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The Bulletin on AIDS Vaccine Research



[SPOTLIGHT]

Brave New World

New tools and strategies for HIV prevention are complicating the business of vaccine development. But, as the Boston conference revealed, HIV vaccine research continues to advance at a torrid pace *By Regina McEneaney*

AIDS vaccine development has never been easy. But with the recent proliferation of new biomedical interventions to prevent HIV, it's getting downright complicated. That much was abundantly clear from the outset at the AIDS Vaccine 2012 Conference, held Sep. 9-12 in Boston, where several satellite sessions examined how the expected rollout of some of those interventions is likely to affect the conduct of AIDS vaccine trials.

More notably, three of the four opening plenary talks had little to do with vaccines. Instead, they covered vaginal microbicides; research into the early initiation of antiretroviral (ARV) treatment to reduce transmission risk; and an overview of what we've learned from trials of pre-exposure prophylaxis (PrEP), the administration of ARVs to HIV-uninfected people to prevent infection. The fourth talk, delivered by Anthony Fauci, the executive director of the US National Institute of Allergy and Infectious Diseases (NIAID)—focused on vaccines in the context of these and other preventive interventions.

"The HIV prevention strategy will in fact," Fauci predicted, "be a unique paradigm of non-vaccine prevention modalities together with a safe and effective vaccine." If properly implemented, he argued, the non-vaccine modalities could by themselves probably turn the tide of the pandemic. Fauci maintained, however, that the only hope the world has of

eliminating—and ultimately eradicating—HIV rests on the development of broadly effective vaccines and their deployment in addition to those other preventive interventions.

Yet making the case for vaccine development isn't likely to get any easier, certainly not in the midst of a global economic downturn. Bill Snow, executive director of the Global HIV Vaccine Enterprise, an organizer of AIDS Vaccine 2012, argued that the AIDS vaccine field needs to redial its message. "We have gotten tired of making our own case," said Snow. "It sounds rote, obligatory and, worst of all, next to impossible. It needn't be that way."

For all the talk of other biomedical interventions, there was ample evidence of the remarkable progress of HIV vaccine design and development at the conference.

Signatures of success

Researchers have been analyzing samples collected in the RV144 trial, which demonstrated 31% efficacy against HIV and remains the only evidence available that vaccines can prevent HIV infection. At last year's AIDS Vaccine meeting in Bangkok, investigators shared the first set of results from such analyses, identifying what they called "correlates of risk" associated with the Thai regimen—a vCP1521 canarypox viral vector prime followed by a gp120 B/E AIDS VAX boost. Subsequently published in the *New England Jour-*

nal of Medicine, those studies revealed, surprisingly, that one antibody response correlated with a reduced risk of HIV, while another correlated with an increased risk of infection (see VAX Sep. 2011 *Spotlight* article, *More Surprises Stem from RV144*).

Scientists have since turned their attention to the antibody responses that correlated with a reduced risk of infection—namely, immunoglobulin G antibodies that bind to the V1/V2 region of HIV's Envelope protein. They have examined whether those vaccine-induced antibody responses selectively blocked certain HIV variants, and what genetic changes allow the virus to elude that targeting. Scientists refer to such escape as a "sieve effect."

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Led by researchers at the US Military HIV Research Program (MHRP), a key collaborator in the RV144 trial, the team examined nearly 1,000 HIV genetic sequences from 110 volunteers who became infected over the course of the RV144 trial—44 who received the candidate vaccine regimen and 66 who received a placebo vaccine. They then examined the viral sequences for evidence that the V2 region plays a major role in the modest protection seen in the trial. And, indeed, in a study presented in Boston, researchers detected two genetic signatures in the V2 region that closely correlated with vaccine efficacy.

That is, viruses that bore certain sequences in two stretches of the Envelope gene appeared to be vulnerable to vaccine-induced immune responses; viruses with mutations in those regions of the gene tended to evade such responses. One of the genetic signatures appeared to be associated with an efficacy as high as 78%. “This is an independent assessment that the V2 region is important,” said Morgane Rolland, lead author of the study and a scientist at MHRP.

The findings, published in *Nature* the same day they were presented at the Boston conference, buttressed the credibility of the Thai trial results—adding to the molecular evidence that the observed protection was real, and not just a statistical anomaly.

On the other hand, they also underscored just how difficult it will be to design a broadly effective AIDS vaccine—a point raised by Jon Cohen, a reporter for the journal *Science*, at a press conference where Rolland presented the results of her study. “In the real world, what practical application would this have? I can see why you can use it as an argument that the efficacy [in RV144] was real, but it is far away from what people dream of—which is a vaccine [that protects] against many strains.”

