular immunology at the California Department of Public Health.

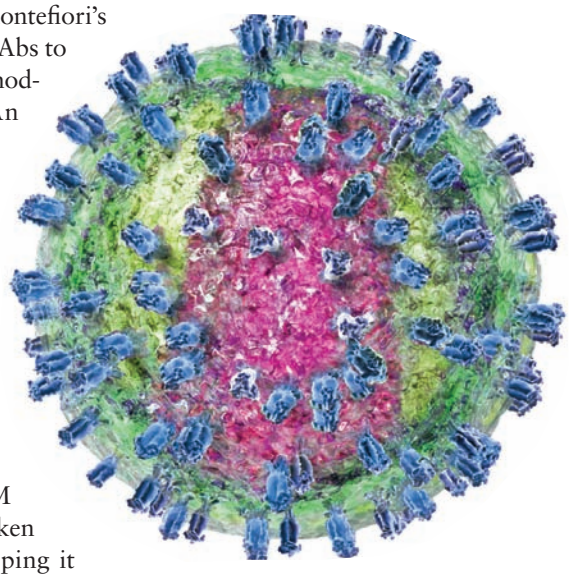
Cao, who conducted the T-cell analysis, said the trial was significant in that it showed that "neonates were able to mount an immune response that is comparable to adult populations." Until now, ALVAC cp1521 had only been tested in clinical trials in combination with other vaccines, but other ALVAC formulations have been tested alone and were found to be poorly immunogenic. *—RM*

The renewed interest in Vax004 began earlier this year when a team of researchers released results of a study that analyzed the neutralizing antibody (NAb) responses against highly sensitive tier 1 and moderately sensitive tier 2 strains of HIV among vaccinated volunteers (*J. Infect. Dis.* **202**, 595, 2010). A subset of plasma samples from over 100 randomly selected vaccine and placebo recipients were tested for their ability to neutralize HIV subtype B strain MN, which was part of the vaccine; a heterologous but readily neutralized tier 1 strain, SF162; as well as a panel of 12 tier 2 viruses that represent a spectrum of clade B viruses, including transmitted founder viruses. A comparison of responses among vaccinated and placebo recipients showed that the vaccine elicited high titers of NAbs against HIV_{MN} and SF162 and weak overall neutralizing antibody responses against tier 2 viruses. The study's authors concluded that the lack of neutralization of tier 2 viruses was consistent with the lack of protection seen in the trial and suggested that, "one way to improve the efficacy of current HIV-1 vaccines may be to elicit stronger NAb responses against tier 2 strains of the virus."

Following this study, Korber, who was not involved in the original Vax004 studies, did an additional comparison of NAb responses in vaccinated individuals to see if there was any association between NAb responses to tier 2 viruses and being uninfected. In Atlanta, she reported results from what she called a "preliminary re-examination of the Vax004 data." Korber found that essentially none of the 21 vaccinated males that were subsequently HIV infected had a detectable tier 2 neutralizing antibody response; their tier 2 responses were comparable to placebo recipients. In contrast, only about 20% of the uninfected vaccinees had a low-level tier 2 antibody response. Vaccinated individuals with detectable tier 2 responses remained uninfected. The difference in the tier 2 responses between infected and uninfected vaccinees was "highly significant," said Korber. This observation was confirmed by Peter Gilbert, research professor in biostatistics at the Fred Hutchinson Cancer Research Center, and David Montefiori, director of the laboratory for AIDS vaccine research and development in the department of surgery at Duke University Medical Center.

A larger study including new samples from male and female subjects from the Vax004 study is being planned to see if the results from Korber's and Gilbert's analyses can be confirmed, but Korber said these findings are intriguing, while also urging caution about over-interpretation of the data. "It suggests that maybe the level of tier 2 responses observed, or some surrogate they are a marker for, did provide some protection, but individuals who made these responses were too infrequent to give an overall vaccine effect." Montefiori said that based on Korber's "interesting and provocative" data there is now "greater confidence that there is a significant association between the level of neutralizing antibody response and the risk of HIV acquisition." Though, he added, "what that means, we still are not certain of."

Korber said these observations suggest that it is worth looking at the tier 2 antibody responses among uninfected and infected vaccinees in RV144 to see if there is a similar correlation. However, a poster presented in Atlanta by Montefiori's group suggests that the level of NAbs to tier 2 viruses may not explain the modest protection seen in RV144. An analysis of RV144 samples by Montefiori's laboratory found that the NAb responses to tier 1 and 2 viruses are actually lower than what was seen in the Vax004 trial. Montefiori cautioned that the populations enrolled in RV144 and Vax004 were considerably different—one involved low-risk heterosexual men and women from Thailand and the other high-risk MSM from the US—yet he was still taken aback by the results. "I was hoping it would be the opposite," said Montefiori. "The fact that the response in RV144 is weaker suggests it might not be neutralizing antibodies that are providing efficacy."



Adapting to more flexible clinical trials

Given the valuable data being extracted from these and other trials, many researchers are calling for more clinical trials and more efficient ways of conducting these trials. "We start an efficacy trial every five years," said Corey, who suggested AIDS vaccine researchers need to get past their twin afflictions: a fear of failure after a few candidates were unsuccessful, and a fear of success that has led to long development timelines for each product. "I don't know, maybe what the field needs is a psychiatrist," he joked.

Corey lamented that the first follow-up efficacy trials to RV144 will not begin before 2013. "The reality is we've been pleasantly surprised by RV144 but it's taken us a lot of time to really line up what's

next," he said. In contrast, Corey pointed out that the microbicide field is preparing to begin a confirmatory trial of the 1% tenofovir vaginal microbicide gel just four months after the first trial results were released in July, showing it was 39% effective in protecting women from HIV infection (see *Microbicides Finally Gel, Securing Spotlight at the International AIDS Conference*, *IAVI Report*, July-Aug. 2010; *Vaccine Briefs*, page 18). Corey acknowledged that with the antiretroviral-based microbicide researchers have the advantage of knowing the mechanism of protection. Still, he said, "we're falling behind other prevention modalities. The number and the pace [of trials] needs to be increased."

The exploration of new trial design strategies was one of the objectives outlined by the Global HIV Vaccine Enterprise in its 2010 Scientific Strategic Plan, published last month and officially launched at the conference (*Nat. Med.* **16**, 981, 2010). At a special session focused on the plan, Bernstein said that efficacy trials should not be viewed as the culmination of a "series of basic science experiments," but as part of the entire vaccine discovery process. The present system of moving candidates through efficacy trials is "not the most productive way of going forward," he added.

During his plenary address, Corey argued that the best way to increase the pace and number of clinical trials is to employ adaptive trial designs that can test multiple vaccine candidates concurrently or sequentially in randomized, blinded, placebo-controlled trials that are conducted as standard Phase IIb trials with prevention of infection as an endpoint. Each candidate would be compared to placebo, and although investigators could not directly compare one candidate to another, Corey said investigators would have some ability to rank the efficacy of the different candidates.

Adaptive trials would be designed with more frequent analyses, or looks at the data, by the data safety monitoring board, allowing them to adapt the trial while it's underway based on how the vaccine candidates are performing. This approach would permit investigators to identify candidates with low or high efficacy more quickly and give researchers the flexibility to add another arm or even trial if a positive signal is detected. In a trial population with a 4% HIV incidence rate and 2,000 volunteers per group, Corey said it would be possible to reach a decision point on whether a vaccine candidate is working in approximately 20 months, as long as volunteers are rapidly enrolled into the trial. If this type of adaptive trial design was employed in past efficacy trials, Corey said the four-and-a-half-year Vax004 trial could have been stopped two to two-and-a-half years earlier, the STEP trial could have been stopped nine months earlier, and RV144 could have been stopped two-and-a-half years earlier. For candidates with an efficacy in the range of 40%-50%, Corey said the trial would have to run the full duration. One important distinction of these adaptive trial designs is that they are not considered licensure trials because the more frequent data analyses while the trial is underway reduce the overall power of the study, making it more of a research tool that allows investigators to rapidly prioritize candidates for further study.

