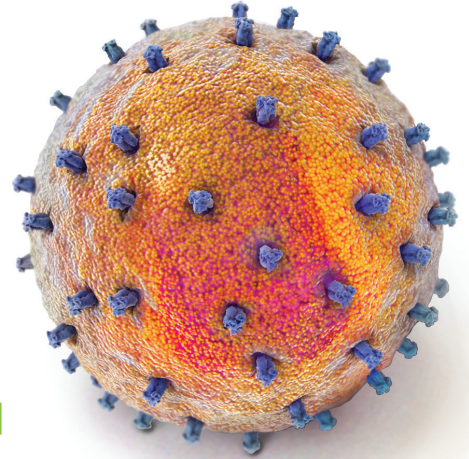


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The Bulletin on AIDS Vaccine Research

[SPOTLIGHT]

Mon Dieu! 30 Years of HIV Science

Leading researchers praise the field's many successes—but remind us the war on AIDS is far from over *By Regina McEnery*

Now in its fourth decade, the HIV pandemic already ranks among the most devastating in recorded history. And the scientific response has, in some ways, been historic as well. Scientists now know far more about the canny virus that causes AIDS than they do about any other viral pathogen. Even better, their discoveries have led directly to the development of a robust arsenal of antiviral drugs that have transformed both the treatment and the prevention of HIV. So the 500 scientists gathered at the Institut Pasteur in Paris for the 30 Years of HIV Science meeting May 21-23—marking the 30th anniversary of the discovery of HIV at that storied institution—had reason to feel at least a little proud.

But their discussions focused much more on what the next 30 years might look like. After 30 years of HIV science, researchers have a profoundly detailed understanding of how HIV hijacks the immune system and exacts its deadly toll, and have figured out how to tame the virus after it has established infection. What they haven't yet worked out is how to stop it before it sets up shop in the body, or how to clear it completely once it has.

Part of the problem is that scientists still don't know how to make a vaccine candidate that elicits broadly neutralizing antibodies, which many researchers believe an AIDS vaccine must induce to prevent infection by the many genetic variants of HIV. And despite growing evidence that HIV might be

curable, scientists are just beginning to get a handle on viral reservoirs, the pool of cells that harbor silent HIV in their genomes, and so seed a lifelong infection.

Longtime director of the US National Institute of Allergy and Infectious Diseases (NIAID) Anthony Fauci praised both the intensive research that has generated powerful antiretroviral therapies, and the global public health response the drugs have enabled, such as the President's Emergency Plan for AIDS Relief and the Global Fund to Fight AIDS, Tuberculosis and Malaria. "But as we celebrate extraordinary accomplishments," he cautioned, "it is important to keep our eye on the target. Much needs to be done."

Cells on high alert

That is indisputably true. One phenomenon that continues to puzzle HIV scientists is the chronic state of immune activation that occurs during HIV infection and leads to disease progression (see *VAX* Jan. 2009 *Primer* on *Understanding the Conundrum of Immune Activation in HIV/AIDS*). Apart from targeting and destroying T cells of the immune system—setting off a destructive cycle that leads to AIDS—HIV appears to induce immune dysfunction in other ways as well. Some scientists believe the virus also overstimulates the immune system, keeping it in such a constant and drawn out state of high alert that it loses its ability to produce

immune responses that might control the rapid replication of the virus.

Scientists would like to find new drugs or perhaps therapeutic vaccine candidates that dampen or eliminate the effects of immune activation. So far, however, their efforts have been impeded by an incomplete understanding of the mechanisms of that activation.

Daniel Douek, chief of the Human Immunology Section at NIAID's Vaccine Research Center has been at the forefront of research linking immune activation and AIDS. At the conference, he provided a retrospective on how his laboratory has investigated the biological products associated with microbial translocation—the leakage of toxins and other microbial products across the gastrointestinal barrier and into systemic circulation. The phenomenon, he said, is a key driver of immune activation and disease progression in people infected with HIV. He and

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his colleagues have also identified several biomarkers that appear to fuel the process.

But their attempts at dampening the effects of such activation in rhesus macaques have so far proved disappointing, said Douek. Specifically, he and his colleagues wanted to see what would happen if they dampened the signals emitted by a member of a class of secreted immune factors known as type 1 interferons (IFNs). While these proteins have long been known to suppress viral replication, their chronic signaling is also associated with immune activation and disease progression in HIV infection.

Douek and his collaborators treated six monkeys with a drug designed specifically to interfere with the signaling of type 1 IFNs. The researchers then challenged the animals rectally with a pathogenic strain of simian immunodeficiency virus (SIV), the monkey form of HIV. They hypothesized that the drug might benefit SIV-infected animals. In fact, the opposite proved to be the case. Within two weeks, the six animals given the drug had higher SIV RNA levels than a matched group of infected monkeys who had not been given the drug. And it only got worse from there. The treated animals rapidly progressed to AIDS and died within eight months, while the untreated macaques remained alive after 13 months.

