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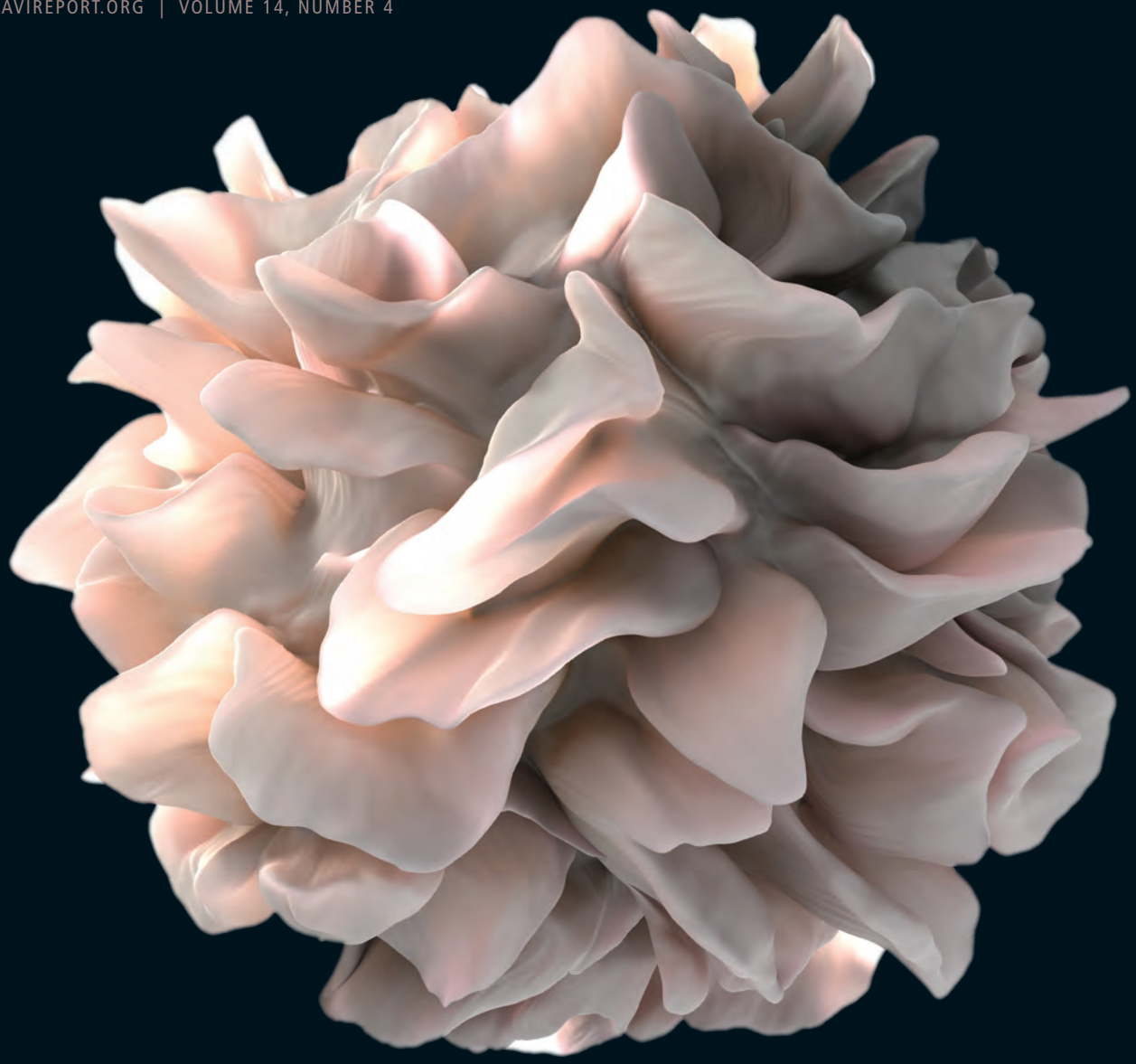

INTERNATIONAL AIDS CONFERENCE

MICROBICIDES GRAB THE SPOTLIGHT IN VIENNA

PLUS

Using Systems Biology to Understand Vaccines

Affinity Maturation of HIV Antibodies: A Potential Obstacle to Vaccine Development?

EDITOR'S LETTER

Plenty of good news has emerged from AIDS conferences in recent years. It is fair to say, however, that not since the dawn of highly active antiretroviral therapy has an advance been greeted with as much unabashed optimism as the announcement that an antiviral-based microbicide candidate reduced the HIV infection rate in a cohort of South African women by 39%. This was, without a doubt, the biggest news to emerge from the XVIII International AIDS Conference that took place in Vienna in July (see *Microbicides Finally Gel, Securing Spotlight at the International AIDS Conference*, page 10), and was a major boost to the previously beleaguered microbicide field.

The other topic that garnered the most discussion, and debate, in Vienna was the future of HIV/AIDS funding. There were calls for more efficiency in the way money is spent and discussions about new innovative financing proposals that aim to bring in additional funds.

Although there wasn't much new information on HIV vaccine research, several speakers commented on recent progress in the field, notably, the RV144 trial that showed a prime-boost strategy could reduce HIV infection risk by approximately 31%, and the recent spate of discoveries of new and more potent broadly neutralizing antibodies.

These antibodies, as well as others previously identified, seem to have a high degree of affinity maturation, which means that the antibodies have accumulated several mutations from their germline sequence (see *Vaccines to Antibodies: Grow Up!*, page 4). Researchers are now beginning to understand how affinity maturation affects the ability of the antibodies to neutralize HIV so well and how this may affect the design of vaccine immunogens.

Meanwhile, researchers are also beginning to employ an evolving repertoire of tools that enable them to study the system-wide response to vaccination (see *A Systems Approach to Understanding Vaccines*, page 16). This systems biology approach, which has been used widely in cancer research, is now being used to evaluate existing vaccines, as well as HIV vaccine candidates.

More news from the AIDS vaccine field is expected in September, when researchers will gather in Atlanta for AIDS Vaccine 2010. For more timely updates on the research presented there, visit the recently launched *IAVI Report* blog (www.iavireport.org/blog). We look forward to your comments.

KRISTEN JILL KRESGE

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International AIDS Vaccine Initiative

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

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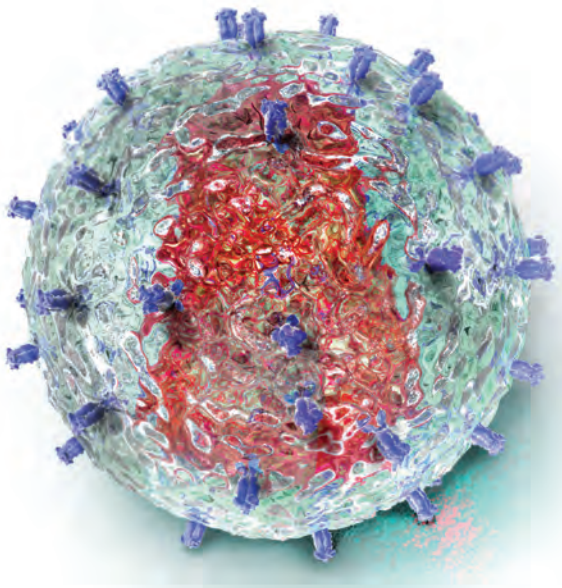

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[ON THE COVER]

DENDRITIC CELL REVEALED. Artistic rendering of the surface of a human dendritic cell illustrating the unexpected discovery of sheet-like processes that fold back onto the membrane surface. When exposed to HIV, these sheets entrap viruses in the vicinity into "virion-channels" that retain continuity with the extracellular milieu. Ion abrasion scanning electron microscopy of contacts formed between dendritic cells and T-cells shows that HIV transfer to T-cells takes place by contact of T-cell filopodia with HIV sequestered deep within these channels.

Image courtesy of Donald Bliss and Sriram Subramaniam, NCI. Previously published on the cover of *Proc. Natl. Acad. Sci.* **107** [30], 2010.

Vaccines to Antibodies: Grow Up!

Through a process called affinity maturation, all of the HIV-specific antibodies identified so far have accumulated multiple mutations, some of which are required for them to bind to and neutralize HIV. Researchers are now beginning to grapple with what this means for vaccine design.

By Andreas von Bubnoff

Recently, researchers from the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID) reported the isolation of three new broadly neutralizing antibodies (bNAbs) against HIV, as well as the structure of the antigen binding portion of one of the bNAbs called VRC01 bound to the HIV gp120 core protein (*Science* **329**, 811, 2010; *Science* **329**, 856, 2010). These are just the latest in a slew of recently isolated bNAbs (see *Research Briefs, IAVI Report*, Jan.-Feb. 2010).

VRC01 neutralizes 91% of the HIV strains tested, making it the most broadly neutralizing antibody isolated so far. Researchers found that VRC01 is so efficient at neutralizing HIV because part of the antibody mimics the way the CD4 co-receptor—the main receptor HIV uses to infect $CD4^+$ T cells—binds to gp120. "[HIV] has to keep part of itself absolutely constant in order to recognize CD4, [and] the immune system has sort of counter-attacked that one part of conservation to neutralize the virus," says Peter Kwong, chief of the structural biology section at the VRC and lead author of the study that described the VRC01 structure.

That study also showed that VRC01 has an unusually high degree of affinity maturation, which indicates how many amino acid residues of an antibody's variable region differ from the genes that antibody is derived from in the germline as the result of a process called somatic hypermutation (see *How Somatic Hypermutation Leads to Affinity Maturation*, page 5, for details). In VRC01, about 30%, or more than 60 amino acids of the variable region, differ from the germline sequence, much more than the 5% to 10% of affinity maturation observed in most other antibodies, says Kwong. VRC01's affinity maturation also seems important for binding—a version of VRC01 with all of the affinity-matured amino acids reverted to germline could not bind gp120 well anymore. "If you reverted them you got really, really low affinity," Kwong says.

VRC01 is the HIV-specific bNAb with one of the highest degrees of affinity maturation

known. But, in general, many HIV-specific bNAbs show a high degree of affinity maturation, and recent studies suggest that affinity maturation is also important for Env binding of several HIV-specific bNAbs other than VRC01. Earlier this year, Kwong's group reported that the bNAbs PG9 and PG16, which were isolated last year by researchers at IAVI and The Scripps Research Institute (TSRI), show about 20% affinity maturation and that versions of these bNAbs that were almost completely reverted to germline show no (PG16) or poor (PG9) breadth and potency of neutralization of a panel of HIV isolates (*J. Virol.* **84**, 8098, 2010). And last year, Dimiter Dimitrov, a senior investigator at the National Cancer Institute (NCI) in Frederick, Maryland, reported that germline-like versions of the bNAbs b12, 2F5, and 2G12, which according to Dimitrov also differ by about 20% from germline in their variable region, don't show any measurable binding to HIV Env (*Biochem. Biophys. Res. Commun.* **390**, 404, 2009).

