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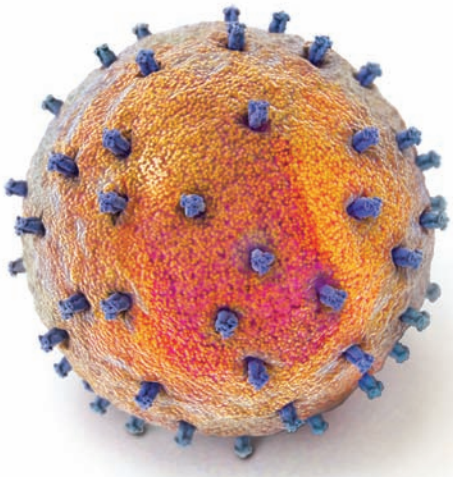

[SPOTLIGHT]

Canvassing CROI

HIV prevention strategies stoke excitement at recent scientific meeting *By Kristen Jill Kresge and Regina McEnery*

At the opening session of the 16th Conference on Retroviruses and Opportunistic Infections (CROI)—which was held this year from February 8-11 in Montreal, Canada—the two opening lectures focused, at least in part, on the success of antiretrovirals (ARVs) in treating HIV/AIDS. Indeed it seems much hope in combating HIV these days is pinned to ARVs, whether it is in expanding access among HIV-infected individuals worldwide, developing microbicide gels based on existing ARVs, or using them prior to exposure as a means of pre-exposure prophylaxis (PrEP) to block HIV infection.

Without doubt, there is still much to be done to accomplish any one of these goals, but this year's CROI showcased some promising results from both clinical trials and animal studies evaluating microbicides—both ARV-based and the non-specific PRO 2000 candidate—and PrEP, providing a burst of enthusiasm around new HIV prevention strategies. Data was also presented on studies relating to control of HIV infection that may help inform future vaccine design.

First hint of microbicide efficacy

Some of the more encouraging data at CROI came from clinical and nonhuman primate studies with new HIV prevention strategies. The first study, known as HPTN 035, evaluated the safety and efficacy of the microbicide candidate PRO 2000, a topical gel composed of a synthetic compound non-specifically designed to block attachment of HIV to host cells and thereby prevent infection.

This Phase IIb study, which was funded by the US National Institutes of Health and conducted by the HIV Prevention Trials Network and the Microbicide Trials Network, enrolled 3,099 women at seven clinical trial centers in Africa and the US and evaluated the efficacy of PRO 2000, as well as a second topical microbicide called BufferGel, which is designed to boost the natural acidity of the vagina in the presence of seminal fluid. The study also had two control groups—one received a placebo gel and the other, which was unblinded, received only condoms and no gel. A no-gel arm was included in the trial over concerns that the placebo gel might have antimicrobial properties that could have a protective effect against HIV.

The study showed that women who randomly received both PRO 2000 gel and condoms had 30% fewer HIV infections than those using a placebo gel and condoms. At the conclusion of this three-year trial, there were 36 HIV infections among women in the PRO 2000 group, compared to 54 in the BufferGel group, 51 in the placebo gel group, and 53 in the no gel group.

However, Salim Abdool Karim, a clinical infectious disease specialist who led this study, cautioned that the PRO 2000 results were not statistically significant compared to either the placebo gel or no gel groups. "This could be a chance finding," he said. Therefore additional evidence would be necessary to "conclusively determine whether PRO 2000 is an effective microbicide," said Karim.

Researchers also analyzed the data based on how often women in the PRO 2000 trial reported using the gel. Among those who said they applied the microbicide candidate at their last sexual act at least 85% of the time, there was an overall 44% reduction in HIV infection compared to the placebo gel. And in women who reported using the gel that often, without regularly using condoms, there was a 78% reduction in HIV infection compared to placebo gel users.

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IAVI is a global not-for-profit organization working to speed the search for a vaccine to prevent HIV infection and AIDS. Founded in 1996 and operational in 24 countries, IAVI and its network of partners research and develop vaccine candidates. IAVI also advocates for a vaccine to be a global priority and works to assure that a future vaccine will be accessible to all who need it. For more information, go to www.iavi.org.