MHRP Director Nelson Michael acknowledged the challenges that HIV’s genetic diversity and mutability present to vaccine designers, but was optimistic those

challenges can be resolved. “We are making substantive progress in understanding what it will take to develop a more effective HIV vaccine, which will ultimately help us end this pandemic,” he said.

In a separate talk, Rolland reported results from a monkey study that found additional evidence supporting the importance of vaccine-induced responses against the V2 region.

Stalled trials

Researchers hope to improve upon the results of RV144. But at a separate satellite session, Jerome Kim, deputy director of science at MHRP, discussed issues impeding two such planned studies—one involving men who have sex with men (MSM) in Thailand and a second among heterosexual men and women in South Africa.

The Pox Protein Public-Private Partnership, or P5, launched a year ago to boost the vaccine efficacy seen in the RV144 trial to at least 50%, had hoped to start both studies by late 2012. But a number of setbacks ranging from money to laboratory infrastructure to manufacturing have scuttled P5’s plans, said Kim. The earliest start date for the Africa trial, now expanded to include southern Africa, is now pegged at late 2014, and it is unclear when the MSM trial in Thailand will get off the ground.

The vaccine regimen slated to be tested in a Phase IIB licensure trial in southern Africa includes an ALVAC viral vector vaccine candidate as the prime, followed by a gp120 protein boost containing a well characterized adjuvant known as MF59. The efficacy trial among MSM in Thailand, meanwhile, is to test an ALVAC prime coupled with a gp120 boost that is also formulated with MF59.

Kim said a major challenge in the MSM trial in Thailand has been finding a manufacturer for the proposed gp120 boost. The company that owns the intellectual property rights for the protein boost in the RV144 trial is unable to produce enough for another trial,

which means a new manufacturer must be found. Novartis Vaccines and Biologics, located in Massachusetts, has the contract to make the protein boost for the southern Africa trial. Kim said discussions with Novartis are ongoing to see if they will make protein for a Phase IIB trial in Thailand as well.

Broadly speaking

The expanding arsenal of bNAbs isolated from chronically HIV-infected individuals dominated the conference, reflecting growing optimism that their analysis will yield clues to the design of a broadly effective HIV vaccine. Several talks dealt with novel approaches to visualizing the interactions of these antibodies with their targets on the HIV Envelope trimer, a three-legged spike-like protein complex that has long proved resistant to structural analysis (see *VAX Primer*, this issue).

Researchers described how they are applying computer modeling to reverse engineer immunogens based on the molecular structures—or epitopes—targeted by bNAbs on the Env protein. One approach involves scanning a vast database of known protein structures to identify those that might hold such epitopes in an orientation and context optimal for bNAb binding. When used as immunogens, such structural mimics of bNAb targets ought to elicit similar antibodies against HIV and so confer broad immunity to circulating virus strains. That, at least, is the theory.

And it would appear to be on sound footing: Bill Schief, an associate professor of immunology at The Scripps Research Institute in La Jolla, CA, and a member of IAVI’s Neutralizing Antibody Center, presented a proof of concept for the strategy. He and his colleagues devised a candidate immunogen for a vaccine against the respiratory syncytial virus (RSV)—a major cause of respiratory tract infections in infants. Using computational modeling and scaffolding, they created a molecular mimic of the epitope targeted by a known neutralizing antibody against the

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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. For more information, see www.iavi.org.

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virus. When monkeys were immunized with this candidate immunogen, they produced antibodies that neutralized laboratory strains of RSV. Schief and other researchers, most notably at NIAID's Vaccine Research Center and IAVI's Neutralizing Antibody Consortium, are using this approach to devise novel immunogens for HIV vaccine candidates.

Mosaic antigens

Researchers in Boston also presented new animal data on mosaic antigens, which are immunogens that have been computationally designed to address the overwhelming diversity of HIV. Most vaccine inserts contain HIV gene sequences from a single virus found in a certain region of the world, or a single

sequence shared by a variety of circulating viruses. Mosaic antigens, however, are cocktails of several intact, full-length or near full-length proteins that are created by tiling together genetic sequences that not only represent several HIV variants but have also been optimized for their potential to induce vigorous and effective immune responses against the diverse viruses in the HIV pandemic.