We simply don't have germline antibodies in our naive B cell repertoire [that] can bind the highly conserved structures, which are exposed, like the CD4 binding site.
— Dimiter Dimitrov

The observations that HIV-specific bNAbs show a high degree of affinity maturation and that their germline reverted versions don't seem to bind HIV Env have led to concern that current strategies to develop vaccines that are based on HIV Env as an immunogen might not work, because if Env does not bind to the germline precursors of bNAbs, it won't induce the affinity maturation process that is necessary to make the bNAbs. "Vaccines based on HIV Envelope may not initiate [an] immune response [that elicits bNAbs]," says Dimitrov. "We simply don't have germline antibodies in our naive B cell repertoire [that] can bind the highly conserved structures, which are exposed, like the CD4 binding site."

To address these concerns, researchers are looking for immunogens that can bind to the germline precursors of bNAbs like VRC01 to be able to develop vaccines that can kick-start the affinity maturation process, though not everyone is convinced this will be necessary. Researchers are also taking a closer look at how the B cell immune response and affinity maturation develop in HIV-infected people starting soon after infection.

Kick-starting affinity maturation

To kick-start the affinity maturation process, Dimitrov argues that a vaccine would have to contain immunogens that are different from HIV proteins, or immunogens that are rare variants of HIV proteins, which can bind the germline precursors of bNAbs. To direct the affinity maturation process toward antibodies that can bind the native HIV Env protein, a vaccine would also have to contain the native Env or gp120, he suggests. "We need [a] variant of the Envelope or another protein which is different from the Envelope to initiate the immune response to reach some intermediate degree of

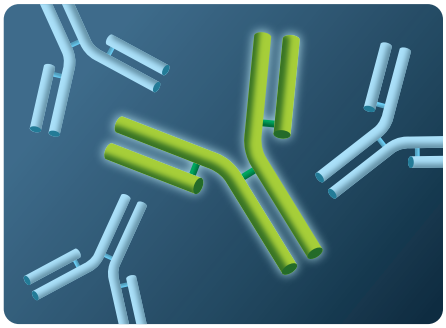
How Somatic Hypermutation Leads to Affinity Maturation

In the bone marrow, the body makes millions of different versions of B cells by rearranging antibody genes. These are called naive B cells because they haven't yet encountered antigen.

Once the B cells come into contact with antigen in lymphoid organs, such as lymph nodes, and that antigen can bind to their B cell receptor, they start to multiply and begin undergoing a process called somatic hypermutation, which causes additional mutations in the antibody genes. The B cells with mutations that result in the most improved binding—or affinity—to antigen multiply again and start another round of somatic hypermutation. These repeated cycles of somatic hypermutation and selection result in increasingly affinity-matured antibodies.

This affinity maturation process enables the body to generate a much larger number of different antibodies than can be generated by shuffling around the body's germline repertoire of antibody genes. —*AvB*

somatic hypermutation," Dimitrov says. "Then [native] Envelope comes, and this intermediate antibody binds the [native] Envelope [efficiently]." Then, he adds, the intermediate antibody matures further, eventually resulting in bNAbs. If this is not enough to help guide the affinity maturation process, Dimitrov suggests a vaccine might also have to contain one or more additional immunogens that have a structure in between the native gp120 or Env and the version that binds to the germline version of the bNAb the vaccine is intended to induce. He cautions, however, that while this is in principle how it could work, it's far from proven. "We need a much deeper understanding of how our immune system works," he says. "Affinity maturation is very complex and guiding immune responses has not been achieved."



Dimitrov, who says he was the first to publish this immunization strategy last year (*Viruses* **1**, 802, 2009), remembers that initially, his talks on this subject often got little attention. Even though the sequence of mature bNAbs such as b12, 2F5, and 2G12 had been known for many years, he says, "there were no published studies to show how important it is that these bNAbs are so somatically hypermutated. There were no reports that this could be critical."

But in light of the recent observations that show bNAbs such as VRC01 have a high degree of affinity maturation that is important for binding, that seems to be changing. "It was discovering these very highly affinity-matured antibodies [like VRC01] that had us focus on affinity maturation," says Lawrence Shapiro, an associate professor of biochemistry and molecular biophysics at Columbia University, who works with Kwong and is thinking about immunization strategies similar to the ones Dimitrov is suggesting. "It's clear that the focus has moved on to affinity maturation more than it ever has."

Yet not everyone is certain that finding an immunogen that can bind to germline-reverted versions of bNAbs is really necessary. The germline-reverted precursors of bNAbs might actually bind gp120, albeit at a very low level that might be sufficient to kick off an immune response, says Dennis Burton, a professor at TSRI. "What we don't know is what the threshold of binding affinity is in order to kick off an antibody response," Burton says. John Mascola of the VRC agrees. "While it's true that the germline antibody doesn't bind well to gp120, it's still possible and perhaps even likely that [the naive B cell] recognizes HIV in a more physiological setting," says Mascola. "It may be that a fairly low affinity interaction of HIV with the naive B cell is all that it takes to get the B cell stimulated and get going." Still, he adds, the idea that an immunogen that shows better binding to germline antibodies might give better initial immune stimulation "is a reasonable hypothesis, and it's testable."

To see if the hypothesis is correct, Dimitrov is screening protein libraries to identify proteins that can bind to germline-reverted versions of bNAbs. Sanjay Phogat, a principal scientist at IAVI's AIDS Vaccine Design and Development Laboratory, says that IAVI will be involved in screens that will look for proteins that can bind versions of the PG9 and PG16 antibodies, which have been largely reverted to their germline versions.

Kwong and colleagues are also trying to engineer an Env-derived immunogen that can bind better to the germline precursor of VRC01. They have already found that a version of gp120, which has been stabilized by adding chemical bonds into the configuration it has when it initially binds to the CD4 receptor, is able to bind the germline precursor of VRC01 better than the natural gp120 (*Science* **329**, 811, 2010). This shows that, in principle, engineered changes in gp120 can improve its binding to the germline precursor of gp120.

Currently, Kwong and colleagues are working on solving the structure of the germline-reverted version of the VRC01 antibody. Knowing that structure will allow them to engineer a version of gp120 that can bind the germline-reverted VRC01, says Shapiro, who

works with Kwong on the project. "It's a well-defined problem. Can we design a gp120 variant that's still a gp120 but is different enough to actually bind to the genomic version of these antibodies and activate the maturation process?" Shapiro asks. "One thing we can do is design mutations in the gp120 surface to change it enough so that it's recognized by the immature antibody." Such an engineered protein, he adds, could then be developed into an antigen, for example by masking with sugars the unimportant parts on its surface that could distract the immune system.

We don't know if it's just a couple of changes that are required or if extensive affinity maturation [of VRC01] is needed. We haven't fully answered that question.

— Peter Kwong

Once an experimental vaccine candidate has been developed, the challenge is to find or develop an animal model that is similar enough to the human immune system to test the candidate and see if it can indeed induce the right antibody responses. "There is some question about how exactly to proceed in terms of getting an animal system to work," Kwong says. According to Dimitrov, several groups are planning to use mice that express human antibody genes. "If we test [this] in mice with the human germline antibodies, maybe we can get a proof of concept," he says.

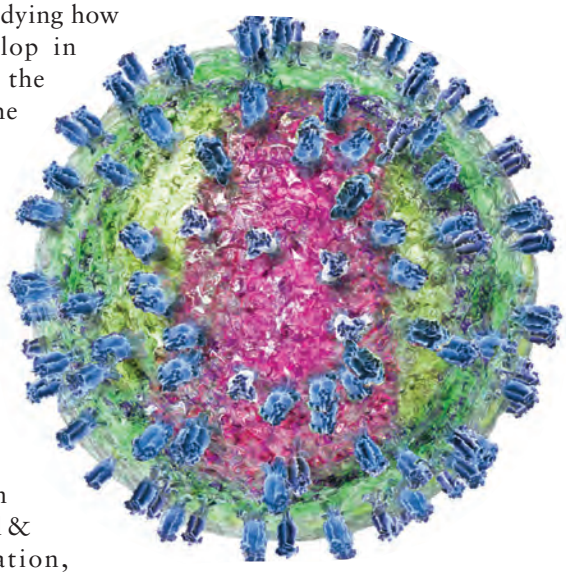
Meanwhile, Burton has been developing transgenic mice that only express the genes for the bNAbs b12, 2F5, or 4E10, respectively. He plans to use these mice to test if weak binding of normal gp120 to the germline versions of these bNAbs might be enough to induce the affinity maturation process. If so, then efforts to look for or develop immunogens that can bind the germline reverted versions of bNAbs might be unnecessary.

A recent study from the labs of Burton and Ian Wilson at TSRI suggests that immunogens different from HIV could also drive the affinity maturation process toward an HIV-specific bNAb. The researchers found that an affinity maturation change that enables the 2G12 antibody to bind to clusters of mannose residues on HIV Env does not necessarily have to have been driven by an interaction with HIV. Instead, it could have come from yeast infection because yeast, like HIV, has clusters of mannose residues on its surface (*J. Virol.* 2010, doi:10.1128/JVI.01110-10).

Understanding the process

The isolation of VRC01 from an HIV-infected person shows that in principle, certain people can make this antibody. But researchers are just starting to understand how common such antibodies really are, and exactly how they actually develop over time in HIV-infected people.

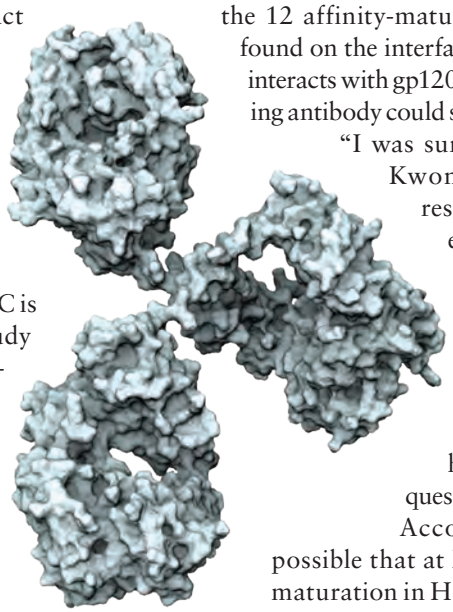
"We already know that [the immune system] can make VRC01, although we are not sure that it can be made in everyone," Kwong says, adding that searches to see how common VRC01-like antibodies are in HIV-infected people are underway at the VRC. "Once you know that many people make VRC01-like antibodies that means that the machinery is there [and] it can be made. [Then] it's just [about] what are the right triggers of that machinery."