Rather than provide a protective effect, Douek said, inhibiting type 1 IFN signaling in acute infection led to long-term loss of viral control and more rapid disease progression in the animals. The big question is, why? Douek's lab is analyzing the data but has no immediate answers. "It's difficult to make sense of this," said Douek. "Clearly, our hypothesis was wrong."

Mucosal immunity

Several talks in Paris also centered on eliciting immune responses in mucosal tissues, the soft lining of inner body cavities. Vaccines

that stimulate such responses could be highly effective against HIV, as the sexually transmitted virus establishes a beachhead in mucosal tissues in the early stages of infection.

Ashley Haase, a researcher at the University of Minnesota, is using an unusual monkey model—one vaccinated with a live-attenuated virus (LAV) SIV vaccine candidate—to study mucosal immunity. The study could have special implications for the development of vaccines that target gp41, one of the components of the viral spike—or Envelope protein—that HIV and SIV use to infiltrate cells. Haase's rhesus macaques were immunized intravenously with a version of the SIV LAV that lacks the gene named *nef*. They were then challenged vaginally with a high dose of SIV.

The vaccine regimen itself has a checkered history. In 1992, studies of rhesus macaques suggested that vaccination with a LAV might protect them from SIV. But four years later, hopes were dashed when the attenuated strain of SIV used in the vaccine regimen mutated into a virulent form, and caused disease and death in infant macaques. An LAV vaccine candidate was, it seemed, too risky for consideration in human trials. LAV candidates have since virtually disappeared from the list of strategies favored by AIDS vaccine researchers.

Indeed, Haase is not interested in developing LAVs for vaccines. Rather, his primary interest is in using the macaque model to study mucosal transmission, the most common mode of HIV infection, by sampling tissue directly after viral challenge. One of the goals of his recent study, he said, was to identify potential correlates of protection—a phrase used to describe the currently unknown array of immune factors and phenomena that might prevent the establishment of HIV infection.

Haase said the vaccinated macaques were protected from SIV within 20 weeks of vaccination, and displayed sterilizing immunity by week 50. When Haase and his col-

leagues then searched for antibodies in the vaginal tissue of the macaques, they found a striking five-fold increase in Immunoglobulin G (IgG) antibodies to certain forms of gp41. This region of the Envelope is considered something of a wasteland for vaccine targets. While two broadly neutralizing antibodies (bNAbs) are known to target the part of gp41 that lies in the membrane proximal external region (MPER) of the Envelope, scientists haven't been able to induce such antibodies through immunization.

"So for vaccine design, one of the things we think we need to reproduce are antibodies to this trimeric gp41," said Haase. "But [the model] also shows us that we need to understand the rules that regulate how the mucosal epithelium is [established as] the frontline of the immune system and how active a role it plays in shaping the antibody response to concentrate antibodies on the path of virus entry."

While a LAV vaccine candidate probably wouldn't survive regulatory review for human evaluation, Haase said what they're learning from the monkey model could advance the field. "It may be possible to figure out new rules by which the mucosal immune system concentrates its resources where they are needed."

Chasing the cure

Many of the talks in Paris reported on the recently invigorated search for a cure, including the March report that a toddler in Mississippi appears to have been cured by early and aggressive antiretroviral therapy (see VAX Mar. 2013 *Spotlight* article, *A Functionally Cured CROI Baby?*). Talks recapped studies of patients who started antiretroviral therapy early and then appeared to control HIV after treatment was stopped, a feat often referred to as a functional cure (see IAVI Report blog, *Cure research: An update and a roadmap*, July 27, 2012).

Another hot topic related to cure research

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was the use of drugs to roust HIV from its reservoirs in latently infected CD4⁺ T cells. The idea is that once HIV has been drawn out of its hiding places, the assault of the immune system combined with therapy might suffice to completely clear infection, especially if a therapeutic vaccine—which, of course, has yet to be developed—can be added to the mix (see VAX Mar. 2013 *Primer on Understanding Therapeutic Vaccination*).

Steven Deeks, a professor of medicine at University of California-San Francisco, said there is reason to be optimistic that a cure is feasible, at least under the right conditions. His laboratory is currently monitoring Timothy Brown, the so-called Berlin patient, who was cured of HIV after receiving a stem cell transplant from a donor naturally resistant to HIV. Deeks cited data from a Thai study of HIV-infected adults, which found that the initiation of antiretroviral therapy within the first weeks of infection can prevent HIV from taking up latent residence in memory cells of the immune system, which can harbor the virus for decades. Deeks said this might also explain why the US toddler, who was treated very quickly following birth, seems to have been functionally cured.