Researchers have found that some prime-boost regimens containing mosaic antigens reduced the risk of infection by 90% per exposure in monkeys. Bette Korber, who oversees the HIV Database and Analysis Project at the Los Alamos National Laboratory in New Mexico, said mosaic inserts are now being made for inclusion in Phase 1 clinical trials. ■

Q&A WITH BILL SNOW



Talks at the annual AIDS Vaccine Conference, held this year in Boston, reflected dramatic shifts in the HIV prevention landscape. The conference also stressed the work of young and early investigators. VAX Science Writer Regina McEnery sat down with longtime AIDS vaccine advocate Bill Snow, appointed six months ago to lead the Global HIV Vaccine Enterprise Secretariat, for his perspective on the conference.

This year, the opening plenary talks didn't focus solely on vaccines. Is this the first time the conference has included talks on other strategies?

People have always had to consider what's going on in the epidemic. You can't run vaccine trials in a vacuum. Anything that has to do with the clinical end of things has had to be responsive pretty much on the spot to changes like [antiretroviral drugs] getting distributed more widely and voluntary male circumcision. It just didn't show up in the titles as much as it did this year.

This conference also had a considerable number of young and early career investigators taking on leadership roles or presenting data. What kind of impact did this have on the conference as a whole?

I think it had a huge impact. If you look at the program, there were a lot of speakers who had not spoken at previous conferences or held such a high visibility spot. What that does is it makes the conference much more interesting. You hear the people who are doing the work instead of the people who are managing the work, and you tend to hear more specifics.

In your talk opening night you said the field is "short on research and long on little tweaks and new ideas that make for indecision and conflict." Can you elaborate?

It really has been an insider's game. The few 1,000 [scientists] who work on this are excited; they can gauge the progress and they know the joy of the little victories and the disappointment of little defeats. But in the end, in the outside world, we have to justify what we are doing and the best way to do that is to let people know what we are really accomplishing along the way and not just talk about the pot of gold at the end of the rainbow.

You pressed the field to either validate or cast aside the results of the RV144-correlates analysis. Were you chastising the field for taking too long?

No. What I meant is that we're halfway to answering that question and we might be able to answer it without an efficacy trial if we are really smart about it. Right now, people are chasing after what they think happened. We don't know whether what they think happened is really causative. We need to know that before we start redesigning all our vaccine studies.

GLOBAL NEWS

New lab facility in South Africa will focus on TB and HIV

The University of KwaZulu-Natal (UKZN) in South Africa and the Howard Hughes Medical Institute (HHMI) opened a seven-story, 40,000 square-foot research center in early October that will primarily focus on the twin scourges of tuberculosis (TB) and HIV. The KwaZulu-Natal Research Institute for Tuberculosis and HIV—or K-RITH for short—will occupy five of the seven floors and has already recruited eight of its 10 principal investigators. The Centre for the AIDS Programme of Research in South Africa (CAPRISA) and UKZN will occupy the remaining two floors. The building is on the campus of the Nelson Mandela School of Medicine in Durban. K-RITH, established in 2008, had been in temporary space on the Durban campus.

HHMI supplied US\$40 million for K-RITH—\$30 million for construction of the facility and an additional \$10 million to equip the floors, which include biosafety level-3 laboratories that will enable scientists to do hands-on research on *Mycobacterium tuberculosis*, drug-resistant *M. tuberculosis*, and HIV. In addition, HHMI has committed \$3 million a year for 10 years to fund operating costs and additional funds for recruitment. UKZN contributed \$10 million to the K-RITH facility as well.

K-RITH's initial focus has been in five research areas: the development of faster diagnostic tests for TB; characterizing drug-resistant strains of TB; analyzing and characterizing complex immune responses to TB; the study of recurrent TB infections in HIV-infected individuals; and improving the treatment of TB. But the research facility also plans to participate in TB and AIDS vaccine trials.

Jill Conley, program director in the science department at HHMI, said K-RITH was a major leap forward for the Maryland-based research institute. "This is our first real international partnership," she said. "It was new for the whole organization and it took a lot of ground work." —Regina McEnery

Understanding How Researchers Visualize the HIV Spike

What tools are researchers using to bring HIV's Envelope protein into focus and what have they learned about its structure?