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At the conclusion of Karim's presentation, there was a palpable level of excitement among attendees, with many rushing to the microphones to congratulate the researchers on the conduct and results of the trial. Karim said this excitement was understandable given recent results from trials of two other microbicide candidates. Carraguard, made from a seaweed derivative, was found to have no effect on HIV acquisition in a three-year Phase III study of 3,200 women from South Africa. And a Phase III trial of cellulose sulfate that enrolled 1,333 women was discontinued in December 2007 after early data suggested that the candidate might be contributing to an increased risk of HIV infection.

"We are at the end of a series of disappointments," Karim said. "We need something that gives us hope. The HPTN 035 trial results represent that hope." A Phase III trial of PRO 2000 involving 9,000 women is nearing completion in South Africa, Tanzania, Uganda, and Zambia, and results from this trial are expected later this year.

New animal data on PrEP

Other excitement came from two separate studies in rhesus macaques—both conducted by the US Centers for Disease Control and Prevention (CDC)—which provided additional evidence for the effectiveness of PrEP. One study evaluated intermittent use of oral PrEP—a strategy referred to as iPrEP.

Researchers administered the human equivalent doses of oral Truvada—a combination pill of two ARVs, tenofovir and emtricitabine (FTC)—at various times both before and after rectal exposure to a simian immunodeficiency virus (SIV)/HIV hybrid virus known as SHIV. All animals were exposed to SHIV weekly over a 14-week period. It took on average two exposures to infect a group of 32 untreated control animals. However, three of six animals that received Truvada just two hours before and 22 hours after SHIV exposure remained uninfected, and three of the six macaques that received the drugs seven days before and two hours after exposure to SHIV, were protected against infection.

The best results were seen in the group that received Truvada either 22 hours before and two hours after, or three days before and two hours after SHIV exposure. In these two groups, five of the six animals were completely protected against infection over the entire study period.

All of the ongoing clinical PrEP trials are testing the efficacy of a daily dose of either Truvada or tenofovir but there is also interest in iPrEP because of concern that adherence could prove to be a major barrier to the effectiveness of this intervention. Intermittent use would also slash the cost of providing PrEP.

Results were also presented from another study, which compared the effectiveness of two different topical PrEP gels. Two groups of six female pigtailed macaques received either a tenofovir gel or a tenofovir/FTC combination gel. These groups, as well as two animals that received no gel and nine that received a placebo gel, were then exposed to a low-dose vaginal SHIV challenge twice a week. Both animals that received no gel became infected, and eight of the nine animals that received the placebo gel were infected after an average of four exposures to SHIV. However, both groups of six animals that received either the tenofovir or tenofovir/FTC combination gel were completely protected against SHIV infection throughout the duration of the 10-week study.

There are currently six clinical trials of PrEP involving nearly 21,000 volunteers. One of these trials known as the VOICE study, which involves 4,200 women in Africa, is comparing the safety and acceptability of oral PrEP to a topical microbicide formulation. The first data on the effectiveness of PrEP from clinical trials will be available in 2010. "It's an exciting time in the prevention field," said Sharon Hillier, vice chairman of the department of obstetrics, gynecology, and reproductive sciences at the University of Pittsburgh.

Clues from controllers

At a symposium titled "Learning from Negative Trials," Emory University researcher Eric Hunter said the STEP trial—the recently conducted Phase IIb trial of Merck's adenovirus serotype-5 based vaccine candidate that showed the candidate offered no protection against HIV—has provided an opportunity to explore the basis for this lack of protection, which could help inform the design of future vaccine candidates.

Researchers are also carefully analyzing long-term nonprogressors (LTNPs) and more specifically elite controllers—individuals who can control HIV infection so that it is undetectable by standard tests for an extended period of time without ARV ther-

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– Salim Abdool Karim

apy—to mine for clues that may indicate the types of immune responses a vaccine candidate should induce. David Heckerman, a researcher at Microsoft Research, in collaboration with Bruce Walker, director of the newly formed Ragon Institute and Harvard AIDS researcher Florencia Pereyra, analyzed a group of LTNPs and mapped the specific regions on HIV that were targeted by their cellular immune responses.

They then analyzed a sub-group of vaccinated volunteers from the STEP trial who became HIV infected, despite vaccination, from natural exposure to the virus, to see if individuals with immune responses that targeted these same regions of the virus were better able to control HIV infection. Heckerman reported that this was indeed what they found. When the immune responses in STEP trial volunteers targeted one of what Heckerman identified as the six critical regions on the virus, it correlated with their having lower levels of HIV in their blood.