Researchers are also studying how antibody responses develop in HIV-infected people from the time of transmission to the time they have bNAbs. Barton Haynes, a professor at Duke University Medical Center and director of the Center for HIV/AIDS Vaccine Immunology (CHAVI), is leading such a study, which also involves Dimitrov, as well as Scott Boyd and Andrew Fire of Stanford University. The study, a collaboration between CHAVI and the Bill & Melinda Gates Foundation, involves next generation 454 sequencing of millions of antibody genes of memory B cells taken from HIV-infected people as they develop bNAbs at different time points after infection. The goal is to determine at least part of what Dimitrov has dubbed the "anti-

bodyome"—the sequence of all or most of a person's antibody genes. Haynes hopes that this will make it possible "to figure out what might have driven [the antibody response] and therefore how you might drive it in a vaccine."

Pascal Poignard, an adjunct professor at TSRI, also plans to study how bNAbs develop over time by selecting HIV-infected people with bNAbs and then sequencing antibody genes of memory B cells from previous samples at various time points starting early after infection. Mascola says the VRC is also planning a project to study how the bNAb response develops in HIV-infected people, although details still need to be worked out.



Haynes is also involved in studying how B cell immune responses are developing in rhesus macaques after immunizing them over long periods of time with the same or different Envs. One goal, he says, is to "determine if repetitive stimulation over the same length of time that it takes to get broad neutralizing antibodies in humans will induce those types of antibodies in nonhuman primates."

A daunting challenge?

Although affinity maturation has received more attention from researchers recently, it's still unclear exactly how much of an obstacle it represents for HIV vaccine design. For example, current data suggest that it seems to take several years for affinity-matured bNAbs to develop in HIV-infected people. If so, the question is whether it will take that long to induce such antibodies with a vaccine. Some researchers say that there are reasons to believe that it won't.

One reason is that not all of the affinity maturation that is observed in bNAbs like VRC01 may be required for broad and potent neutralization of HIV. "If you came with a vaccine that was exquisite or an immunogen that was very well designed, it's possible that you could induce the corresponding antibody with much less somatic mutation," says Burton, adding that he has found that many affinity-matured residues in the b12 antibody can be mutated without affecting binding and neutralization of HIV.

This may also be true for VRC01. When Kwong and colleagues did germline reversions of the 12 affinity-matured amino acids that are found on the interface of VRC01 that directly interacts with gp120, they found that the resulting antibody could still bind gp120 pretty well. "I was surprised to see this," says Kwong, adding that more research is needed to know exactly how much affinity maturation is required. "We don't know if it's just a couple of changes that are required or if extensive affinity maturation [of VRC01] is needed. We haven't fully answered that question."

According to Shapiro, it is possible that at least some of the affinity maturation in HIV-specific bNAbs is simply the result of chronic HIV infection, during which constant interaction with HIV causes B cells to continuously change. "Does it require this much affinity maturation to make an antibody that's effective like this?" he asks. "Or is it simply something that happens because these people have been chronically infected for so many years once they get these antibodies?"

A recent study from the labs of Burton and Wilson suggests that it does not take much affinity maturation to induce important functional changes in the bNAb 2G12 (*J. Virol.* 2010, doi:10.1128/JVI.01111-10). They found that surprisingly few of the over 50 affinity maturation changes are required for 2G12 to assume its unique shape of interlocked or exchanged V_H domains, which enables it to bind a cluster of mannose residues on HIV Env. This "suggests that the evolution of a domain exchanged antibody response *in vivo* may be more readily achieved than considered to date," the authors concluded. These results, Wilson says, support the view that many of the affinity mutations that are acquired on the pathway to final affinity-matured bNAbs may have little or no residual functional significance in the final end product, as they neither enhance nor destroy antigen binding.

And while currently available data suggest that it appears to take years for these bNAbs to develop in HIV-infected individuals, a closer look might reveal that it doesn't have to take that long, Phogat says. A recent study in macaques infected with a simian immunodeficiency virus (SIV)/HIV hybrid known as SHIV showed induction of highly potent neutralizing antibodies to an epitope on the native trimeric Env spike after just nine months (*J. Virol.* 84, 3443, 2010). This suggests, Phogat says, that a SHIV with the PG9/16 epitope, which is also specific for the native trimeric Env spike, should elicit PG-like antibodies in macaques rather quickly as well, and that it shouldn't take that long to induce PG-like antibodies in humans. "It did not take a very long time in macaques," Phogat says. "Why should it in humans?"

In addition, Phogat says, it may not take uninfected people, who would be the recipients of a preventive vaccine, as long to develop affinity-matured antibodies as HIV-infected people. The reason is that HIV infection probably inhibits or slows down the process of affinity maturation because B cells need the help of $CD4^+$ T cells, the primary target of HIV, to undergo affinity maturation. "We are talking about a screwed up immune system [in HIV infected people]," says Phogat. "They don't have proper T [cell] help because those [are the] cells HIV feeds [on]." That's possible, says Burton, but he cautions that not enough is known yet. It's also possible that "some feature of [HIV] infection favors the induction of [broadly neutralizing] antibodies in some individuals," he says. "That's why it's important to understand the origins of broadly neutralizing antibodies in natural infection."

While there is still much to understand, the path forward is pretty clear, Shapiro says. "At the VRC it's a time of really extraordinary excitement," he says. "We have what looks to us, at least until we are proven wrong, like clear targets and clear ways to approach them. Right now the path looks pretty well defined." ■

[UPCOMING CONFERENCES]

40TH ANNUAL CONFERENCE OF THE GERMAN SOCIETY OF IMMUNOLOGY ***Sep. 22-25*****, Leipzig, Germany.** Website: www.immunologie2010.de

AIDS VACCINE 2010 ***Sep. 28 - Oct. 1, 2010*****, Atlanta, Georgia, USA.** Website: www.hivvaccineenterprise.org/conference/2010/index.php

4TH VACCINE AND ISV ANNUAL GLOBAL CONGRESS ***Oct. 3-5, 2010*****, Vienna, Austria.** Website: www.vaccinecongress.com

1ST INTERNATIONAL WORKSHOP ON HIV AND AGING ***Oct. 4-5, 2010*****, Baltimore, Maryland.** Website: www.virology-education.com/index.cfm/t/1st_INTERNATIONAL/vid/513D2261-FF57-A7E0-434955A2F5F5FC07

MODERN VACCINES/ADJUVANTS FORMULATION 2010: IMPACT ON FUTURE DEVELOPMENT ***Oct. 13-15, 2010*****, Cannes, France.** Website: www.meetingsmanagement.com/mvaf_2010/index.htm

3RD BOTSWANA INTERNATIONAL HIV CONFERENCE ***Oct. 13-16, 2010*****, Gabarone, Botswana.** Website: www.botshiv.org.bw

AUSTRALASIAN HIV/AIDS CONFERENCE 2010 ***Oct. 20-22, 2010*****, Sydney, Australia.** Website: www.hivaidsconference.com.au

IMMUNOLOGICAL MECHANISMS OF VACCINATION ***Oct. 27 - Nov. 1, 2010*****, Seattle, Washington.** Website: www.keystonesymposia.org/meetings/viewMeetings.cfm?MeetingID=1108

MEEGID X: 10TH INTERNATIONAL CONFERENCE ON MOLECULAR EPIDEMIOLOGY AND EVOLUTIONARY GENETICS OF INFECTIOUS DISEASES ***Nov. 3-5, 2010*****, Amsterdam, The Netherlands.** Website: www.conferencealerts.com/seeconf.mv?q=ca1msxxi

GENE-BASED VACCINES 2010 ***Nov. 8-10, 2010*****, Cannes, France.** Website: www.meetingsmanagement.com/gbv_2010/index.htm

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MICROBICIDES FINALLY GEL, Securing Spotlight at the International AIDS Conference

The efficacy of a microbicide candidate was the definitive bright spot at this year's conference, while the lingering economic slowdown and its impact on HIV/AIDS funding were dark clouds on the horizon

By Kristen Jill Kresge

2010 WAS TO BE A LANDMARK YEAR in the global response to HIV/AIDS. Following the endorsement by the member states of the United Nations (UN) at the 2005 UN Millennium Summit, this year was when the international community was to achieve universal access to HIV treatment, prevention, and care. But it came as no surprise to the more than 19,000 delegates from 193 countries who gathered in Vienna from July 18-23 for the XVIII International AIDS Conference (IAC) that this goal is far from being met, despite substantial progress in delivering antiretroviral therapy (ART) to those in need.

"We are nowhere near delivering on the promise of universal access," said Julio Montaner, outgoing president of the International AIDS Society, which hosts the biannual IAC.

Some researchers even speculated that the hardest battles in delivering treatment are yet to be fought. "It is possible we've done the easiest part of the job," said Yves Souteyrand, coordinator of the strategic information unit in the HIV/AIDS department at the World Health Organization (WHO). Reports of the escalating HIV/AIDS epidemic in Eastern Europe and Central Asia, where HIV continues to spread within marginalized populations, such as injection drug users, migrants, and sex workers, were a harbinger of how difficult it may be to achieve universal access. "In the past five years, the coverage of HIV treatment in low- and middle-income countries has increased ten-fold," said Brigitte Schmied, president of the Austrian AIDS Society at the conference's opening ceremony. Yet, only 23% of people in need of ART in Eastern Europe and Central Asia have access, according to Schmied.

These regions are now home to a "volatile and increasing" number of new HIV infections, according to the WHO. Although the number of new HIV infections in 2008 was relatively stable in many parts of the world, Europe now has the fastest growing HIV epidemic due to the growing number of new infections in Eastern Europe, primarily in the Ukraine and the Russian Federation.

The hopes of achieving universal access any time soon were also dampened by the lingering economic slowdown that has gripped many of the nations that are the biggest funders of HIV/AIDS treatment and prevention. Concerns about future financing of the HIV/AIDS response dominated this year's IAC, with many keynote speakers, including former US President Bill Clinton and Bill Gates, co-chair of the Bill & Melinda Gates Foundation, calling on organizations to be more efficient in their use of donor money.

But amidst the clouds of economic uncertainty, a bright spot emerged in HIV prevention efforts

with the results of the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 trial, which showed that a microbicide candidate consisting of a 1% tenofovir gel was able to reduce the HIV incidence in a group of South African women by 39% (*Science*, doi:10.1126/science.1193748). "The implications of this are really enormous," said Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases. Even with this level of efficacy, one of the trial's principal investigators, Salim Abdool Karim, said that mathematical models indicate "this gel could prevent 1.3 million new HIV infections and over 800,000 deaths in South Africa alone."

Updates on other prevention strategies, including adult male circumcision, HIV vaccines, and the use of antiretrovirals (ARVs) for prevention were also provided.