If HIV vaccine investigators have been acting like serial monogamists, Corey is advocating that they become speed daters, viewing trials more as experiments that allow them to quickly assess which candidates to court further. "We need to initiate one to two such trials each year for the next four years," said Corey.

But exactly how these adaptive trials would be structured won't be known until regulators and investigators are able to sort through logistical and ethical questions. The Joint United Nations Programme on HIV/AIDS (UNAIDS), the World Health Organization (WHO), and IAVI will be hosting a meeting in February 2011 to discuss the challenges and advantages of adaptive clinical trial designs. Catherine Hankins, chief scientific officer of UNAIDS, said the biggest challenge will be communication. "People will need to understand why one candidate gets dropped and another does not and that when it is dropped it doesn't mean that it is a bad product, just that it is not doing as well as others."

How full is the pipeline?

When asked if there were enough candidates in the pipeline to support adaptive clinical trials that call for testing multiple candidates simultaneously, Corey answered, without hesitation, "yes." Some of these candidates will be different combinations of the now familiar cast of viral vectors. Giuseppe Pantaleo, principal investigator of the poxvirus vaccine development consortium of the Collaboration for AIDS Vaccine Discovery, provided an overview of viral vectors now in development.

The RV144 follow-up trials will involve canarypox vectors, either ALVAC, the vector developed by Sanofi Pasteur that was tested in RV144, or NYVAC, another canarypox vector that has been tested as part of a prime-boost regimen in Phase II trials by EuroVacc. Michael said ALVAC has a commanding position among viral

[BETTER TOGETHER?]

Things are looking up. In the past year, the HIV prevention field has been buoyed by results from trials that show a prime-boost vaccine regimen and an antiretroviral-based microbicide gel are both partially effective at blocking HIV infection. And, as early as February of next year, the results from the first efficacy trials of oral pre-exposure prophylaxis (PrEP) as a means of HIV prevention will be released.

While trials to both confirm and improve upon these results are being planned, researchers are also considering ways to evaluate the possible synergy of partially effective HIV prevention strategies in clinical trials. At the AIDS Vaccine 2010 conference, Steven Self, co-director of the Vaccine and Infectious Disease Institute at the Fred Hutchinson Cancer Research Center, noted that there is a limited window of opportunity for such trials, including one that would evaluate the combined efficacy of the partially effective vaccine regimen tested in the RV144 trial in Thailand (see page 4) and the 1% tenofovir microbicide gel that was recently shown to block acquisition of HIV among women in the recent CAPRISA 004 trial in South Africa (see *Microbicides Finally Gel, Securing Spotlight at the International AIDS Conference*, *IAVI Report*, July-Aug. 2010). Self said that within two or three years the opportunity to conduct such trials could pass as efficacy data on both oral PrEP and ARV-based microbicides becomes robust enough to perhaps warrant licensure of these modalities. "You really want to do them sooner rather than later," he said.

Mathematical models suggest varying degrees of efficacy when two partially effective interventions—such as a vaccine or a PrEP regimen—are offered in combination, according to Self. One model predicted a combined efficacy as high as 70% when a vaccine with 30% efficacy and a PrEP regimen with 40% efficacy were given in combination. —*RM and KJK*

vectors because it is the only one to have shown any efficacy and joked that Stanley Plotkin, a veteran vaccinologist and advisor to Sanofi Pasteur, calls ALVAC the "Rodney Dangerfield of vectors because it doesn't get much respect."

Other replication-defective vectors in development include alternate serotype Ad vectors, including an Ad35 candidate developed by IAVI, and an Ad26 candidate developed by Dan Barouch, an associate professor of medicine at the Beth Israel Deaconness Medical Center (BIDMC) and Harvard Medical School. Both of these candidates have been tested alone in Phase I safety and immunogenicity studies and preliminary results suggest they were both safe and immunogenic, and a Phase I trial of an Ad35/26 prime-boost regimen recently started in Boston (see *Vaccine Briefs*, page 18).

There are also plans to begin a Phase I trial next year to test Barouch's Ad26 candidate in a prime-boost combination with a modified vaccinia Ankara (MVA) vector-based candidate developed by MHRP. Michael presented results in Atlanta from a nonhuman primate (NHP) study of Ad26/MVA conducted by MHRP and the Integrated Preclinical/Clinical AIDS Vaccine Development program, funded by a NIAID grant and led by Barouch. In this study, five groups of eight macaques were immunized with placebo, MVA/MVA, DNA/MVA, Ad26/MVA, or MVA/Ad26, encoding Gag, Pol, and Env from simian immunodeficiency virus (SIV)smE660. Michael showed that the Ad26/MVA prime-boost regimen was a more potent inducer of Gag- and Env-specific antibody responses by ELISA, and Gag-, Pol-, and Env-specific $CD8^+$ and $CD4^+$ T cell responses as measured by IFN-γ ELISPOT than DNA/MVA, MVA/MVA, or MVA/Ad26 prime-boost regimens.

To see how well these regimens protected, all animals received six low-dose, intra-rectal, heterologous SIVmac251 challenges. After only one challenge, 50% of the macaques in both the placebo and MVA/MVA groups were infected. Two challenges were required to infect 50% of the animals in the DNA/MVA group, and three challenges were required to infect an equivalent percentage of monkeys in both the MVA/Ad26 and Ad26/MVA groups. Michael noted that the DNA/Ad5 prime-boost regimen developed at the VRC, which is currently being tested in a Phase II trial, did not show any protection against heterologous SIVmac251 challenge in NHP studies. Four of the eight monkeys in the Ad26/MVA group that were infected had an approximately 1 log lower set-point viral load than infected animals in the other groups.

Michael said additional NHP studies would be conducted to see if the level of protection in this study can be augmented by adding an Env protein boost to the Ad26/MVA regimen and to evaluate the immunogenicity of the vectors formulated with mosaic inserts. There are also plans to test the Ad26/MVA prime-boost regimen with mosaic inserts in a Phase I clinical trial starting next year that is a collaboration of MHRP, BIDMC, NIAID, and Crucell. Mosaic antigens are computationally designed to achieve optimal coverage of the many different versions of HIV circulating globally. NHP studies have shown that mosaic antigens induce T-cell responses with greater breadth (number of epitopes recognized) and depth (number of viral variants recognized) than either natural proteins or consensus antigens (*Nat. Med.* **16**, 324, 2010).

The enhanced breadth of T-cell responses that is achieved with mosaic antigens directly translates into the ability of the T cells to recognize more epitopes in circulating strains, according to Korber, who designed the mosaic antigens. "Breadth correlates with viral control in monkey studies," she said. "Although we don't have a single clade vaccine yet, a global vaccine is a worthy target."

Two other Phase I clinical trials involving mosaic antigens are also planned to start in the next few years. One, which is being conducted by Barton Haynes, director of the Center for HIV/AIDS Vaccine Immunology (CHAVI), will compare a wild type clade B HIV Env, a trivalent mosaic Env, and a group M consensus Env in a DNA /NYVAC prime-boost regimen. This trial is expected to begin in 2012 and is being sponsored by the Bill & Melinda Gates Foundation and NIAID. Dan Barouch is also collaborating with IAVI and Crucell to manufacture Ad35/26 vectors encoding mosaic antigens in preparation for clinical trials.