By Regina McEnerly

One of the most exciting developments in AIDS vaccine research in recent years has been the isolation of dozens of broadly neutralizing antibodies (bNAbs) from the serum of a minority of HIV-infected individuals. In the lab, at least, these antibodies are capable of neutralizing many of the HIV strains currently in circulation by binding a three-legged, spike-like protein on the surface of the virus called the Envelope trimer, or Env. Scientists are now applying what they are learning from these antibodies to design vaccine candidates that might elicit similar antibodies in people before they are exposed to HIV, and so block infection (see VAX May 2010 *Primer on Understanding if Broadly Neutralizing Antibodies are the Answer*).

The bNAbs elicited by any such vaccine would attack HIV before it can invade its target cells. Ideally, they would not only target a broad spectrum of HIV variants, but do so potently—in other words, at very low concentrations.

But eliciting such antibodies isn't easy. The regions of Env most susceptible to neutralizing antibodies are hidden by a thick coat of sugars. These sugars restrict antibody access to the underlying protein surface. Many of the protein targets—or epitopes—that are accessible to antibodies, meanwhile, do not elicit neutralizing antibodies, and act as decoys that confound the immune response. But perhaps most importantly, large swathes of the trimer change constantly due to HIV's extraordinary mutability. This allows the virus to continuously evade immune recognition.

Scientists have sought to overcome these challenges by studying how bNAbs latch onto the Envelope trimer (see VAX March 2011 *Primer on Understanding HIV's Envelope Protein*). But determining the shape of the trimer—with or without an antibody bound to it—has been an uphill battle.

One way to do so is by X-ray crystallography, which involves beaming X-rays

through a crystal of the purified protein and reading how the X-rays are scattered by that passage. This allows researchers to determine the precise spatial arrangement of atoms that make up the protein molecule. If the molecule—or the relevant part of it—can be co-crystallized in complex with an antibody that recognizes it, crystallography reveals in exquisite detail how the two interact with each other. Researchers attempting to reverse engineer novel HIV vaccine candidates rely heavily on such imaging.

But the HIV trimer poses unique challenges to that approach. Because the complex is structurally dynamic and highly unstable, researchers have had great difficulty crystallizing it in its functional (or “native”) state.

Freezing the trimer

To circumvent these problems, some laboratories employ a newer generation imaging technology called cryo-electron microscopy, or cryo-EM, to study the trimer's structure. Cryo-EM involves snap-freezing a protein in liquid nitrogen. This freezes Env in its natural state. Scientists then use an electron microscope to capture thousands of images of the protein from different angles. These images are then merged to reconstruct a high-resolution, 3D image of the frozen protein's fine structure.

Cryo-EM has often been used to study the Env trimer on the virus. Today scientists are also using a more advanced version of cryo-EM—single-particle cryo-EM—to analyze Env in isolation, affording a higher resolution snapshot of its structure. In one recent study, researchers used this approach to describe Env in its earliest native state, before it binds receptor proteins on the target cell. They found that Env, in its unbound state, has a “doughnut hole” in its center and is quite different from the densely packed structure that emerges at the end of the viral entry process. The images also capture an unusual, cage-like architecture, which likely helps HIV evade the immune system, and

show how the triangular pyramid structure of Env hampers access by antibodies.

In another recent study, researchers used single particle cryo-EM to take a look at Env at a later stage of the infection process, just after it has docked with its protein receptor on the target cell. They found that Env partially opens up at this stage, exposing parts of its inner surface that it deploys to fuse the virus membrane with the cell membrane. This exposes the inner surface of Env to attack by antibodies.

While these cryo-EM models of Env are not as vivid as those obtained by X-ray crystallography, scientists have found them instructive and suspect they could help in the design of HIV immunogens—the active ingredients of vaccine candidates—that induce bNAbs. Already, they are planning to synthesize molecules that mimic the partially opened trimer, a conformation it assumes after it has bound its cellular receptors. A vaccine bearing such an immunogen might induce antibodies that prevent membrane fusion, which is essential to the viral life cycle.

Meanwhile, researchers are learning more about how some of the bNAbs prevent viral entry by interacting with other regions of the HIV Envelope trimer. In one recently completed experiment, researchers mixed HIV particles with two bNAbs—b12 and VRC01—and used cryo-EM to study if and how the binding of bNAbs changes the structure of the Envelope spike. They found that VRC01 did not require any changes in Env structure to bind its target; b12, however, did. This might explain why the VRC01 antibody neutralizes a broader range of HIV strains than b12.

To learn more about how researchers obtain models of the HIV Envelope glycoprotein, link to this video <http://www.jove.com/video/2770/determination-molecular-structures-hiv-envelope-glycoproteins-using> that details work from the laboratory of Sriram Subramaniam at the US National Cancer Institute. ■