A victory in South Africa

After successfully hosting this year's World Cup, South Africa, or at least HIV prevention researchers in that country, had another reason to celebrate. "Today we celebrate the proof-of-concept of microbicides," said Gita Ramjee, director of the HIV prevention research unit at the South African Medical Research Council, who spoke at the session in which the CAPRISA 004 results were presented.

Delegates and researchers were unabashedly gleeful about the first efficacy trial of any microbicide candidate to show a statistically significant reduction in risk of HIV infection. The audience at the standing room only session, which occurred the day after the news dominated the headlines, greeted the data and the trial's co-principal investigators, the husband and wife duo Quarraisha and Salim Abdool Karim, with multiple standing ovations. Researchers also expressed their excitement. "These groundbreaking results mean a lot to me personally," said Ramjee.

The proof-of-concept, double-blinded, placebo-controlled trial tested the safety and efficacy of a coitally dependent, vaginal application of a 1% gel formulation of the ARV tenofovir in 889 women at high risk of HIV infection.

Women in the trial received regular HIV prevention counseling and were instructed to apply the gel up to 12 hours before sex and as soon as possible following intercourse, but within 12 hours—a regimen referred to as BAT24. The overall retention rate was 95%, with 422 women who received the microbicide candidate and 421 women who received the placebo gel completing the trial.

The pre-determined endpoint for the trial was the accumulation of 92 HIV infections. After 30 months, there were 60 HIV infections among placebo recipients and only 38 among the women who received the tenofovir gel, corresponding to a statistically significant 39% lower HIV incidence in the microbicide group (p-value of 0.017, confidence interval of 6%-60%).

However, when researchers looked at earlier time points in the trial, they found that the HIV incidence rate in the microbicide group was as much as 50% lower than in the placebo group after just 12 months. The effectiveness of the tenofovir gel seemed to slowly decline over the subsequent year and a half of the study (see Table 1). "Whichever way you analyze this data, we have a range of protection with a statistically significant result," said Quarraisha Abdool Karim.

This was a relief to some researchers who were familiar with the results released last year from RV144, the efficacy trial of a prime-boost HIV vaccine candidate that provided the first evidence of vaccine-induced protection against HIV. The results stirred some skepticism within the AIDS vaccine field because only one analysis of the data yielded a statistically significant result (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009). "There was a certain feeling of ease and pleasure in looking at data that no matter how you slice it are statistically significant," said Fauci.

Researchers analyzed how the effectiveness of the microbicide candidate correlated with adherence to the prescribed BAT24 dosing regimen. To evaluate adherence, they asked women to return their used plastic applicators to the clinics. Each month, study participants returned an average of six used applicators and reported on average having five sex acts.

Researchers classified women who used the gel more than 80% of the time as "high adherers." Among this group, the efficacy of the tenofovir gel was a statistically significant 54%, as compared to placebo, higher than the overall efficacy of 39%. The efficacy was markedly lower, only 28%, among the group of "low adherers," who used the gel less than 50% of the time.

Over the course of the study, researchers found that the number of returned applicators declined among the women who eventually became HIV infected, suggesting that gel use over time tapered off. This could be a cause for concern, as adherence will obviously impact the real-world efficacy of any behaviorally dependent intervention. And adherence rates outside of the

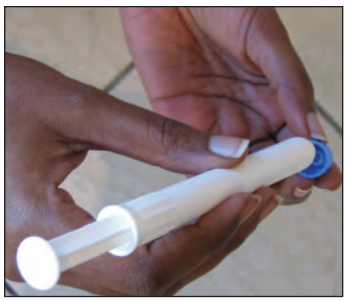
TABLE 1

Time (months)	Effectiveness*	p-value
6	47%	0.064
12	50%	0.007
18	47%	0.004
24	40%	0.013
30	39%	0.017

** Effectiveness of microbicide candidate as compared to placebo*

setting of a clinical trial might differ. "I think we will see very different gel use outside of a trial setting," says Salim Abdool Karim. He thinks gel use may actually be higher in a real-world setting because women would be receiving more positive messages. In a clinical trial, Salim Abdool Karim said women are repeatedly told that they might be getting placebo or that the gel might not have any effect.

In addition to its ability to protect against HIV infection, researchers also determined that the tenofovir microbicide gel reduced the incidence of herpes simplex virus (HSV)-2 by 51% (p-value 0.003, confidence interval 22%-70%) in a subset of women who weren't already infected with HSV-2 at the start of the study. Previous studies have shown that individuals infected with HSV-2 have twice the probability of acquiring HIV (see *HIV Prevention in a Pill?*, *IAVI Report*, Sep.-Oct. 2005). But the ability of the tenofovir gel to block HSV-2 transmission is not responsible for the protection against HIV seen in this trial, according to investigators. "HSV-2 does not account for the effect on HIV," said Salim Abdool Karim. Tenofovir, which was developed by the biopharmaceutical company Gilead, comes from the same precursor compound as a sister drug that has been shown to have potency against HSV-2.



▲ Applicator used to dispense either the microbicide candidate or placebo gel. *Photo courtesy of Centre for the Programme of Research in South Africa*

Gilead still holds licensing rights for the use of tenofovir for HIV treatment, but provided the drug for use in CAPRISA 004 and has granted CONRAD—which collaborated with Family Health International and CAPRISA to carry out this trial—and the International Partnership for Microbicides co-exclusive rights to develop a tenofovir-based vaginal microbicide. About 90% of the funding for the trial came from the United States Agency for International Development, with the remainder contributed by the South African Department of Science and Technology.

CONRAD has given the rights to manufacture tenofovir gel to the South African government, which means that if a tenofovir gel microbicide is eventually licensed, it can be manufactured and distributed directly in South Africa. However, as Salim Abdool Karim explained, the CAPRISA 004 results are really just the "first step and additional studies are needed to confirm findings from this trial."

Luckily, one of those trials is already underway. The Vaginal and Oral Interventions to Control the Epidemic (VOICE) study is comparing daily application of a tenofovir gel to oral administration of either tenofovir or Truvada (a single pill combination of tenofovir and another antiretroviral emtricitabine) in 5,000 women in southern Africa. Results from this trial are expected in 2013. "We're going to get data that will hopefully confirm this and push the field further ahead," said Fauci.

In the meantime, researchers are studying the drug levels in both blood and cervicovaginal fluid among CAPRISA 004 volunteers, and looking for possible explanations for why some women who used the gel still became infected. Angela Kashuba, an associate professor of pharmacotherapy and experimental therapeutics at the University of North Carolina, reported that tenofovir concentrations in blood plasma were <1 ng/ml in women using the microbicide gel. This may be in part why researchers did not detect any tenofovir-associated resistance mutations in women who received the tenofovir gel and eventually became HIV infected. "I was very struck by the lack of any degree of resistance," said Fauci. "That was really quite encouraging."

In contrast, tenofovir concentrations in the cervicovaginal fluids in the first few days following dosing were above 1,000 ng/ml. "The concentrations in the vagina compared to blood showed you could get a whopping concentration at the portal of entry," said Fauci, who called this "the best of all possible worlds." Kashuba also showed that the tenofovir concentrations in the cervicovaginal fluid correlated with HIV and HSV-2 infection status. She said that it would be difficult to achieve such high drug concentrations if tenofovir were taken orally rather than topically, raising questions about how effective oral pre-exposure prophylaxis (PrEP) will be in blocking HIV transmission.

Update on other prevention strategies

While the field eagerly awaits the first efficacy results from oral PrEP trials, which are expected as early as this year, researchers in Vienna reported some interim results from smaller safety studies. Data from a placebo-controlled Phase II trial in the US indicate that there were no significant safety issues associated with administration of once-daily tenofovir to HIV-uninfected men who have sex with men (MSM). The overall number of adverse events that occurred in men receiving tenofovir was similar to that in the placebo group.

Although tenofovir is generally safe and well tolerated in HIV-infected individuals, use of the drug has been associated with some more serious side effects, including compromised kidney function and a reduction in bone mineral density. However, in this study, there was no significant

difference in bone density between the tenofovir and placebo groups, and none of the individuals had elevated creatinine levels, which are an indication of compromised kidney function.

The study, conducted by the US Centers for Disease Control and Prevention, enrolled 400 MSM in Atlanta, San Francisco, and Boston. The men were randomized to receive a once-daily dose of 300 mg of tenofovir or placebo either at the start of the trial, or after a nine-month delay. This allowed researchers to compare the risk behaviors between the two groups during the nine months when only half of the volunteers were taking a daily dose of either tenofovir or placebo. In the analyses conducted so far, researchers reported that there was no significant difference in risk behaviors between the two groups of men during that nine-month period.

Data on the safety of intermittent dosing of Truvada in five female sex workers and 67 MSM in Kenya, as well as 36 serodiscordant couples (where one partner is HIV infected and the other is not) in Uganda were also presented in a poster at the conference. In this study, researchers compared daily dosing of Truvada or placebo with an intermittent dosing schedule consisting of a fixed dose on Mondays and Fridays and a dose following each sex act, without exceeding one pill per day. At the conclusion of the four-month study, researchers found that the safety profiles of the daily and intermittently scheduled dosing of Truvada were similar.

Researchers also compared the adherence rates for volunteers in the daily and intermittent groups. Adherence to Truvada or placebo was primarily assessed using the medication event monitoring system (MEMS), an electronic pill bottle cap that records each date and time that the bottle is opened. Investigators also calculated adherence based on self-reported behavior, collected either through questionnaires or though an interactive short message service (SMS). The overall adherence rate to the daily dose was high—83% among the MSM and female sex workers at two sites in Kenya, and 96% among discordant couples in Uganda. Adherence in the intermittent-dosing group was lower with an overall adherence rate of 68% in Kenya and 80% in Uganda. In both groups, adherence rates among discordant couples were significantly higher than in MSM or female sex workers.

When researchers analyzed the adherence rates to the fixed versus post-coital doses in the intermittent group, they found the adherence rates to the post-coital dose were much lower—26% in Kenya and 45% in Uganda for the post-coital dose, compared to 55%, and 91% respectively for the fixed doses. The self-reported behavior differed drastically—volunteers reported taking the post-coital doses 105% of the time in Kenya and 103% of the time in Uganda. Although the investigators acknowledge that it was difficult to accurately measure adherence to the post-coital dosing, they suggest this may be a challenge in these settings and that fixed-dosing schedules may be preferable.

This study, conducted by IAVI in collaboration with the Kenya AIDS Vaccine Initiative, the Kenya Medical Research Institute, the Medical Research Council in Uganda, and the Uganda Vaccine Research Institute, was the first to compare the safety of and adherence to an intermittent PrEP regimen with daily use.