Korber reported that her team is also working on optimizing B cell mosaic antigens. "We've done it for the entire Envelope but you could also do it for a specific region such as the CD4 binding site," said Korber. This, she suggests, may be one way that researchers would be able to guide antibodies along the affinity maturation process that creates mutations in the variable regions of an antibody. This may be important given the observation that many of the HIV-specific broadly neutralizing antibodies have an unusually high degree of affinity maturation compared with other antibodies (see *Vaccines to Antibodies: Grow Up!*, *IAVI Report*, July-Aug. 2010). Korber said that the hypothesis is that most good neutralizing antibodies develop in chronic

infection because of continuous exposure to virus variation. A vaccine could mimic that diversity in target epitopes using B cell mosaics either all at once or as a series of immunizations, she posited.

Other viral vectors in various stages of preclinical development include a Lymphocytic Choriomeningitis Virus vector, which is being developed by the VRC, and several replication-competent viral vectors. Pantaleo said a replication-competent form of NYVAC is in preclinical testing and should enter clinical trials by 2012.

One replication-competent virus vector that has shown promise is a rhesus cytomegalovirus (RhCMV) vector developed by Louis Picker, associate director of the Vaccine & Gene Therapy Institute at Oregon Health & Science University. At last year's meeting Picker showed that 54% (13 of 24) of macaques vaccinated with the replicating RhCMV vector exhibited immediate viral control upon infection with SIVmac239 (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009). In fact, 12 of the 13 animals maintained undetectable plasma viral loads for a year with occasional blips of detectable virus decreasing in frequency. Picker reported results in Atlanta from a study designed to further analyze what was happening in these 12 animals. He showed that depleting either the $CD8^+$ or $CD4^+$ T cells in these animals had no effect on viral replication, in contrast to control animals.

Picker and colleagues then sacrificed four of the RhCMV vector vaccinated macaques to conduct extensive tissue analysis of the animals using ultrasensitive polymerase chain reaction to try to detect SIV DNA or RNA in the spleen, liver, tonsils, jejunum, ileum, colon, lymph nodes, bone marrow, or thymus of the animals. They found no inducible, replication-competent SIV in any of these tissues, indicating to Picker that the RhCMV vector-based vaccine is controlling SIV infection in these animals over the long term, and may possibly even lead to eventual clearing of the virus altogether.

Picker contends that this observation provides strong support for the hypothesis that vaccine-elicited cellular immunity, if present early at the sites of SIV infection and replication, could prevent or abort infection, or provide complete control of the infection before massive systemic viral replication occurs. He compared the control afforded by the RhCMV vector to that seen with live-attenuated SIV or SHIV, an SIV/HIV hybrid virus, which when administered either intravenously or mucosally can protect against highly pathogenic SIV challenge. Picker noted that the protection seen with live-attenuated SIV and SHIV occurs in the absence of any SIV-specific antibodies, and that successful live-attenuated vaccines induce persistent replication and a high frequency of SIV-specific T-cell responses in tissues.

The bigger question, it seems, is whether CMV vectors can be safely tested in clinical trials, or as Picker asked, "is this protection without practicality," much like the live-attenuated vaccine concept. To this end, Picker is developing CMV vector-based candidates that retain the immunogenicity of wild-type CMV, yet are attenuated enough so that they are safe enough to be tested in CMV seronegative volunteers in clinical trials. He called this work "quite promising."

Antibody frenzy continues

Over the past year there has been a flurry of new HIV-specific broadly neutralizing antibodies (bNAbs) isolated from chronically HIV-infected individuals (see *Research Briefs*, *IAVI Report*, Jan.-Feb. 2010). And it seems that now that researchers have developed the technology to pluck them out quite efficiently, there will be many more additions to the antibody armamentarium.

In Atlanta, researchers from the VRC reported for the first time the isolation of two bNAbs, referred to as VRCPG04 and VRCPG05 because they were isolated from samples collected from IAVI's protocol G cohort of chronically HIV-infected individuals that also led to the isolation of PG9 and PG16 (*Science* **326**, 285, 2009). John Mascola, deputy director of the VRC, said both of these antibodies target the CD4 binding site and were identified using the same technology that allowed them to isolate VRC01-03 (*Science* **329**, 811, 2010). The identification of VRCPG04-05 shows that VRC01-like antibodies are not limited to a single donor.

Pascal Poignard, principal scientist at IAVI's Neutralizing Antibody Center at The Scripps Research Institute (TSRI), reported the isolation of 13 new monoclonal antibodies from four so-called elite neutralizers—individuals whose sera can neutralize a large number of HIV isolates—identified from IAVI's protocol G cohort. Three of these antibodies target a collection of overlapping, highly conserved epitopes on the viral spike not targeted by any of the other bNAbs described so far.

And researchers from CHAVI also reported isolation of new neutralizing antibodies from their cohorts of both acutely and chronically HIV-infected individuals. Georgia Tomaras, associate director of research at the Duke Human Vaccine Institute, reported that bNAbs targeting the membrane-prox-

[A CHEAPER OPTION]

Amid the talk of biomedical HIV prevention strategies, there was one voice at the AIDS Vaccine 2010 conference in Atlanta, calling for a much more low-tech strategy—behavior change. Susan Allen, who directs the Rwanda Zambia HIV Research Group, presented a mathematical modeling study that shows behavior change is not only a more cost-effective approach, it could avert more new HIV infections than test and treat, a strategy that calls for universal testing and immediate treatment for HIV-infected individuals as a way of reducing HIV transmission.

Allen used mathematical models to compare the impact of couples voluntary counseling and testing (CVCT)—the type of counseling she has pioneered in her well-established cohorts of HIV serodiscordant couples—with test and treat for HIV-infected partners of serodiscordant couples in Mozambique.

The model took into account that 20% of the HIV-infected partners among serodiscordant couples already meet the criteria for treatment and that an additional 5% of HIV-infected partners per year would require treatment.

The models predict that a five-year, nationwide rollout of CVCT for 3.3 million couples in Mozambique would cost about US$115 million and avert between 180,000 and 580,000 new HIV infections. Whereas, the cost of a five-year rollout of test and treat for 551,525 couples would avert only 41,364 infections and cost $372 million. The model only included the cost of treatment for those HIV-infected individuals who did not yet meet the criteria for starting treatment. "Why test and treat continues to be promoted in this economic climate is beyond me," she said. —*RM*

imal external region (MPER), which is also the target site for the bNAb 4E10, have been detected in three individuals from the CHAVI cohorts. The epitope targeted by these antibodies "binds to a similar but not identical epitope as 4E10," said Tomaras. She and Mattia Bonsignori, also with CHAVI, also reported that five new monoclonal antibodies dubbed CH01-CH05 that target quaternary epitopes on the V2 and V3 loops of HIV Env were isolated from a single individual in the CHAVI cohorts.

Despite this windfall, the generation of bNAbs to HIV is still uncommon. "Individuals can make these antibodies, but they are a relatively rare occurrence," said Haynes, who noted that while approximately 20% of chronically infected individuals have some degree of antibody neutralization breadth in plasma, only 1% -2% of individuals make very broadly neutralizing antibodies. "Multiple factors may predispose people to being able to make bNAbs," said Haynes. One of these factors might have to do with lax tolerance controls that otherwise would delete such antibodies, he suggested. Haynes showed that in knock-in studies in mice in which the 2F5 heavy chain is expressed, the B cells that express the 2F5 antibody either get eliminated or are controlled by tolerance mechanisms.

The evolution of antibodies

As researchers home in on the structures of the new crop of antibodies, they are developing a clearer picture of some of their unique attributes (see *Research Briefs*, *IAVI Report*, May-June 2010; *Science* **329**, 856, 2010). Studies have shown that these bNAbs are poly-reactive, have long CDR H3 regions (particularly PG9 and PG16, see image at right), and are extremely somatically hypermutated, according to Haynes.