Yet, given the many caveats associated with cure strategies, Deeks said a safe, scal-

able intervention may prove impossible and, in any case, will take decades to develop. He said current antiretroviral therapy is not fully suppressive in many, and perhaps most, people. Nor have any tests yet been developed to measure viral reservoirs in individuals. But he noted that researchers have made considerable progress in unraveling the mechanisms of HIV persistence. “There is,” he feels, “reason to be optimistic.”

One of the HIV cure strategies being studied by Deeks’ lab is the use of drugs to reduce the activation and proliferation of T cells, and their expression of CCR5—a surface protein HIV uses to enter and infect T cells. One such drug, sirolimus (a.k.a. Rapamycin), which is used as an immunosuppressant to prevent organ rejection, is being tested in HIV-infected individuals who have also undergone a kidney transplant.

In a study measuring HIV persistence in 91 HIV-infected kidney recipients, Deeks said they found exposure to sirolimus is in some individuals associated with relative reductions in HIV DNA. This suggests it helped shrink the viral reservoir, though Deeks said the reductions were not dramatic. He also said the approach needs to be used with caution. “This is not a benign drug,” he said.

Nonetheless, he said plans are underway

for a new study to see if such drugs—known as immune modulators—can be used to block T cell proliferation. “Bob Gallo would have loved this story,” said Deeks, referring to the co-discoverer of HIV, whose research helped lead to the discovery of CCR5.

In fact, Robert Gallo—who led a National Institutes of Health team that co-discovered HIV 30 years ago—was at the Paris meeting to deliver a much anticipated dinner talk. Part of the buzz stemmed from the presence at the conference of the former Institut Pasteur scientist whose lab also isolated HIV, in 1983. The two labs feuded for a while over who actually discovered the virus first and whose test won the first patent. In 2008, the French team of Montagnier and Françoise Barré-Sinoussi were awarded the Nobel Prize for the discovery of HIV. Gallo was left out.

If there are any lingering bad feelings on Gallo’s part, they weren’t apparent at the dinner. Gallo talked briefly about his early recollections of the AIDS crisis, when he was drawn into studies of what was then a mysterious and entirely new syndrome. “Scientists got involved quite by chance,” he said. “I know I did. When someone challenges you, you take the call.”

Those challenges, it is clear, persist even today. ■

GLOBAL NEWS *by Regina McEnery*

Old vaccine trial draws new scrutiny

On the heels of the premature termination of a Phase IIb HIV vaccine trial in the US known as HVTN 505 (see *IAVI Report* blog, *Large AIDS vaccine trial shudders to a halt*, Apr. 26, 2013) comes fresh scrutiny of an earlier Phase IIb study from South Africa that was halted a few months after it started in 2007. The cause: Follow-up studies of participants in that earlier study—HVTN 503, or the “Phambili trial”—3 ½ years after it was terminated, suggest that volunteers who received the vaccine candidate might have contracted HIV at higher rates than placebo recipients.

The history behind this unhappy discovery is a bit convoluted. Briefly: The Phambili trial was itself halted when another Phase IIb study named Step, which was testing the same vaccine candidate, was found in early analyses of trial data to be ineffective (see *IAVI Report* article *Special Report: ‘Stopping a Steam Train’*, Sep.-Dec. 2007). That analysis also revealed a trend of more HIV infections among certain sub-groups of Step volunteers who received the vaccine candidate.

All three of the trials used the same viral vector—a weakened form of a common respiratory virus known as adenovirus serotype 5 (Ad5)—to deliver genes encoding HIV antigens to vaccine recipients. This, of course, means that the US National Institute

of Allergy and Infectious Diseases (NIAID) must now take a close look at the use of all adenovirus vectors in AIDS vaccine studies, even though most are biologically distinct entities.

HVTN 503 was designed to assess an investigational vaccine developed by Merck & Co. for its ability to prevent HIV infection in heterosexual men and women at high risk of infection, or to reduce viral load in those who later became infected. After immunizations were terminated, the 801 participants who had already enrolled in the trial were immediately told whether or not they had received the vaccine candidate, and were asked to undergo HIV testing and counseling every three months for the next 3 ½ years.

An analysis of the full 3 ½ year follow-up period reveals that of the 100 monitored enrollees who contracted HIV, 63 came from the vaccine arm. Most of them were men, and the disparity between vaccine and placebo groups was more pronounced 30 months after the initial vaccination. Still, the investigators have been unable to draw any firm conclusions about the findings, largely because the HIV infection status of 189 participants (88 participants in the vaccine group and 101 participants in the placebo group) is not known. NIAID says study investigators will attempt to call other former volunteers to clinical sites for testing. ■

Understanding How a Vaccine May be Designed to Induce Broadly Neutralizing Antibodies

Could recent breakthroughs in research be applied to make broadly effective vaccines against HIV? *By Andreas von Bubnoff*

Antibodies are among the most effective weapons the body deploys against invading pathogens. These roughly Y-shaped proteins, which can bind to viruses and inactivate them, are thought to be essential to the protection afforded by most, if not all, existing vaccines (see *VAX* Feb. 2007 *Primer on Understanding Neutralizing Antibodies*).