One month, 36,000 circumcisions

While several trials of microbicides and PrEP are underway, Bill Gates reminded the delegates in Vienna that several proven HIV prevention strategies, including adult male circumcision, the prevention of mother-to-child transmission, and syringe exchange still need to be implemented more widely. He said these strategies were "so cheap and effective it's more expensive not to pursue them," and he emphasized the need to scale up strategies as soon as they're proven to work. "Male circumcision is an amazing advance in prevention," said Gates.

Efforts to scale up adult male circumcision, which was found to be about 60% effective in reducing HIV infection risk among heterosexual men in three large trials, have been underway in several African countries. But perhaps nowhere has there been more progress than in Kenya, where between November and December 2009, the country launched a campaign in 11 districts of Nyanza province to perform voluntarily circumcisions on 30,000 men in 30 days.

After one month, researchers actually exceeded this target—36,077 voluntary medical male circumcisions were performed. On average, the team performed 10 male circumcisions per day, which reduced the overall cost of the intervention because the staff achieved optimal efficiency, according to Elijah Odoyo June, of the Nyanza Reproductive Health Society, who presented on the success of this circumcision campaign.

"I was doubtful a large number of men would sign up for it, [but] I was wrong," said Gates. June said the demand for male circumcision by parents of boys younger than 12 was higher than expected. Of the 36,077 males who were circumcised, nearly

[THERAPEUTIC VACCINE CANDIDATE SHOWS PROMISE]

Results from a Phase II trial show that multiple intramuscular injections of a DNA-based vaccine candidate, developed by the biotechnology company FIT Biotech, reduced average HIV viral load by 0.5 log compared to placebo in 60 clade C HIV-infected, treatment-naive volunteers. The viral load reduction was statistically significant and was sustained for at least two years.

The trial was conducted at a single clinical trial center in Soweto, South Africa, and was the first therapeutic vaccine trial to be conducted in the country. All volunteers were HIV-infected for at least three years, had HIV viral loads greater than 38,000 copies per ml of blood plasma, and CD4⁺ T-cell counts above 500 cells per µl, making them ineligible to receive treatment based on South Africa's guidelines.

The vaccine candidate, referred to as FIT-06, utilizes a proprietary gene transfer unit (GTU) technology that allows for more long-term gene expression, and therefore requires lower doses of DNA. "So far, DNA vaccines haven't been so successful, so the technology makes a difference," says Joep Lange, head of the Amsterdam Institute for Global Health and Development.

Researchers speculate that the CD4⁺ and CD8⁺ T-cell responses induced by the candidate helped control viral replication. In this trial, an increase in antigen-specific CD4⁺ and CD8⁺ T-cell responses was detected in 55% of volunteers.

FIT Biotech plans to initiate a Phase I trial in the US to test this vaccine candidate as a preventive approach. *—KJK*

half (45%) were under 15 years of age.

In advance of the campaign, the team accelerated public education efforts on male circumcision and strengthened referral services in the target districts. Yet uptake was still slow at the start of the campaign. "Despite a lot of preparation, it took two weeks to attain optimal client flow," said June. Given the overall success of the campaign, June said the team recommends using this approach to scale up male circumcision in other areas.

Less enthusiasm for test and treat

The use of universal testing and immediate antiretroviral treatment for those infected, what is referred to as the test and treat strategy, has received growing attention in the past year. However, in Vienna, questions were raised about this approach, much to the chagrin of Montaner, who said he hoped it would be the emerging issue of the conference. "We have abundant evidence that ARV treatment is prevention," he said.

But others were more circumspect about the potential benefits of test and treat. Myron Cohen, director of the Institute for Global Health at the University of North Carolina, speaking at a session on ARV-based prevention, declared himself a big advocate of treatment and prevention, but said "the details really matter."

A study, presented by Jing Luo of the University of Illinois Chicago College of Medicine, found ARV therapy had no effect on HIV transmission rates during a three-year study in nearly 2,000 serodisordant couples in Henan province,

A Summary of Progress in the HIV Vaccine Field

"We need a big game changer and that can only be a vaccine," said Peter Piot, a professor at Imperial College London and chairman of the board of the Global HIV Vaccine Enterprise, speaking at the satellite session "The Search for an HIV Vaccine: Where are we, where are we going, and how can we get there faster," which was held on the opening day of the XVIII International AIDS Conference in Vienna.

During the session, almost everyone commented on the significant progress in the field, and in particular the results of the RV144 efficacy trial, which, when released last September, marked the first evidence of vaccine-induced protection against HIV to emerge from clinical trials (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct., 2009). According to Piot, these results "put HIV vaccine development back on the world stage."

Another area of progress in the HIV vaccine field was the recent identification of several new broadly neutralizing antibodies (bNAbs) against HIV (see *Adding to the Armamentarium of Broadly Neutralizing Antibodies*, *IAVI Report*, Jan.-Feb. 2010). Seth Berkley, president and chief executive officer of IAVI, which isolated two of the new more potent bNAbs (dubbed PG9 and PG16) last year in collaboration with The Scripps Research Institute, said these discoveries have contributed to what he called a renaissance in AIDS vaccine research and development.

The discovery of three other new bNAbs, identified by researchers at the Vaccine Research Center at the US National Institute of Allergy and Infectious Diseases, was officially reported just before the start of the conference in Vienna (*Science* **329**, 856, 2010). Berkley said there are also "more than a dozen new antibodies that are in the process of discovery," and are now being characterized.

Robin Shattock, a professor of cellular and molecular infection at St. George's, University of London, said these new antibodies "open up a real hope for identification of novel immunogens." But Shattock also pointed out that these bNAbs only develop in HIV-infected individuals after several years of infection, suggesting that the maturation of antibody responses may contribute to their potency and ability to neutralize so broadly (see *Vaccines to Antibodies: Grow Up!*, page 4). Shattock said that researchers may have the challenge of developing "vaccine approaches that could mimic that maturation without multiple injections."

This work is now underway as researchers try to reverse engineer vaccine immunogens based on this new crop of bNAbs. A proof of principle for this reverse vaccinology approach was recently demonstrated by scientists at Merck Research Laboratories (*Proc. Natl. Acad. Sci.* **107**, 10655, 2010). The researchers identified an engineered peptide that mimics the N-terminal heptad repeat (NHR) region of the pre-hairpin intermediate that is transiently exposed during the binding of HIV's gp41 protein to cell membranes. X-ray crystallographic studies showed that this peptide could bind to a monoclonal antibody referred to as D5 that can neutralize various HIV isolates *in vitro*.

Vaccination of guinea pigs with two of the most promising immunogens developed based on this peptide elicited neutralizing antibody responses against some tier 1 viral isolates, but not against any tier 2 viruses, which are generally more difficult to neutralize. The neutralization potency and quantity of the D5-like antibodies induced in rabbits were much lower than in guinea pigs, leading the study's authors to conclude that this "may point to increased difficulty in eliciting protective neutralizing antibody titers in higher species, including primates."

While there are still many obstacles to eliciting broadly neutralizing antibodies against HIV through vaccination, many researchers believe this is still an important goal. "If we could achieve broadly neutralizing antibodies or broad cellular immunity, those would undoubtedly add to the efficacy we observed [in RV144]," said Merlin Robb, HIV program director for the US Military HIV Research Program.

In his special plenary session during the conference, Bill Gates spoke about the need to speed up the HIV vaccine development process, citing the fact that only three vaccine candidates have undergone efficacy testing so far. "The ultimate prevention tool is a vaccine," said Gates, and "it is possible." —*KJK*

China. However, one limitation was that researchers did not collect adherence or viral load data, which could have elucidated whether the HIV-infected partners had stopped therapy or were on sub-optimal therapy due to development of drug resistance. "Most likely the patients were intermittently taking therapy," suggested Cohen. Luo acknowledged the study's limitations, but still concluded that "antiretroviral treatment alone may not be sufficient to reduce HIV transmission among serodiscordant couples in real-world, resource-poor settings."

"We need to deal with these issues and flesh this out going forward," said Cohen. He also said more long-term studies are needed to see if the prevention benefits of ARV therapy, which have been seen in other studies, are durable. Cohen is heading up a study of nearly 1,800 serodiscordant couples to see if there is a prevention benefit over five to seven years if the HIV-infected partners are taking ARVs.

Studies have indicated that during acute or early HIV infection, a person is at least 26 times more infectious, and other studies have estimated that between 9% and 90% of infections are the result of HIV transmission from someone who is acutely infected. Cohen called this an "inconvenient truth," and said this may mean that initiating treatment immediately regardless of $CD4^+$ T cell count may not have as great of an effect on preventing the spread of HIV as treating people either very early or late in the course of HIV infections when people are most infectious.

The funding forecast

Looming over nearly all the discussions at the IAC this year were dark clouds of economic uncertainty. In 2008, the global investment in HIV/AIDS reached a record high of US$15.6 billion, a 39% increase in funding from the previous year, according to a recent report issued by the Joint United Nations Programme on HIV/AIDS (UNAIDS).

But following the recent economic crisis, many countries are freezing their investments in global health. "The idea that we should cut back now is ridiculous," said Michel Sidibé, executive director of UNAIDS. An analysis by UNAIDS and the Kaiser Family Foundation, which was released in Vienna, found that contributions from the Group of Eight Nations, the European Commission, and other governments provided $7.6 billion for AIDS in developing countries in 2009, nearly level with their $7.7 billion contribution in 2008. This was the first year of flat funding, following a substantial increase in donor support for AIDS since 2002 when the total contribution was only $1.2 billion.

Funding for HIV vaccine research was also flat in 2009. According to the HIV Vaccines and Microbicides Resource Tracking Working Group, the total global investment in HIV vaccine research and development held steady last year at $868 million. And according to Seth Berkley, president and chief executive officer of IAVI, the only reason it stayed flat rather than dipping was because of the economic stimulus funding the US government provided, some of which went to preventive HIV vaccine research projects funded by the US National Institutes of Health.

"We're not seeing the increases we've seen in the past," said Gates. "Today, skeptics say we can't beat AIDS because of these financial limitations," he added. But while acknowledging that these are tough economic times, Gates said he is still an optimist and emphasized the need to "push for efficiency in both treatment and prevention."

For treatment, Gates said organizations should share best practices and strive to lower the cost of delivering ARVs. "If we could limit the delivery costs to $300 per person per year we could treat twice as many people [with existing funding]," Gates said.

Peter Mugyeni, director and founder of the HIV/AIDS Joint Clinical Research Centre in Uganda, said that by doing fewer $CD4^+$ T-cell tests in developing countries, researchers could save money that could be used to provide treatment to more people. "We're not arguing for second-rate HIV monitoring but we could treat more people by eliminating non-essential tests," he said.