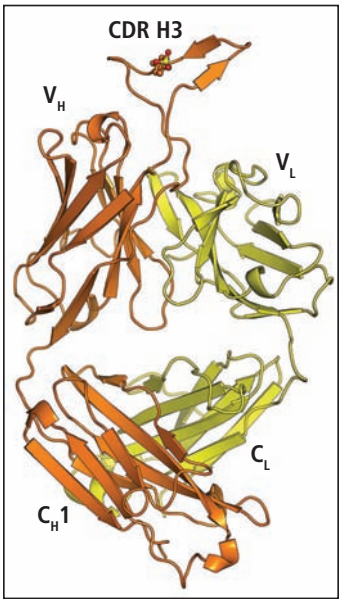
The high level of affinity maturation is one attribute of these HIV-specific bNAbs that has been receiving more attention (see *Vaccines to Antibodies: Grow Up!*, *IAVI Report*, July-Aug. 2010). "The persistent nature of HIV may lead to affinity maturation," said Peter Kwong, chief of the structural biology section at the VRC. Even the most recently identified antibodies such as VRCPG04 have accumulated a large number of mutations in their variable regions. For VRCPG04, which can neutralize approximately 90% of circulating isolates, 29% of its sequence differs from the germline version of the antibody. "This antibody again is highly affinity matured," said Mascola. The sequence of VRCPG04 is only 51% homologous to VRC01 in its heavy chain, but "despite substantial sequence diversity, they have very similar modes of recognition by their structures," said Mascola. This suggests to him that there may be more than one path to achieve this high level of affinity maturation. "Antibodies all develop in different ways," added Kwong.

To better understand how germline antibodies evolve to attain the affinity maturation needed for potent neutralization, researchers at the VRC are beginning to track the precursors of the bNAb VRC01 by applying genomic technologies to the analysis of antibody development. Mascola, Kwong, and colleagues have used 454 Sequencing to identify more than 250,000 antibody heavy chain sequences from peripheral blood mononuclear cells of the same donor from whom the VRC01-03 antibodies were isolated. From these sequences, they have categorized a family of 20,000 based on how much they diverge from the germline precursor and how their sequence is similar to VRC01. One antibody they have studied is 59% homologous to VRC01 and only 9% divergent from the germline sequence. "This antibody is nowhere near as affinity matured as VRC01 and neutralizes quite potently," said Mascola, adding, however, that it does not neutralize as potently or with the same breadth as VRC01. He said this method can be used to potentially identify the genetic precursors of VRC01. This information could reveal how the immune system got there, said Mascola, which "may provide insights on how to design immunogens and which strategies to use for vaccination."

Haynes and colleagues are also exploring computationally derived, reverted versions of bNAbs to understand how affinity maturation and/or tolerance controls impact the ability of naive B cells to make bNAbs. Haynes referred to the germline ancestors of 2F5 as putative antibodies because there are several unmutated germline versions that can be inferred. "These are only approximations," he said. "We don't have the actual naive B cell in our hands."

Effector functions of antibodies

In addition to the focus on bNAbs, there is also a more concerted effort to understand the role of Fc-receptor mediated antibody function, including antibody-dependent cellular viral inhibition (ADCVI), or what Don Forthal, associate professor of medicine at the University of California Irvine School of Medicine, referred to as "an immune function in search of a better acronym." ADCVI is a measure of the overall antiviral effects due to the Fc-receptor effector functions of antibodies, including antibody-dependent cellular cytotoxicity (ADCC), which occurs when antibodies coat an HIV-infected cell and recruit innate



PG16 Fab structure with variable (V) and constant (C) parts of the heavy chain in orange and the variable and constant parts of the light chain in yellow. The CDR H3 can be seen on top.

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

immune cells to kill the infected cell. "The Fc region is really the link between the innate and the adaptive immune system," said Galit Alter, principal investigator at the Ragon Institute.

Interest in ADCVI has been fueled by both animal studies and results from clinical trials. There is direct evidence from studies by Ann Hessell, a staff scientist at TSRI, and colleagues that shows Fc-receptor mediated antibody functions play a role in protection against SHIV infection in rhesus macaques. In another NHP study, Genoveffa Franchini, chief of the animal models and retroviral vaccine section at the National Cancer Institute, has been able to mimic the RV144 results in rhesus macaques immunized with an ALVAC prime/AIDSVAX boost and challenged rectally with repeated low-doses of SIVmac251. In this study, highlighted by Forthal in Atlanta, three of the 12 animals were completely protected. Two of the protected animals had good ADCVI responses, while the other had no ADCVI activity. Forthal said some of the variability in ADCVI responses is due to levels of immunoglobulin G2, which correlate inversely with ADCVI activity. And in Vax004, the Phase III clinical trial of AIDSVAX, there was higher ADCVI activity among uninfected subjects (*J. Immunol.* **178**, 596, 2007). "ADCVI seems to play an important role in this study," said Forthal.

All of this suggests to Forthal that improved Fc-mediated antibody function should improve vaccine efficacy. "This might be achieved by having the right adjuvant, which shouldn't be that hard, and the right immunogens, which might be harder," said Forthal.

Guido Ferrari, a CHAVI investigator, and colleagues are studying the development of ADCC and ADCVI responses in natural HIV infection. In two individuals from CHAVI's cohort of acutely infected individuals, Ferrari reported that both ADCC and ADCVI mediating antibodies develop rather quickly against the autologous transmitted virus, and at least three months earlier than neutralizing antibody responses against heterologous virus.

Meanwhile, Justin Pollara, from the Duke University Medical Center, and colleagues are studying the role of ADCC-mediating antibody responses in nine elite neutralizers selected from a CHAVI cohort of 308 chronically HIV-infected individuals. Pollara found that these elite neutralizers had a significantly higher magnitude of ADCC mediating antibodies than neutralizing antibodies. Pollara said this suggests that antibodies capable of mediating ADCC are more commonly produced in natural infection and therefore may be more readily induced by vaccination. Future studies will focus on what makes a good ADCC response in natural HIV infection and on mapping the epitopes recognized by ADCC mediating antibodies to identify the HIV Env epitopes that may be important to include in vaccine candidates.

Structure-based design

One obstacle to developing vaccine immunogens that could induce bNAbs against HIV is the unstable trimeric structure of HIV's Envelope spikes, what Rich Wyatt, director of viral immunology at IAVI's Neutralizing Antibody Center at TSRI, called "a well-shielded, shape-shifting target." In the absence of stable, engineered trimers that mimic the structure of HIV's functional spikes, researchers have taken a different tack to developing immunogens. This approach, referred to as epitope scaffolding, marries the precision of structural biology to elucidate the epitopes on HIV that bNAbs bind to, with computational biology, which allows researchers to manipulate and design protein structures.

Just before the conference in Atlanta, progress was reported in the use of epitope scaffolds to elicit antibodies (*Proc. Natl. Acad. Sci.* doi:10.1073/pnas.1004728107). Bill Schief, a research assistant professor at the University of Washington, along with Kwong, Wyatt, and others, grafted the epitope of the HIV gp41 transmembrane glycoprotein that is the target for the bNAb 2F5 onto a heterologous protein scaffold using techniques of computational protein design. Immunization of guinea pigs with either a single epitope scaffold or a prime-boost combination of two different epitope-scaffold platforms elicited structure-specific antibodies that mimic 2F5. Crystal structures of the antibodies elicited by the 2F5 epitope-scaffolds in complex with gp41 showed that these antibodies were able to induce the gp41 conformation that allowed the antibodies to bind to the 2F5 epitope. In his opening remarks, Fauci trumpeted this approach to immunogen design, calling these "very exciting results."