This suggests that these bulls-eye regions on the virus may be important for generating an immune response that could control HIV infection, and could be used in the design of future AIDS vaccine candidates.

Several other studies were also presented on the unique characteristics that lead to control of HIV infection. Mark Connors, chief of the HIV-Specific Immunity Section at the US National Institutes of Allergy and Infectious Diseases said, in his estimation, it is likely that there will be evidence from clinical trials in the near future showing that candidate vaccines can induce similar T-cell responses to those seen in elite controllers. ■

GLOBAL NEWS *by Regina McEnery*

$100 million Gift Creates New AIDS Vaccine Research Institute

The Phillip T. and Susan M. Ragon Institute, a unique collaboration of engineers, biologists, and doctors, was recently established at Massachusetts General Hospital (MGH) in Boston with US$100 million in funding from technology magnate Phillip Ragon to explore how the immune system combats disease, with an initial focus on developing an AIDS vaccine. The gift is unprecedented for MGH and the newly established Ragon Institute will be headed by Bruce Walker, an immunologist and director of the Partners AIDS Research Center, which is now part of the Ragon Institute.

Ragon, who has a degree in physics from MIT, became drawn to the field of AIDS vaccines after meeting Walker and hearing about his research. Two years ago Walker suggested that Ragon visit AIDS clinics in South Africa and this affected him deeply. "I began to talk with Bruce about what I could do to help," says Ragon.

"What this money means is that we can launch new collaborations in new areas with people with new perspectives, and do that immediately," says Walker. The funding will be used to attract researchers from MGH as well as Harvard University and the Massachusetts Institute of Technology (MIT). "What we are going to be able to do is track a lot of talented people and give them license with flexible funding—the license to be innovative and creative and to take some bold chances."

The Ragon Institute is also partnering with IAVI to conduct preclinical and clinical evaluation of AIDS vaccine concepts developed at the Institute.

Two Phase I Trials Launched

The Indian Council of Medical Research and IAVI have launched a Phase I trial to test the safety and immune responses elicited by two AIDS vaccine candidates administered sequentially in a prime-boost regimen. The trial known as P001 will enroll 32 volunteers at clinical trial centers in Pune and Chennai to evaluate different doses and vaccination regimens of the vaccine candidates. One candidate, TBC-M4, utilizes a modified vaccinia Ankara virus vector to deliver non-infectious HIV fragments in the hope of inducing an immune response against HIV. The candidate was developed in collaboration with the National Institute of Cholera and Enteric Diseases in India and was tested previously in a Phase I trial conducted in Chennai. In this trial, administration of TBC-M4 will be preceded by a prime vaccination with ADVAX, a DNA-based vaccine candidate, which was developed at the Aaron Diamond AIDS Research Center in New York City in collaboration with Rockefeller University and IAVI. Neither of the candidates being tested in this trial can cause HIV infection.

IAVI is also planning to begin enrolling volunteers in a Phase I trial of its adenovirus serotype 35 (Ad35)-based vaccine candidate. The trial will enroll 42 volunteers at the University of Rochester Medical Center who will be randomly selected to receive either two intramuscular injections of the Ad35-based vaccine candidate or placebo at three different doses. Clinicians will first administer the lowest dose and will review the safety data before proceeding to the next higher dose.

Ad35 is a serotype or strain of the common cold virus that researchers are using as a vaccine vector in this candidate to shuttle non-harmful fragments of clade A HIV, which is the predominant strain circulating in East Africa. The prevalence of naturally circulating Ad35 is much lower worldwide than the prevalence of adenovirus serotype 5, which was the virus used as a vector in Merck's AIDS vaccine candidate that was tested in the STEP trial. By using Ad35, it may be possible to circumvent issues involving pre-existing immunity to the viral vector (see *VAX* February 2005 *Primer* on *Understanding Pre-existing Immunity*).

[PRIMER]

Understanding How Immune Responses to AIDS Vaccine Candidates are Measured

What are the limitations of current methods used to analyze immune responses to AIDS vaccine candidates and what new strategies are being explored? ***By Regina McEnery***

Researchers do not measure the efficacy of a vaccine candidate—its actual ability to protect against HIV infection or control disease progression in individuals who become HIV infected despite vaccination—until the candidate is tested in large trials that involve thousands of volunteers who are potentially at risk of acquiring HIV. Instead, during the early stages of clinical evaluation, researchers primarily evaluate the safety of the candidate as well as its ability to trigger an immune response against HIV. The ability of a candidate vaccine to induce immune responses is referred to as its immunogenicity, and evaluating immunogenicity is one way that researchers can determine which candidates are worth pursuing in larger trials.