Former president Clinton also called for more efficiency in HIV/AIDS programs. "We cannot get to the end of this epidemic without more money and changes in the way we spend it." Clinton said more of the money spent on AIDS needs to go directly to helping people rather than on the apparatus or establishment that provides the services. "Every dollar we waste today puts a life at risk," he said. Clinton also suggested that programs should shift tasks from doctors to nurses wherever possible to cut costs, and that donors should only support organizations that do things better, faster, and at a lower cost.

"We're at a turning point. We can push ourselves to make the most out of every dollar of funding," said Gates, who also spoke about his foundation's commitment. "We give more to AIDS than any other disease and we'll be here until this thing gets finished and keep it as our top priority." ■

A SYSTEMS APPROACH
To Understanding Vaccines

Researchers are starting to apply the tools of systems biology to better understand human immune responses to vaccination, including HIV vaccine candidates

By Andreas von Bubnoff

Increasingly, researchers are using a more integrative approach to understand complex biological systems, such as the human immune system. Systems biology, as this approach is called, has only recently become possible because of the availability of technologies such as microarrays that can simultaneously measure the expression of many of an organism's genes. This results in the so-called transcriptome, the level of RNA transcripts of some or all of the genes expressed in a cell. It is now also possible to measure the levels of all of the proteins or metabolites in a cell, resulting in the proteome or the metabolome, respectively.

One advantage of a system-wide approach is that it collects a massive amount of data without requiring researchers to operate on hypotheses. "You accumulate a lot of data [in an] unbiased way, which I think is a wake-up call compared to what everybody has been doing," says Rafick Sékaly, scientific director of the Vaccine & Gene Therapy Institute in Florida. Michael Katze, a professor of microbiology at the University of Washington, has a similar view. "The traditional reductionists will say that [we will] get the answers by doing empirical reductionist biology. The people who are more converted to systems biology like me will say we will only get the answers when we have enough data. In my mind it's an unacceptable failure that over 25 years after the discovery of the AIDS virus and all the billions of dollars that have been spent on it we don't understand what a good vaccine is. My argument, my hypothesis, is that we don't know anything, so let's discover what's going on. Let's take more of a holistic rather than a reductionist approach."

But just getting people to agree on the definition of systems biology can be a challenge. "It means different things to different people," says Stephen Popper, a research associate at Stanford University. "To some people, doing microarrays or some other sort of expression profiling is systems biology," he says. To others, he adds, it is integrating different types of information such as gene expression and proteomics, or building dynamic networks.

Bali Pulendran, a professor of immunology at Emory University, says systems biology is much more than microarrays and genomics. "The real goal is to use high throughput data such as microarrays to understand the behavior of complex biological systems and ultimately predict the behavior of such systems, including vaccine-induced host defenses against HIV."

Other areas of biomedical research, such as cancer research, have been using the tools and concepts of systems biology for some time. To encourage the HIV vaccine field to explore possible uses

of this new technology, the Global HIV Vaccine Enterprise in 2008 helped organize one of the first meetings that brought together systems biologists and HIV vaccine researchers. "I think there has been a silo between people who do systems biology and people who do HIV vaccinology, and I think one of our jobs is to help bring people together and encourage collaboration," says Alan Bernstein, executive director of the Global HIV Vaccine Enterprise. Katze agrees. Systems biology projects, he says, involve "a lot of people who don't normally talk to each other—clinicians, biostatisticians, virologists. It defies the way classical experimentalists have grown up."

Researchers are now using the tools of systems biology to characterize the immune responses induced by existing vaccines and identify the gene expression changes that occur following vaccination. They are also beginning to apply systems biology approaches to the development of HIV vaccine candidates and to better understand HIV pathogenesis.

Responses to existing vaccines

Recently, Pulendran's and Sékaly's labs used a systems biology approach for the first time to study the immune response induced by a vaccine (*J. Exp. Med*. **205**, 3119, 2008; *Nat. Immunol*. **10**, 116, 2009; see *Research Briefs, IAVI Report,* Jan.-Feb. 2009). Using microarray analysis, they measured gene expression changes in the innate immune response to the yellow fever vaccine, starting days after vaccination. The vaccine against yellow fever is one of the most effective vaccines ever developed. "The reason why we decided to do yellow fever was because it works, so we wanted to understand what a good vaccine looks like," says Alan Aderem, director and cofounder of the Institute for Systems Biology in Seattle, who collaborated on Pulendran's study.

The studies found a group of genes, or a signature, which was consistently expressed more strongly after yellow fever vaccination. Pulendran's group also showed that this signature could be used to predict the level of the later adaptive B- and T-cell immune response to the vaccine.

Researchers in Sékaly's group have also identified a similar gene expression signature in yellow fever vaccinated rhesus macaques and plan to test whether this signature can predict protection. They are also trying to see if the gene expression signatures induced by the yellow fever vaccine are absent in people who got sick despite yellow fever vaccination. This is not easy, however, because almost all yellow fever vaccinees are protected.

The yellow fever findings show that, in principle, it is possible to use gene expression signatures as an early biomarker to indicate whether a vaccine will work. This could save time in vaccine development. "The idea is a biomarker that [lets] you have an earlier endpoint for measuring how well your vaccine is working," Popper says. "[It] gives you a readout that you can use as you go [to] some next stage of development. If you collect all the right samples and identify a relevant signature, then the next round of development will profit from increased information and hopefully shorten the time cycle." Aderem agrees. "[A signature] will be critical because you'd be able to assess early on whether or not a vaccine is a good one or not," he says.

The real goal is to…understand the behavior of complex biological systems and ultimately predict the behavior of such systems. *– Bali Pulendran*

Researchers have also begun to study other licensed vaccines to determine whether they induce similar gene expression signatures to those induced by the yellow fever vaccine. Influenza vaccination is the focus of the first major project at the Center for Human Immunology, Autoimmunity and Inflammation (CHI), that was recently established by the US National Institutes of Health (NIH) to use systems biology tools to analyze, in unprecedented detail, the normal and perturbed state of the human immune system. CHI-affiliated researchers are collecting data from more than 150 people of different ages and ethnic backgrounds who have been vaccinated with the killed flu virus. Researchers will collect data prior to vaccination to establish baseline measurements and help define the normal so-called "immunome." They will also collect post-vaccination data on day one for the innate response, on day seven for the adaptive response, and on day 70 for the memory response, according to Ron Germain, deputy chief of the Laboratory of Immunology at the National Institute of Allergy and Infectious Diseases (NIAID), and an associate director of CHI.

In each individual, researchers will analyze, among other things, genome-wide gene expression, more than 60 cytokines in blood, and

700,000 gene variants, known as single nucleotide polymorphisms (SNPs), to help track the effects of host genes on the immune response. In a related study, researchers are also looking at the early innate immune response in blood of flu vaccinees as early as four hours after vaccination. CHI is also considering assessing the immune status of people who are given the live-attenuated flu vaccine, as well as other vaccines with or without adjuvants, Germain adds. "Over the next year or two we will be getting much more information at a much larger scale that will let us set the baseline standards to try to understand what it [looks] like when you have a normal human immune system [or] when you begin to perturb it with various vaccine challenges," he says.

Pulendran and colleagues are also studying gene expression changes after vaccination with the inactivated or the live-attenuated seasonal influenza vaccines. They have found that gene expression signatures at days three and seven after vaccination with the inactivated flu shot predict the magnitude of the later antibody responses to the flu protein hemagglutinin. They also found that the expression level of a gene called tumor necrosis factor (TNF) receptor superfamily 17, which promotes B-cell maturation, predicts the later antibody response after both yellow fever and flu vaccination. "This is quite exciting to me because it hints at the likelihood that there might be some common predictors in different vaccines," says Pulendran. "It's possible that [such genes] may also be predictive of antibody responses against HIV."

Recently, Pulendran was awarded a grant by the NIH to establish a Center for Systems Vaccinology at Emory University, which will study whether this approach can be used to predict the effectiveness of vaccines, including those against influenza, pneumococcal disease, and shingles. The US$15.5 million center will be established as part of a $100 million nationwide research initiative by the NIH to define changes in the human immune system in response to infection or vaccination.

Pulendran has also shown that gene expression changes can be used to predict the kind of immune cells that are induced by the flu vaccine. Using publicly available microarray data, Pulendran has compiled libraries of genes that are typically expressed in different types of immune cells such as macrophages, B cells, or $CD4^+$ and $CD8^+$ T cells. He has validated these cell-type specific signatures in gene expression data from whole blood in people vaccinated against flu. For example, flu vaccinees express many genes that, according to the library, belong to plasma B cells, and as predicted, Pulendran found more B cells in these vaccinees.

Pulendran also plans to study people who received pneumococcal vaccine, which is given to the elderly to prevent pneumonia, or herpes zoster vaccine, which is given to the elderly to protect against shingles. Because these vaccines don't protect all vaccinees, it is possible to compare gene expression signatures in protected and unprotected vaccinees. "In the elderly population, a substantial number of people are not protected," Pulendran says. "We want to use the biomarker approach to identify those people."

According to Germain, there are also discussions about comparing hepatitis B vaccinees, 80% to 90% of whom are protected, to recipients who receive the standard hepatitis B vaccine with an adjuvant called CpG (an artificial DNA oligonucleotide containing the CpG motif), all of whom are protected. If CHI can participate in

Building Models of Complex Biological Systems

One goal of systems biology is to develop models of complex biological systems. "It may be quite challenging to develop all-encompassing models for complex physiological systems such as the immune system," says Leor Weinberger, a biochemist and virologist at the University of California in San Diego. "But a number of researchers have already developed simple models that are effective and predictive."

Weinberger has used computer models to predict how HIV Tat protein expression influences the expression of HIV genes and then verified the predictions in experiments. Also, Alan Aderem, director and co-founder of the Institute for Systems Biology in Seattle, and colleagues have analyzed and simulated the signaling cascade activated by Toll-like receptors, which is part of the innate immune response to bacteria and viruses.

Others are using computer models that simulate components of the immune system itself. Vladimir Brusic, director of the cancer vaccine center bioinformatics core at the Dana-Farber Cancer Institute, has been involved in a project that used a model of the immune system that contains about 50-100 different elements such as the major cell types, cytokines, and other molecules of the immune system to predict the immune responses to breast cancer vaccine regimens in mice. The model predicted that the number of vaccinations could be reduced by about 40% without a loss in vaccine efficacy. Subsequent experiments showed that the prediction was largely correct. In principle, Brusic says, this approach could also be used to assist in the development of HIV candidate vaccine regimens.

A recent study led by Arup Chakraborty, a professor of chemistry, chemical engineering, and biological engineering at the Massachusetts Institute of Technology, and Bruce Walker, a professor of medicine at Harvard Medical School, used computer models of relevant parts of the immune system to predict that $CD8^+$ T-cell repertoires that are more cross reactive to different HIV peptides lead to better control of viral load (*Nature* **465**, 350, 2010; see *Research Briefs*, *IAVI Report*, May-June, 2010). The study then validated the prediction with data from elite controllers and HIV-infected people with progressive infection. —*AvB*

such a study, it would include gene expression analysis and genomic analysis of SNPs to see if there are any genetic variants that are associated with a lack of response to the vaccine.