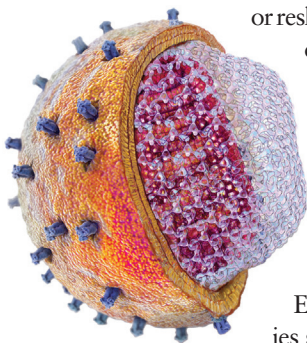
In recent years, researchers have isolated dozens of antibodies from the blood of HIV-infected individuals that can inactivate, or neutralize, most HIV strains in laboratory tests (see *VAX* Mar. 2010 *Primer on Understanding Advances in the Search for Antibodies Against HIV*). These antibodies are called broadly neutralizing antibodies (bNAbs). Some of them disable HIV at very low concentrations in lab studies, suggesting that they are very potent.

AIDS vaccine researchers have in recent years exhaustively studied the structure, biochemistry, and genetics of bNAbs in an effort to design vaccine candidates that might coax the body's immune system to make similarly potent antibodies against HIV.

But that's easier said than done. In uninfected people, the B cells that produce antibodies only come with a certain inherited set of

antibody genes; by combining, or reshuffling, them in many different ways, the immune system generates a variety of B cells that express any one of more than a million different antibodies on their surfaces.

Each of those antibodies specifically binds to a particular molecular shape—or an epitope—that is foreign to the body, as are the epitopes found on most pathogens. When such binding occurs, the B cell expressing the binding antibody proliferates and begins secreting millions of copies of that antibody.



Although the body can make a variety of antibodies against the Envelope protein found on the surface of HIV, most of them do not neutralize the virus. Meanwhile, bNAbs are only found in a minority of HIV-infected people, and can take years to develop. To better understand why that is the case, researchers have been studying just how bNAbs evolve in HIV-infected people. They have found that B cells need to come in repeated contact with the virus over years to accumulate changes, or mutations, in their antibody genes that enable their antibodies to bind epitopes on the HIV Envelope more efficiently. Eventually, this so-called affinity maturation process leads to B cells that can produce HIV-specific bNAbs that are very different from their unmutated precursors (see *VAX* Jan. 2011 *Primer on Understanding How Broadly Neutralizing Antibodies Evolve*).

Biological quandary

This poses a problem. It suggests that researchers will need to design immunogens—the active ingredients of vaccines—that can drive the required affinity maturation process. Such a vaccine regimen would need to deliver two types of immunogens: those that can bind the unmutated precursors of bNAbs to kick-start the affinity maturation process, and those that can subsequently guide that process toward the desired bNAbs.

That is a formidable challenge. But recent research suggests that, at least in principle, developing such a vaccine regimen might be possible. For example, researchers have made immunogens that can bind the unmutated precursors of a potent HIV-specific bNAb known as VRC01. They have made these immunogens either by modifying the naturally occurring HIV Envelope protein, or by creating an artificial molecule that resembles certain parts of HIV Envelope.

Researchers have also charted, in unprecedented detail, how bNAbs evolve

over the course of two and a half years in an infected patient. They isolated not only the final mature bNAb, but also its unmutated precursors and several intermediate antibodies generated along the way to the bNAb. They found that the primary ancestor of the antibody lineage that led to the bNAb had already appeared 14 weeks after infection. This suggests that a vaccine might be able to induce the kinds of antibody responses that eventually lead to bNAbs faster than previously thought.

In the same patient, researchers also isolated many of the viruses that developed mutations in response to the increasingly mature antibody responses. They found that the earliest of these viruses (the version that presumably infected the patient), could bind to the unmutated precursors of the bNAb that eventually developed in this patient. This suggests that this virus kick-started the affinity maturation process that eventually led to the development of bNAbs in this patient.

For the first time, researchers can use the series of HIV variants observed in this patient as a blueprint for a series of immunogens that might induce bNAbs like the one isolated from the patient. Researchers plan to make such immunogens to study whether this is the case, first in animals and, eventually, in humans. Combining the right immunogens in the right order, they hope, should eventually induce the maturation of bNAbs in uninfected people much faster than it occurs in infected patients.

While this analysis only addressed one type of bNAb directed at one part of the HIV Envelope in one patient, researchers are planning to perform similar analyses of antibody and virus co-evolution in 16 additional patients to understand what kinds of immunogens are needed to induce bNAbs directed at other parts of the HIV Envelope protein.

Taken together, such studies could pave the way to the design of vaccines that elicit potentially protective responses against HIV. ■