Researchers utilize different tests known as assays to determine the immunogenicity of AIDS vaccine candidates and different types of assays are used to measure different types of immune responses. Antibodies—Y-shaped proteins that latch on to the virus and stop it from infecting human cells—are most commonly measured using an ELISA or enzyme-linked immunosorbent assay (for more on how ELISA works, see *VAX* August 2007 *Primer* on *Understanding Immunogenicity*).

But many of the vaccine candidates that are currently undergoing clinical testing induce primarily cellular immune responses—both CD4+ and CD8+ T cells—against HIV, and not antibodies. Researchers measure and categorize the cellular immune responses induced by a vaccine candidate in many different ways.

Cytokine secretion

To study HIV-specific CD4+ and CD8+ T-cell responses, researchers isolate these cells from blood samples taken from volunteers in AIDS vaccine trials who received the candidate vaccine. They then expose these cells to the HIV fragment, or antigen, that was included in the vaccine candidate. This stimulates some of the immune cells and causes them to secrete certain proteins, known as cytokines, which can then be measured.

There are many different cytokines that play an important role in the immune response against a virus or bacteria. Some have direct antiviral activity, while others work more indirectly by activating other types of immune cells.

The ELISPOT assay is used to detect secretion of a single cytokine by both CD4+ and CD8+ T cells that are induced by a vaccine candidate. It is most commonly used to measure the release of a specific cytokine called interferon-gamma (IFN-γ; see *VAX* August 2007 *Primer* on *Understanding Immunogenicity*).

Measuring multiple cytokines

Another assay that can measure the ability of CD4+ and CD8+ T cells to secrete a broad range of cytokines is known as multi-parameter flow cytometry. As its name suggests, multi-parameter flow cytometry has a distinct advantage over ELISPOT assays in that it can measure the secretion of multiple cytokines simultaneously. This helps researchers more thoroughly define the cellular immune responses induced by a vaccine candidate.

In flow cytometry, cells or parts of cells are tagged with fluorescent probes that then flow through a beam of light, usually from a laser. Cells with different characteristics scatter the light in different ways, allowing them to be analyzed and sorted based on their ability to secrete different cytokines.

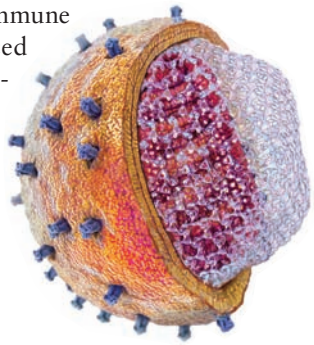
Limitations

While ELISPOT and flow cytometry assays provide useful data, they are not perfect tools. There are some indications, based on the results of clinical trials, that the ability of a vaccine candidate to induce cells that secrete cytokines is not necessarily an accurate predictor of whether an AIDS vaccine candidate will be effective. For instance, in the recently conducted STEP trial that tested Merck's adenovirus serotype 5-based vaccine candidate, the ELISPOT assay analysis showed the candidate induced high levels of T cells secreting the cytokine IFN-γ, but the vaccine was still found not to be effective in preventing or controlling HIV infection.

Functional assays

Another assay now being assessed in clinical trials measures the specific function of immune cells induced in response to an AIDS vaccine candidate, rather than cytokine secretion, which is just a signal of immune activation. One of these so-called functional assays is known as the viral inhibition assay. It measures whether CD8+ "killer" T cells taken from blood samples of volunteers who received an AIDS vaccine candidate in a clinical trial are actually capable of doing their job and killing HIV-infected cells. Researchers isolate CD8+ T cells from blood of a vaccinated trial volunteer and combine them with HIV-infected cells in the lab to see if they are able to inhibit the virus. This approach is just now starting to be utilized in clinical trials of AIDS vaccine candidates.

Since researchers do not know precisely what immune responses against HIV will help control the virus or prevent infection altogether, it is important to study several different assays to infer as much as possible about the immune responses induced by vaccine candidates. ■



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