Systems biology and HIV vaccines

While researchers are collecting clues from studies of existing vaccines, they are also beginning to use systems biology approaches to study experimental vaccines, such as HIV vaccine candidates tested in recent clinical trials. Bob Palermo, a principal research scientist in Katze's lab, is studying genome-wide gene expression in 200 volunteers from the STEP trial, a phase IIb trial of Merck's adenovirus serotype 5 (Ad5)-based vaccine candidate known as MRKAd5. Palermo is looking for hints about why MRKAd5 failed to protect against HIV infection, and initially even seemed to increase the risk of HIV acquisition in vaccinees that were uncircumcised, had a high titer of preexisting Ad5 antibodies, or both.

Samples from RV144—an HIV vaccine efficacy trial conducted in more than 16,000 volunteers in Thailand that showed a modest 31% protection against HIV infection—will also be analyzed for genome-wide RNA transcriptional analysis by Sékaly's lab, according to Nelson Michael, director of the US Military HIV Research Program, one of the agencies that conducted the RV144 trial. The initial analysis will include samples from the time of the first vaccination, six months later (two weeks after the last vaccination), which is close to the peak of immune responses, and one year after the initial vaccination. An initial pilot study will only use samples from HIV-uninfected RV144 trial volunteers, and will include samples from 80 vaccine recipients (40 male and 40 female), and 20 placebo recipients (10 male and 10 female). Afterwards, a case-control study will look at possible gene expression differences between approximately 40 vaccinees who later became infected and 200 who did not.

In most vaccinees, the RV144 candidate vaccine regimen, a canarypox vector-based candidate, ALVAC-HIV, administered in a prime-boost combination with an engineered HIV gp120 protein, AIDSVAX B/E, resulted in gp120-binding antibody responses. Michael says that the titers of antibodies that bind gp120 will be determined in 100 RV144 vaccinees that didn't get infected with HIV. To see if there are any genetic differences between the vaccinees with high antibody responses to the vaccine and those with lower antibody responses, David Goldstein, director of the Center for Human Genome Variation at Duke University Medical Center, will then determine the genome sequence of the 20 RV144 vaccinees with the lowest and the highest antibody titers, respectively. If genetic differences can be identified, they would make it possible to better interpret the efficacy of vaccines in the future, Michael says. "There could be people that just inherently are more able to respond to a vaccine," he says. "It's important to know if the deck is already stacked one way or the other against people."

Researchers are also starting experiments in rhesus macaques to study early gene expression changes in response to experimental vaccine regimens against simian immunodeficiency virus (SIV). One advantage of the animal model is that researchers can later infect, or challenge, the vaccinated animals and compare those that are protected to those that become infected. In principle, this should enable them to find early gene expression changes in the innate immune response to a vaccine regimen that can predict whether an animal will be protected against challenge.

Sékaly's group is collaborating with Adrian McDermott, director of immunology and vaccine design at IAVI, to analyze gene expression changes in macaques four days, two weeks, and three weeks after vaccination with SIVmac239Δ*nef*, a live-attenuated version of SIVmac239. The researchers found that the macaques that were protected from SIVmac251 challenge a year later had a different early gene expression profile from the ones that became infected. "Both the innate and the adaptive immune responses are quite distinct," Sékaly says.

Insights into HIV and SIV infection

Researchers also hope that systems biology tools will give them insight into the effects of HIV infection in unprecedented detail, and at earlier time points than ever before. "We really do not know how any virus triggers the events that ultimately lead to virulence and pathogenesis," Katze says. "Now we have the tools."

Already, Katze and colleagues have infected $CD4^+$ T-cell lines *in vitro* with HIV and have looked at changes in the abundance of more than 1,000 proteins as early as a few hours after infection. "Cell lines are an easy start," says Palermo, adding that ultimately, the goal is to do this kind of analysis with primary human cells. "We would like to do it with truly *bona fide* target cells [of HIV]," he says. The analyses will then also look at gene expression as early as one hour after infection, Palermo adds.

Damien Chaussabel, an associate investigator at the Baylor Institute for Immunology Research, hopes to correlate gene expression signatures soon after HIV infection with the clinical outcome later on. He does expression profiling of blood samples taken from HIV-infected people at five time points from as soon as one to two weeks after infection, and up to 24 weeks later. The samples, provided by the Center for HIV/AIDS Vaccine Immunology (CHAVI), come from HIV-infected people in Malawi, South Africa, and North Carolina.

It's like Christopher Columbus—you can see that there is a whole new world out there but you don't know quite what's out there yet.

– Louis Picker

Systems biology analyses are also underway to better understand why certain people, dubbed elite controllers, can control viral load below detectable levels for decades without treatment. It is already known that their $CD8^+$ T cells function better than those of progressors, which are thought to be deficient in their ability to multiply or to secrete cytokines (see *Research Briefs, IAVI Report*, Jan.-Feb. 2009). Consistent with this, Sékaly and Elias Haddad, an associate scientist at the Vaccine & Gene Therapy Institute in Florida, have done gene expression analyses that suggest that HIV-specific T cells from elite controllers survive longer than the same cells from chronic progressors. Next, they want to see if this difference is also present at the protein level, and if the T cells show corresponding functional differences. If gene expression in elite controllers is found to be similar to gene expression in people vaccinated with yellow fever vaccine, it would suggest that it might be possible to eventually use such gene expression signatures of elite controllers as a guide to assess HIV vaccine candidates.

W. Nicholas Haining, an assistant professor of pediatrics at the Dana-Farber Cancer Institute and Harvard Medical School, says that gene expression analysis of antigen-specific $CD8^+$ T cells from elite controllers and progressors has allowed him to identify new classes of genes that have not previously been associated with T-cell dysfunction in HIV.

Researchers are also using transcriptional profiling to understand why some nonhuman primates (NHPs) such as African green monkeys (AGMs) don't get sick from SIVagm infection, while other NHPs such as pigtail macaques do. Looking at gene expression changes in cells taken from the colon, lymph nodes, and blood, Katze found that while SIV induces immune activation and interferon-stimulated genes in both AGMs and pigtail macaques, these responses only resolve in the AGMs. In addition, AGMs have more active cell survival pathways (*PLoS Pathog.* **5**, e1000296, 2009). Other groups made similar observations when they compared gene expression in SIV-infected AGMs or sooty mangabeys—neither of which get sick when infected with SIV—with rhesus macaques, which do get sick (*J. Clin. Invest.* **119**, 3544 and 3556, 2009). They also observed that activation of interferon activated genes was transient in the species that don't get sick, and chronic in rhesus macaques.

New tools, new questions

As researchers are implementing systems biology approaches, the tools available to them keep evolving. To analyze the expression of thousands of genes, for example, many researchers currently use microarrays, which only probe for the expression of the genes that are known to be encoded by RNAs. But such analyses are already being replaced by another method called RNAseq, which uses ultrafast high-throughput next-generation sequencing to sequence and also count all RNAs in a sample, including ones whose functions are completely unknown.

Recently, Katze used RNAseq to sequence all RNAs expressed in the lungs of severe acute respiratory syndrome (SARS)-infected mice. The results were rather "scary," he told the audience at this year's Conference on Retroviruses and Opportunistic Infections, which took place in February. "You see all these novel RNAs that are not annotated to anything in the genome," Katze said. "Nobody knows what the hell these RNAs do."

Systems biology will probably enable researchers to make a lot of new discoveries, says Louis Picker, a professor at Oregon Health & Science University, who works with Sékaly and Aderem on systems biology analysis of candidate SIV vaccine regimens in rhesus macaques. "It's like Christopher Columbus—you can see that there is a whole new world out there but you don't know quite what's out there yet." ■

Research BRIEFS

3-D Structure of Dendritic Cell-T Cell Virological Synapses Revealed

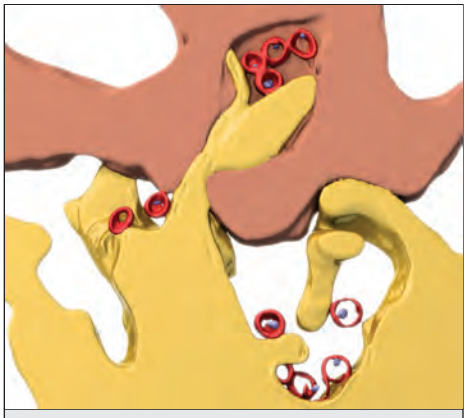
HIV can either be transmitted as free virus or be transferred from infected to non-infected cells through so-called virological synapses. How often HIV transmission *in vivo* results from cell-cell viral transfer is unknown, but it has been shown that this mode of transmission is likely more efficient than cell-free transmission. Previous studies suggest that HIV infects CD4+ T cells about 1,000 times more efficiently in the presence of dendritic cells (DCs), presumably because in this case HIV is transmitted through a virological synapse between the DC and the CD4+ T cell.

Now, a study using electron microscopy has shown the three-dimensional (3-D) structure of DC-T cell synapses in unprecedented detail, providing some clues as to what makes this type of transmission so efficient (*Proc. Natl. Acad. Sci.* **107**, 13336, 2010). The study, led by Sriram Subramaniam, chief of the biophysics section in the laboratory of cell biology at the National Cancer Institute (NCI) in Bethesda, also implies that it may be harder for molecules such as antibodies to reach HIV particles in a DC-T cell synapse.

The researchers cultured human DCs with HIV for one to two hours, then added CD4+ T cells from the same human donor for another two hours before fixing the cells for microscopic analysis. The study is the first that uses a method called ion abrasion scanning electron microscopy (IA-SEM) to view cell-to-cell contacts, Subramaniam says. IA-SEM uses an ion beam that takes slices off the surface of a biological specimen, such as a cell, and captures an image of the surface after each slicing. The resulting images are then reassembled into a 3-D image.

In this study, researchers used light microscopy to show that the HIV particles are at the interface between the DC and the T cell. Closer inspection with IA-SEM and electron tomography revealed that the HIV particles are enclosed in spaces inside DCs that are connected to the surface by channels.

IA-SEM also showed that the DCs had large veil-like extensions on their surface (see cover image), which catch HIV particles from the surrounding medium and enclose them in spaces that remain connected with the surface, says Subramaniam, who last year used IA-SEM to show similar structures in HIV-infected macrophages (see *The Beauty Behind the Beasts, IAVI Report*, Nov.-Dec. 2009).