Schief and colleagues have also developed CD4 binding-site scaffolds that he says are now being tested as immunogens. "Epitope scaffolds have utility as immunogens," said Schief. "We do have a chance to elicit neutralizing antibodies." ■

Luck FAVORS THE Prepared

The success of a young scientist illustrates the valuable role mentors can play in establishing a research career

By Andreas von Bubnoff

The child of a professor of German literature at Brigham Young University (BYU) in Provo, Utah, Brandon Keele knew he wanted a career similar to his father's. "I always wanted to be a professor, which is funny," says the 38 year old, referring to the fact that his current position as head of the viral evolution and genomics core at the National Cancer Institute (NCI) in Frederick, Maryland, does not involve teaching. Instead, it's all about research. "It turns out that I am a fairly good researcher," he says, "so I thought I would stick with what has proven to be successful."

Last year, Keele moved to the NCI after a successful six-year postdoctoral stint in the laboratories of Beatrice Hahn and her husband George M. Shaw, both professors at the University of Alabama in Birmingham. His time there was a success by anyone's estimation. He was first author of several seminal papers, including a 2008 paper that showed for the first time that in most cases, productive HIV infection is the result of transmission of a single founder virus (*Proc. Natl. Acad. Sci.* **105**, 7552, 2008). Other papers he was first author of include a 2006 paper suggesting that the origin of HIV lies in an area in southeastern Cameroon (*Science* **313**, 523, 2006), and a paper published just last year that showed that chimpanzees in Gombe National Park in Tanzania are more likely to die if they are infected with simian immunodeficiency virus (SIV), challenging the view that natural SIV infections don't cause disease (*Nature* **460**, 515, 2009).

While hard work, passion, good hands, and a portion of luck all played a role in Keele's success, his career shows how important it can be for a young researcher to choose the right mentors and lab for their postdoctoral work. "I was in the right environment with very supportive mentors," Keele says.

The makings of a researcher

Keele first got excited about immunology when taking classes as an undergraduate at BYU. "I was interested in what causes human suffering," he says. "I kind of have this soft spot for human problems and I think I really wanted to help the world. I was interested in human diseases, what caused disease, why vaccines worked or didn't work."

In 1997, still an undergraduate at BYU, Keele got his first molecular biology experience as an intern in the lab of Greg Burton, at the time an associate professor in the department of microbiology. "[Brandon] just came to me and said I would like to do something in the lab and work on a project with you," remembers Burton.

Keele says he always wanted to eventually get a PhD degree, but after graduating from BYU, he initially wanted to first obtain a master's degree and wait until his wife, whom he had met at BYU, was ready to move on to another university with him. But Burton convinced him to do a PhD in his lab. "I tried to twist his arm to consider a doctoral program because he [was] an exceptionally gifted student," says Burton, in whose lab Keele started his PhD research in 1999. "Once I had everything running [in Burton's lab], it seemed like a waste of time to start over at another school," Keele says. Keele was studying how HIV accumulates on the surface of so-called follicular dendritic cells (FDCs), which can be found in secondary lymphoid tissue such as lymph nodes. A few years earlier, Burton had shown in mice that HIV stays infectious at the surface of FDCs, suggesting that FDCs can act as a reservoir, keeping infectious HIV particles trapped on their surface (*Nature* **377**, 740, 1995).

Keele's work in mice then showed that HIV on FDCs remains infectious for at least nine months and that FDCs keep HIV particles infectious by holding them in complexes with antibodies on their surface (*J. Immunol.* **166**, 690, 2001; *J. Immunol.* **168**, 2408, 2002). He also sequenced HIV particles isolated from different cells and tissues of infected patients, including FDCs, and concluded that the HIV particles from FDCs were older because their sequences changed less over time than virus in other tissues of the patient. This was further evidence that FDCs keep a reservoir of non-replicating, but infectious HIV particles.

From immunology to viral evolution

It was several years before this HIV sequencing study would eventually get published (*J. Virol.* **82**, 5548, 2008), but working with viral sequences immediately got Keele interested in viral evolution, which he decided he wanted to study in his postdoctoral work. "Sequencing and analyzing the changes that occurred in viruses revealed to me a powerful tool in understanding the virus, how it evolves, and poten-

tially how we can inhibit it," he says.

After receiving his PhD in 2003, Keele began to look for postdoc positions with researchers who studied viral evolution. This led him to Hahn, who was studying the origin of HIV. At the time, Hahn's group had published high-profile studies that suggested that HIV originated from SIV found in chimpanzees in Africa (*Nature* **397**, 436, 1999; *Science* **295**, 465, 2002), although the exact location of the precursor of HIV-1 remained to be found. Keele says he was a little worried that some people might find it odd that he did both his undergraduate and graduate work at the same university, and when he gave a talk at Hahn's lab he felt it went "really poorly," he remembers. "They were just very critical and really wanted to know all the ins and outs of the data. I initially thought they wouldn't hire me." But Hahn felt differently. "I talked to him and I immediately liked him," Hahn recalls, adding that she didn't even look much at Keele's publication record.

As his first project, Hahn asked Keele to work with Mario Santiago, a graduate student in her lab, to test the hypothesis put forward in a 1999 book called "The River" by journalist Edward Hooper that the HIV pandemic originated from SIV-infected cells from chimpanzees used to prepare or grow oral polio vaccine in Kisangani in the Democratic Republic of Congo. Santiago taught Keele how to isolate and analyze SIV RNA from fecal samples from chimpanzees. "We had just gotten some fecal samples from Kisangani from chimps that were sampled directly around where [Hooper] said to look," Keele says. "The idea was to ask what kind of virus they have." In a paper that appeared in *Nature*, with Keele as the third author (Michael Worobey of the University of Arizona in Tucson was the first author), they reported that the SIV in these animals was too different from HIV for Hooper's hypothesis to be true, Keele says (*Nature* **428**, 820, 2004).

This meant that the true SIV precursor for the pandemic HIV-1 group M, which makes up the majority of HIV infections, still remained to be found. "With the help of many great collaborators who spent countless hours in the forest looking for piles of poop," Keele says, he and Santiago kept searching for the origins of HIV in other areas. It seemed an almost impossible task. "We originally thought that maybe those viruses that are most closely related to HIV-1 are not there anymore," Santiago says. "Chimpanzees are hunted like crazy in that area of the world."

But to their surprise Keele found an SIV variant in chimpanzee fecal samples from southeast Cameroon that was more similar to HIV-1 group M than any other known SIV variants, suggesting that SIV from there gave rise to much of the HIV pandemic. "We thought it was really lucky to find something that clustered very closely with HIV-1," says Santiago.

The resulting study, published in 2006 in *Science* with Keele as first author, gave Keele his first big break, attracting calls from many journalists (*Science* **313**, 523, 2006). "This was really big," Keele remembers. "It was new for me to have so much attention. We had the BBC talking to us, we had lots of interviews, we had lots of stuff going on, lots of newsprint, lots of fun." Lots of fun, that is, except for one thing. When it was time to toast the success with champagne, Keele wouldn't drink because he is a Mormon, Hahn recalls. "He will not touch alcohol, not even to toast when you have the best papers," she says.

With hard work comes luck

Discoveries like the one in Cameroon were the result of a combination of hard work and luck, Keele says. "It has to be some luck because it's not every day that you get a *Nature* or a *Science* paper, but it's also a lot of hard work," he says. "Luck favors the prepared."

Luck played a role because Keele was the one who analyzed the fecal sample that contained the relevant SIV strain, Hahn remembers. The sample came from a place called Lobeke in Cameroon. Because Keele analyzed it, the sample came to be known as "LBK," for Lobeke and for "Lucky Brandon Keele," says Santiago. "Luck did play a role," Keele says, but adds that since this was his project, "I analyzed all the samples, so I would have come to it sooner or later."