In this electron tomographic image, a CD4+ T cell (yellow) appears to use filopodia to pick up HIV particles (red) that are buried inside channels in a dendritic cell (brown). *Image courtesy of Donald Bliss and Sriram Subramaniam, NCI.*

For the first time, Subramaniam says, the study also found that these membrane extensions from DCs sometimes enclose entire T cells, shielding off the virological synapse. This, together with the fact that the HIV particles are found in spaces deep inside the DCs, suggests that HIV particles in DC-T synapses may be harder for molecules like antibodies to reach than free virus, Subramaniam says. But this may not be true for all types of virological synapses. A recent study showed that HIV particles transmitted between CD4+ T cells are not harder to reach than cell-free HIV (see *Research Briefs, IAVI Report*, Mar.-Apr. 2010).

The researchers also found that the CD4+ T cells appear to use filopodia to pick up the HIV particles by reaching into the channels and spaces in the DC where the HIV particles are found (see Figure, left). In addition, when they added antibody to the CD4 co-receptor, the main receptor HIV uses to infect CD4+ T cells, they were surprised to find that HIV remained stuck to the DCs. "I thought the viruses would just fall off, but it looks as though they are anchored to the surface of the dendritic cell," Subramaniam says. This suggests that the CD4+ T cells actively pluck the virus off the DCs, ensuring that no virus is just wasted without infecting its target cell, which may in part explain why HIV is transmitted so efficiently via DC-T cell synapses. "The virus is not transferred under conditions when it knows it's not going to infect the T cell," he adds.

Once the HIV particles are picked up by the filopodia, they need to make their way toward the cell bodies of the T cells to eventually enter and infect them. How they do that is unclear, but Subramaniam says that a possible mechanism is a process called virus surfing, by which viruses move along the filopodia of target cells. Walther Mothes of Yale University, who discovered virus surfing several years ago, calls the findings "exciting evidence that raises the possibility that the virus surfing also contributes to the transmission of HIV in lymphocytes." Mothes previously only showed virus surfing of HIV particles between cultured fibroblasts.

The overarching question that remains is just how often HIV transmission of CD4+ T cells *in vivo* occurs because of DC-T cell transmission. Subramaniam therefore wants to take an electron-microscopic look at HIV transmission in cultured tissues, which has not yet been done. "This is where we are headed," Subramaniam says. —*Andreas von Bubnoff*

Vaccine BRIEFS

First US National AIDS Strategy Aims to Cut New Infections and Optimize Care

US President Barack Obama's administration released the nation's first National AIDS Strategy in July that pledges to reduce the number of new HIV infections by 25% within five years. The 60-page report, which was prepared by the White House Office of National AIDS Policy (ONAP), also aims to increase access to care and optimize health outcomes for people living with HIV/AIDS, and reduce HIV-related health disparities by reducing stigma and discrimination.

Approximately 55,000 Americans are newly infected with HIV each year, an estimate that epidemiologists say has remained static for the past 15 years, despite ongoing efforts to try to reduce HIV incidence rates. The US Centers for Disease Control and Prevention estimates that there are now more than one million people living with HIV/AIDS in the US, and that about a fifth of them are unaware that they are HIV infected. The National AIDS Strategy aims to increase the percentage of people living with HIV who know they are infected from 79% to 90%. The strategy also mentions the need to develop, evaluate, and implement a combination of effective HIV prevention strategies, including AIDS vaccines and microbicides, as well as strategic use of syringe exchange and expanded HIV testing to reduce HIV transmission rates.

The Obama administration has designated six federal agencies to execute the strategy and has given the agencies a deadline of January 2011 to submit a report to ONAP and the Office of Management and Budget on the operational plans for implementing the strategy.

US spending for HIV/AIDS programs and services will remain flat in the proposed 2011 budget that kicks in October 1, 2010. This prompted some AIDS advocates to wonder whether the National AIDS Strategy will ultimately be able to have any meaningful influence on the domestic response.

Mark Harrington, executive director of the Treatment Action Group, a New York-based AIDS research and policy organization, says he thought the US aim of reducing new infections by 25% within five years wasn't nearly aggressive enough. "If we really did a good job, we could reduce infections by 50% and really make an impact," says Harrington. "This strategy shows a lack of ambition and a lack of resources. I think we need presidential leadership on this, but Obama hasn't done that, and I'm disappointed. This is not a strategy to end AIDS, this is a strategy to manage AIDS."

> It was striking, not just to Americans, but to the rest of the world that we did not have a singular plan.
>
> *– Judy Auerbach*

Jeff Crowley, director of ONAP, said during a July 18 session at the XVIII International AIDS Conference in Vienna that the US already provides more than US$19 billion in annual funding for domestic HIV prevention, care, and research programs. "We believe we can make significant improvements with existing resources," said Crowley, who moderated a discussion about the community response to the strategy.

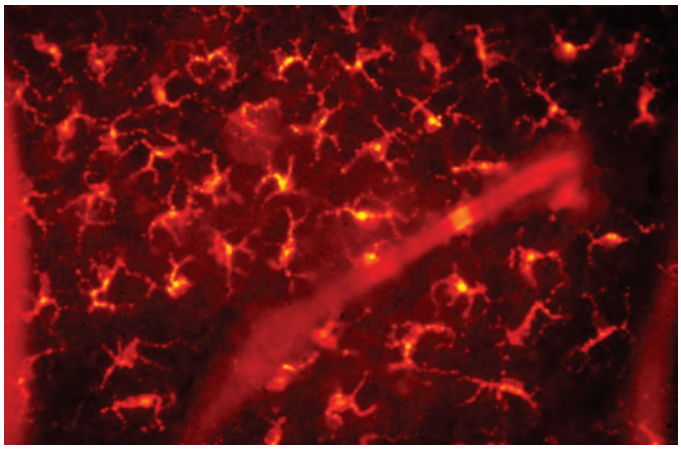
Leading up to the release of the strategy, Crowley said the Obama administration held 14 discussions attended by more than 4,000 people within the AIDS community, and issued an "online call to action" that received more than 1,000 recommendations.

Judy Auerbach, vice president of Science & Public Policy at the San Francisco AIDS Foundation, was one of the founders of the Coalition for a National AIDS Strategy that secured a commitment from Obama to develop such a plan when he was seeking the presidency. "It was striking, not just to Americans, but to the rest of the world that we did not have a singular plan," says Auerbach. "So from my point of view, it's really encouraging that it happened as quickly as it did. I think the plan is quite good in terms of its specificity." —*Regina McEnery*

Vaccine Candidate Targeting Dendritic Cells Enters Clinical Trial

Scientists at Rockefeller University in New York City began testing a novel AIDS vaccine candidate in July that specifically targets dendritic cells in the lymph nodes and other organs of the immune system.

The vaccine candidate contains a monoclonal antibody (mAb) engineered to recognize DEC-205, an endocytic protein found on the surface of dendritic cells that mediates efficient presentation of antigens. DEC-205 was discovered 12 years ago by Michel Nussenzweig, who is head of the laboratory of Molecular Immunology at Rockefeller University. Dendritic cells themselves were co-discovered at Rockefeller in 1973 by Ralph Steinman, the Henry J. Kunkel Professor and Senior Physician of the Laboratory of Cellular Physiology and Immunology at Rockefeller, and his colleague, cell biologist Zanvil Cohn, who died in 1993.



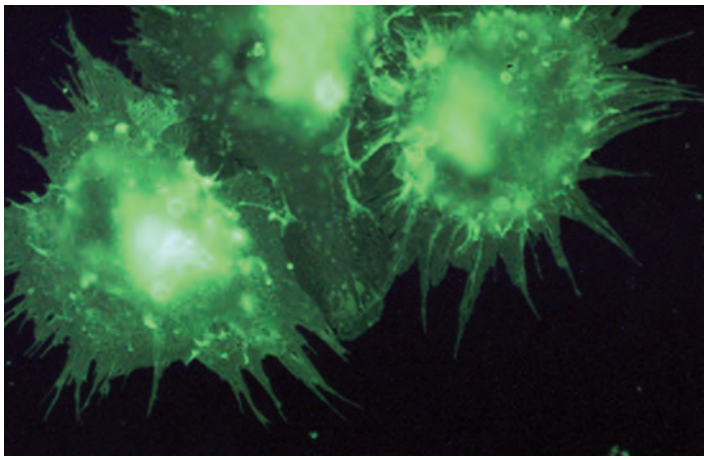
The mAb is fused to an HIV clade B p24 Gag protein. Gag p24 was selected as the vaccine antigen because of its many conserved epitopes, which may induce greater breadth of $CD8^+$ T-cell responses.

The three-year, randomized, placebo-controlled, Phase I trial, known as DCVax-001, will enroll 45 healthy HIV-uninfected volunteers in New York City. Investigators will evaluate both the safety and immunogenicity of the vaccine candidate administered at three different doses, along with a fixed dose of an experimental adjuvant called Poly ICLC (Hiltonol) that was designed to activate innate immune responses. The volunteers will receive three subcutaneous vaccinations of either the vaccine candidate or placebo over 12 weeks, and will then be monitored for 12 months.

Researchers at Rockefeller have been working with Celldex Therapeutics, a biotechnology company headquartered in Massachusetts, on the development of the candidate vaccine. Celldex previously developed a vaccine candidate with a mAb fused to DEC-205 to target a tumor-associated antigen known as NY-ESO-1, but this is the first time an AIDS vaccine candidate has been constructed using this technology.

The experimental adjuvant Hiltonol was developed by the pharmaceutical company Oncovir, based in Washington, D.C. It induces the production of interferon, which interferes with viral growth. Hiltonol was previously evaluated in therapeutic vaccine studies for brain tumors. More recently, researchers have been eyeing Hiltonol as an adjuvant for prostate cancer vaccines. This adjuvant has also demonstrated the ability to improve the capacity of dendritic cells to recognize and present certain viruses, such as Ebola virus and poxviruses, to the immune system, leading researchers to consider using it as an adjuvant in preventive vaccines.

In the DCVax-001 study, Hiltonol is used to help immature dendritic cells—whose job it is to scoop up antigens—mature and present the antigens to $CD4^+$ T cells and B cells. Sarah Schlesinger, the trial's principal investigator, says this candidate vaccine regimen performed well in experiments in Indian rhesus macaques, although she did not disclose specific details because the nonhuman primate findings will be published soon.



Jacques Banchereau, director of the Baylor Institute of Immunological Research in Texas, is developing a therapeutic HIV vaccine candidate that targets dendritic cells. However, the Rockefeller study is the first time a dendritic cell-focused approach is being tested as a preventive HIV vaccine candidate. —*Regina McEnery*

▲ Pictured above is a red fluorescent image of dendritic cells in skin with en face staining; and a green fluorescent image of a splenic dendritic cell taken from a mouse. *Images courtesy of Rockefeller University.*

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