Isolating and sequencing RNA from fecal samples is no easy task, Keele says. One might even call it a shitty job. "It's a lot of hard work because it's isolating RNA from shit," he says. "The fecal samples stink to high heaven and RNA doesn't do well in fecal samples." One important thing that made the 2006 paper possible was Keele's knack for improving experimental approaches or adopting them for new applications. "He started thinking out of the box to try to improve the methods that we started out with," remembers Santiago. Keele, Hahn says, figured out a quick way to test if fecal samples contain SIV-specific antibodies. "I came up with a convenient way to get antibodies out in a fairly high-throughput manner," Keele says. This saved time and effort because it made it possible to focus only on sequencing SIV from fecal samples that were positive for antibodies.

In addition, Santiago says, Keele used genetic tools in a way that made it possible to identify individual chimpanzees in wild living populations that were not habituated, or used to being around humans. This way, researchers could determine how many different individual chimpanzees the fecal samples came from and how prevalent SIV infection really was among the chimpanzees in a given area. It was the first time anyone combined the genetic tools with antibody and viral RNA detection to accurately determine the prevalence of SIV infection in different ape populations, Keele says.

The lab next door

After his success in studying the origins of HIV, Keele began looking to do something different for a second postdoctoral position. It turns out that he didn't have to go far. Shaw's lab, where Keele ended up, is next door to Hahn's. "Our labs are almost joined at the hip," says Shaw.

Working with Shaw, Keele again adapted an existing technology for use in a new context. He was the first to use single genome amplification (SGA) to characterize HIV samples from acutely HIV-infected individuals. In this approach, HIV samples are diluted so much that they likely contain just one copy of the HIV genome, which can then be amplified and sequenced. This makes it possible to determine the proportion of different HIV variants in an infected person.

The SGA project also showed Keele's willingness to look for expertise outside the lab when necessary, Shaw says. "Brandon went to John Coffin's laboratory at NCI Frederick and brought the single genome amplification technology back to Birmingham, modified it, and then rapidly exploited that technology," Shaw says, adding that this was just one of several key things Keele was involved in "that allowed us to realize the potential of SGA and sequencing," in acutely infected people.

The resulting study analyzed *env* sequences of people infected with HIV for just a few weeks to show that the majority of clinically productive HIV infections are the result of a single transmitted founder virus (*Proc. Natl. Acad. Sci.* **105**, 7552, 2008). Other studies had already suggested that there might be a genetic bottleneck that limits the number of transmitted founder viruses that eventually cause productive infection to just one HIV variant out of the many in the donor, but the 2008 study for the first time revealed the exact sequence of the actual founder virus and quantified this genetic bottleneck, showing that in the majority of transmissions there is just one transmitted founder virus. "What we really tried to do was to define in a quantitative way how many viruses initiated infection," Keele says. "For the first time ever we were able to say look, this patient was infected with this exact nucleotide for nucleotide virus and we can see the evolution away from this virus and we can time all of this accordingly."

Cynthia Derdeyn, the first author of a study in 2004 that suggested that a genetic bottleneck exists in HIV transmission, says that prior to Keele's 2008 study, "I don't think the field appreciated that one or a few differences in the viruses present in very early infection could represent adaptation to immune selection and thus were not the actual founder virus."

"I think most regard [the 2008 *PNAS* paper as] a seminal paper in the field," says Bart Haynes, a co-author of the paper and director of the Center for HIV/AIDS Vaccine Immunology (CHAVI), which provided many of the clinical blood plasma samples and some of the funding for the research. Later, Keele also used SGA to show that transmission of just one transmitted founder virus in most cases can be recapitulated in rhesus macaques that are rectally infected with low doses of SIV (*J. Exp. Med.* **206**, 1117, 2009).

Even while Keele was working with Shaw he kept analyzing fecal samples for Hahn as part of a longitudinal study that followed wild, but habituated, chimpanzees in Gombe National Park in Tanzania. Keele determined which chimps became newly infected with SIV using the genetic tools he had developed to identify the individual chimpanzees over the years. The fact that they were habituated also helped in their identification. "There were a lot of other people involved but [Keele] was the one who carried the project almost from the minute he walked into the lab," Hahn says. "He kept track of these chimps, he analyzed the chimp samples as they came in, he knew who became newly infected, he analyzed the viruses, etc." The study, which followed the chimpanzees for over nine years, appeared last year with Keele as co-first author (*Nature* **460**, 515, 2009). It showed that the SIV-infected chimpanzees were 10-16 times more likely to die than uninfected ones, challenging the view that all natural SIV infections are non-pathogenic. Derdeyn, who was not connected to the study, calls it "a paradigm shift in our thinking of 'nonpathogenic' natural SIV infections of nonhuman primate species."

Many reasons for success

Keele's time in Birmingham "would go down in anyone's book as successful," he says, attributing this success in large part to having Hahn and

Shaw as mentors. "They facilitate young people being successful," Keele says. Santiago, who has worked for both Hahn and Shaw and is now an assistant professor of medicine at the University of Colorado in Denver, agrees. "They are just fantastic mentors," he says.

"In addition to my excellent mentors," Keele adds, "I was fortunate enough to be associated with great collaborators. My research has benefited significantly from the generosity and kindness of many in the AIDS research community."

Keele says he aimed quite high when he applied for his first postdoctoral position, but believes that doing so is possible because hiring a postdoctoral researcher carries little risk for a principal investigator. "I think most people ought to shoot really high for their postdoc," he says. "A postdoc is a very easy hire for most people because you are only under a one year contract and the price is fairly low."

Maybe one day when I'm older I'll put on a tweed jacket and stand up in front of budding scientists and tell them how it is.

—Brandon Keele

The size of the lab also plays a role, he adds. "If I were to speak to graduate students throughout America, I would say to find somebody who does excellent science but who doesn't have an enormously large lab, because the enormously large labs [are] how a postdoc or a graduate student can get lost," says Keele.

It's not just that Keele found great mentors in Hahn and Shaw; they found a great postdoc in Keele, who was not only passionate about science, Hahn says, but also unusually productive and reliable. "Whenever you would see him in the hallway or on the weekends or whenever, he'd say, 'Oh, have you seen this?' and he would show me a new piece of data," Hahn says. "He was a quick learner and a great experimentalist. He is passionate about science, that's why he is successful."

Keele was also a good collaborator, Shaw adds. "One of his greatest strengths would be his ability to work very effectively with many different people." Hahn agrees, and says this has contributed to his success. "He gets the most out of people and gets them to do what he wants," she says. "I think everything goes better and the science moves faster when everyone is sharing data and is working together," Keele says.

Last year, Keele moved to the NCI in Frederick to start his first independent position, which was hard to do after so much success, he says. "If you can't prove your independence in a certain amount of time, you are written off as a lifetime postdoc," he adds.

In Frederick, Keele is not working with any students because NCI is not affiliated with a university, but he has two technical employees and works with an animal facility on the main NCI campus in Bethesda. One advantage of being at NCI, he says, is that he doesn't have to apply for grants. "It's great being here because we can just focus on the science. We spend much less time worrying about other things." This way, he also doesn't compete with his former advisors for grant money and instead actually collaborates. "I like working here because I am able to collaborate with lots of people," Keele says, adding that he is now collaborating with over 20 labs.

For his own studies, Keele decided to focus on studying virus transmission in more detail in rhesus macaques to better understand it and also to recapitulate HIV transmission in an animal model. With Chris Miller, a professor in the School of Veterinary Medicine at the University of California at Davis, he has been using SGA to study transmission routes other then rectal transmission in rhesus macaques. This has shown that intravaginal infection of different animals with the same dose of SIV leads to a more variable number of transmitted founder viruses than rectal transmission (*J. Virol.* **84**, 7083, 2010). Keele and Miller are also using SGA to determine the number of transmitted founder viruses in male rhesus macaques whose penises were exposed to SIVmac251.

Keele also wants to understand where along the transmission route the genetic botteneck is located. In collaboration with Jake Estes and Jeffrey Lifson at NCI Frederick, Keele is using a laser to isolate SIV-infected cells from histological sections of the cervix of animals vaginally infected with SIV. They then sequence the integrated proviral SIV from these cells. The goals are to find if there is a specific location where the transmitted founder viruses first take hold in an infected animal, and to see if the location of the transmitted viruses in the infected animals plays any role in how well they replicate and cause systemic infection.

Keele is as busy as ever. "I still work very hard, and I have a hundred things to do as we speak." But he doesn't talk much science at home. "I like to shut off and kick the soccer ball around with my kids," says Keele, who is married with three kids and likes hiking, bike riding, skiing, and photography.

And compared with his postdoc days, he doesn't work every weekend anymore. But he still keeps a stack of Progresso soup cans in his desk drawer so he can grab a quick lunch. And he still thinks it's possible that maybe one day he will follow in his father's footsteps and become a professor. "Maybe one day when I'm older I'll put on a tweed jacket and stand up in front of budding scientists and tell them how it is." ■

Research BRIEFS

Clues Found About How HIV-1 Avoids Triggering Innate Immune Responses

The innate immune response is activated within hours after direct recognition of pathogens. This first-line response helps to contain the pathogens and present them to the adaptive immune system. Many bacteria and viruses can induce innate immune responses, but there is not much evidence for direct innate immune responses to retroviruses, including HIV-1, perhaps at least in part because HIV-1 has evolved elaborate strategies to evade triggering innate immunity.

Just how HIV-1 evades triggering innate immune responses is now becoming clearer from two recent studies in which researchers were able to trick HIV-1 to induce innate immune responses in cells in which it does not normally induce such responses. Dan Littman, an investigator at the Howard Hughes Medical Institute at the Skirball Institute at New York University School of Medicine, and colleagues reported that they were able to induce an innate immune response to HIV-1 in dendritic cells (DCs; *Nature* **467**, 214, 2010), and Judy Lieberman, a professor of pediatrics at Harvard Medical School, and colleagues showed the induction of an innate immune response to HIV-1 in CD4$^+$ T cells and macrophages (*Nat. Immunol.* **11**, 1005, 2010).

The two groups used different methods to trigger an innate immune response to HIV-1 in these different cell types. Because DCs mostly resist productive infection with HIV-1, Littman and colleagues used a previously developed strategy to overcome this resistance by delivering HIV-1 to DCs together with simian immunodeficiency virus (SIV) virus-like particles that carry a protein called Vpx (*Gene Ther.* **13**, 991, 2006). This strategy is based on the finding that in DCs, the viral replication cycle of HIV-1 is blocked at several points, whereas SIV and HIV-2 have a protein called Vpx that can counteract one of these blocks.

When Littman and colleagues used this strategy to productively infect DCs with HIV-1, they found, to Littman's surprise, that the infected DCs produced type I interferon (IFN), a strong inhibitor of viral replication and a sign that the DCs had recognized HIV-1 as dangerous and activated an innate immune response against it.

> [This study] finally opens the door to studying direct innate responses to HIV infection in the host, something that has just not been really observed prior to this in a way that could be significant for controlling the virus
>
> – Dan Littman

Lieberman and colleagues induced a direct innate immune response and IFN production in HIV-1 infected CD4$^+$ T cells and macrophages by inhibiting the expression of a human host cell protein called TREX1, which normally degrades cytoplasmic HIV-1 DNA in the infected cell. This suggests that in CD4$^+$ T cells and macrophages TREX1 helps HIV-1 avoid inducing an innate immune response in infected CD4$^+$ T cells by degrading HIV-1 DNA.

"I think the key point of both studies is that HIV evades triggering innate immunity either by not efficiently infecting dendritic cells, [or] in our case [by getting help from a host gene] we need to knock down to get IFN induction," says Lieberman.

In addition, Littman says the induction of an innate immune response in DCs suggests that it should be possible, in principle, to develop improved HIV-1 vaccine candidates that can induce an innate immune response in DCs. "[This study] finally opens the door to studying direct innate responses to HIV infection in the host, something that has just not been really observed prior to this in a way that could be significant for controlling the virus," he says. "The most interesting implication would be that it may be possible to develop vaccines that specifically target DCs to improve the activation of T cells specific against the virus." Lieberman agrees but adds that "most vaccines that use live vectors or adjuvants should activate innate immunity, which enhances the adaptive immune response."

The TREX1 finding might also be applicable to vaccine development, Lieberman says. "Inhibiting TREX1, which degrades cytoplasmic DNA, might enhance immunity to DNA vaccines possibly by having them trigger innate immunity," she says. "In my opinion one reason they are not that potent is that they don't provide a danger signal."

One reason elite controllers can control HIV-1 infection without treatment could be that somehow these individuals can activate an innate immune response against the virus. "Maybe there is some genetic predisposition in those elite controllers that allows either the virus to gain access to DCs or allows the T cells to mount a type I IFN response following infection," Littman says. "It would be worth exploring whether exposed but uninfected individuals or elite controllers might have differences in some of the genes involved, such as in *TREX1*," adds Lieberman. —*Andreas von Bubnoff*

Vaccine BRIEFS

Trials Planned to Confirm Efficacy of Tenofovir Microbicide Gel

Following the results from the recent Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 trial, which demonstrated the first statistically significant reduction in HIV infection from a microbicide candidate, researchers are planning two confirmatory trials that could lead to licensure of the gel.

At the International AIDS Conference in Vienna in July, researchers reported that vaginal application of a 1% gel formulation of the antiretroviral (ARV) tenofovir in 889 South African women ages 18-40 at high risk of HIV infection reduced HIV incidence by 39% after 30 months of use, with efficacy as high as 50% after 12 months (see *Microbicides Finally Gel, Securing Spotlight at the International AIDS Conference*, *IAVI Report*, July-Aug. 2010). Researchers also found that the tenofovir-based microbicide gel reduced the incidence of herpes simplex virus (HSV)-2 by 51% in a subset of women who weren't already infected with HSV-2 at the start of the study.

Researchers now hope to be able to replicate the results of CAPRISA 004 in a confirmatory trial involving 3,000 women enrolled at six clinical research centers in South Africa. The confirmatory trial known as FACTS 001, which is still pending approval by South African regulatory authorities, will evaluate the same BAT24 dosing regimen tested in the CAPRISA 004 trial—women are counseled to apply the gel up to 12 hours before sex and as soon as possible following intercourse but within 12 hours. Eligibility criteria for enrollment in the FACTS 001 trial will be expanded to include girls ages 16 and 17 because they are considered to be at high risk of HIV infection through heterosexual sex. FACTS 001 would also add prevention of HSV-2 as an endpoint. Salim Abdool Karim, director of CAPRISA, says he hopes to begin the confirmatory trial in early 2011, with results expected in 2013.

A second confirmatory trial is also being considered with the aim of determining whether two doses are necessary to reduce the risk of HIV infection or whether a single dose around the time of intercourse is sufficient. This trial, referred to as MDP 302, will compare the efficacy of the CAPRISA 004 BAT24 dosing regimen with a simplified regimen of one dose of tenofovir gel right before sexual intercourse or, failing that, as soon as possible after intercourse. Some clinicians and public health researchers in South Africa are concerned that a stricter dosing schedule may prove to be impractical in a real-world setting, undercutting the effectiveness of this intervention. There was some indication that adherence to the BAT24 regimen waned over the course of the CAPRISA 004 trial. The single-dose regimen is also less expensive. Plans are to enroll 3,750 women from up to five African countries, among them, Uganda, Tanzania, and Mozambique.

The South African Department of Science and Technology and the US Agency for International Development (USAID), which together funded CAPRISA 004, will be providing the bulk of the funding for the FACTS 001 trial. "There's still a shortfall so there's an ongoing effort to raise those funds," says Karim. The MDP 302 trial will be partly funded by the Medical Research Council in the UK, with other funding sources to be determined.

Additionally, researchers are planning two follow-up studies to determine the best way to deliver the microbicide gel and the effects of tenofovir gel use on the safety and effectiveness of oral tenofovir when used to treat HIV. —*Regina McEnery*

Phase I Trial of Adenovirus-based Prime-boost Regimen Begins in Boston

A Phase I trial to test the safety and immunogenicity of an adenovirus (Ad) serotype 35 vector-based vaccine candidate with an Ad26 serotype vector-based candidate in a prime-boost combination is now underway at Brigham and Women's Hospital in Boston. Both of the candidates express HIV clade A *env* genes. The Ad26.ENVA.01 candidate was developed by Dan Barouch, an associate professor of medicine at the Beth Israel Deaconness Medical Center (BIDMC) and Harvard Medical School, and manufactured by the Dutch biopharmaceutical company Crucell. The Ad35-ENV candidate was developed by IAVI and manufactured by the French biopharmaceutical company Transgene. Volunteers will receive two injections, administered either three or six months apart, of Ad26.ENVA.01 as a prime followed by Ad35-ENV as a boost or vice versa, or repeated vaccinations with one of the two vaccine candidates.

Vaccinations of volunteers in the trial in Boston, known as IAVI B003/IPCAVD-004, began in October, following approval by the US Food and Drug Administration and Harvard's institutional review board. Pending regulatory approval, investigators will also enroll additional volunteers for the trial in Africa. The overall goal is to enroll approximately 212 HIV-uninfected individuals at low risk of HIV infection at as many as six clinical research centers.

"[This] will be the first-in-human evaluation of homologous and heterologous Ad26 and Ad35 regimens in multiple regions of the world, including target populations in Africa," says Barouch, who leads the Integrated Preclinical/Clinical AIDS Vaccine Development program (IPCAVD) team that is developing novel Ad vectors as HIV vaccine candidates. A heterologous Ad26/Ad5 prime-boost vaccine regimen was shown to enhance the magnitude and breadth of cellular immune responses as compared with a homologous regimen in nonhuman primates (*Nature* **457**, 87,

2009). Nonhuman primate studies show the Ad35/Ad26 prime-boost regimen induces a high frequency of antibody and T-cell responses, according to Barouch, who said data from protective efficacy studies in monkeys is expected early next year.

Data from ongoing clinical trials that were presented at the recent AIDS Vaccine 2010 conference in Atlanta suggest that both Ad26 and Ad35 candidate vaccines are safe and immunogenic. Preliminary results of IAVI B001, a double-blind, placebo-controlled, randomized Phase I trial involving 56 volunteers showed that combined vaccination with the clade A Ad35-ENV and an Ad35 vector expressing a fusion protein consisting of parts of HIV subtype A genes *gag*, *reverse transcriptase*, *integrase*, and *nef* (referred to as GRIN) at three different doses was safe and immunogenic, according to Michael Keefer, the principal investigator of B001. In Atlanta, Barouch presented preliminary results of a Phase I trial called IPCAVD 001, which is testing the safety and immunogenicity of Ad26.ENVA.01 in 60 volunteers in Boston. He says results so far suggest that "the vector is safe and immunogenic at all doses tested."

According to Barouch, the Ad26 and Ad35 vectors to be used in IAVI B003/IPCAVD-004 are biologically very different from Ad5, the vector used in the MRKAd5 vaccine candidate, developed by Merck, which failed to induce any protection or control of virus in the Phase IIb STEP trial. Results from the STEP trial showed that uncircumcised, vaccinated male volunteers who had preexisting antibody immunity to naturally circulating Ad5 were at increased risk of HIV acquisition (see *A Change of Tune*, page 4). Individuals with preexisting immunity to Ad26 or Ad35 will be eligible to enroll in B003, according to IAVI. "Given the major biological differences among these Ad vectors, it is not at all clear whether findings with Ad5 from the STEP study will be applicable for biologically very different Ad vectors such as Ad26 and Ad35," Barouch says. In addition, fewer people have preexisting immunity to Ad26 and Ad35 and at lower titers than to Ad5, he adds. The B003 trial will only enroll individuals at low risk of HIV infection, said IAVI's Chief Medical Officer Pat Fast, and the goal of the trial is to advance the development of a vaccine applicable to the general population.

The trial is a joint effort by IAVI, BIDMC, the Ragon Institute, Harvard University, Massachusetts Institute of Technology, the National Institute of Allergy and Infectious Diseases' (NIAID) Division of AIDS, the HIV Vaccine Trials Network (HVTN) and Crucell. It is funded by the HVTN, NIAID's Division of AIDS, the Ragon Institute, and IAVI. —*Andreas von Bubnoff*

Gates Foundation Announces New Round of CAVD Grants

The Collaboration for AIDS Vaccine Discovery (CAVD), an international network launched in 2006 by the Bill & Melinda Gates Foundation to accelerate the development of an AIDS vaccine, recently issued a new request for proposals (RFP).

The HIV vaccine approaches being considered within this RFP include: HIV vaccines for containment at portal of entry, novel HIV vaccines to elicit protective antibodies, replicating viral vectors for an HIV vaccine, and passive immunization with human monoclonal antibodies for HIV prevention. The CAVD awards will range from US$500,000 to $3 million per year, per grant, over approximately three years. Additional funding may be awarded to successful projects that are on a clear product development pathway.

Letters of inquiry can be submitted through March 31, 2011. Grants will be awarded from June 2011 through June 2012. For more information on the grants, visit www.cavd.org. —*Regina McEnery*

NIAID Unveils Plans for CHAVI 2.0

At the AIDS Vaccine Research Subcommittee Meeting of the US National Institutes of Health (NIH), which was held Sep. 21-22 in Rockville, Maryland, plans were unveiled for the second iteration of the Center for HIV/AIDS Vaccine Immunology (CHAVI), a virtual research consortium established five years ago by NIAID to coordinate research and promote big science efforts to overcome roadblocks to HIV vaccine development.

The second iteration of CHAVI, called CHAVI Immunogen Design (ID), will focus on understanding why broadly neutralizing antibodies are not readily induced, dissecting the interplay between innate and adaptive immune responses, rapidly translating new knowledge into novel immunization approaches, and finally moving these approaches into clinical trials. "Clearly the focus is on prevention of acquisition [of HIV]," said Peggy Johnston, director of the Vaccine Research Program in NIAID's Division of AIDS.

NIAID is seeking US $31.5 million for CHAVI-ID in 2012, including up to $20 million in direct costs for the first fiscal year, though this amount could change based on the final NIAID budget. This is about $15 million less in total costs ($10 million less in direct costs) than what was allocated annually to CHAVI beginning in 2005. Still, the award is the largest single grant administered by NIAID for AIDS vaccine research.

CHAVI-ID will be awarded for seven years, but this time it may be divided into two awards. NIAID says the decision about whether to fund one or two awards will be made during the peer review process of the CHAVI-ID applications. NIAID says a new request for applications (RFA) for the second generation CHAVI should be formally announced within six to eight months.

The current CHAVI is led by Barton Haynes, who also directs the Human Vaccine Institute at Duke University. CHAVI's network is comprised of more than 60 investigators at 28 institutions in Africa, Europe, Australia, and North America. —*Kristen Jill Kresge*

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