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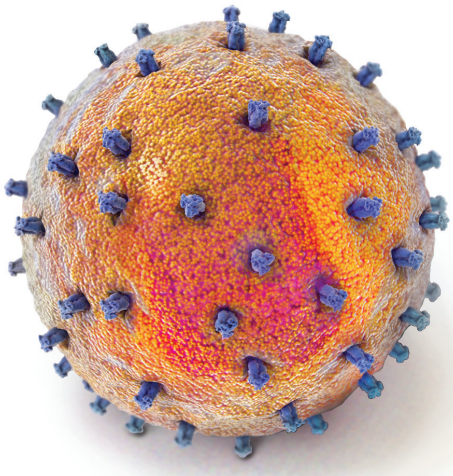

[SPOTLIGHT]

Vaccine Research Gains Momentum

The AIDS Vaccine 2009 conference drew a record number of attendees to what some researchers called the most positive meeting for the field in years ***By Kristen Jill Kresge***

During this year's annual AIDS vaccine conference, which was held from October 19-22 in Paris, there was a renewed sense of optimism among the nearly 1,000 researchers and policymakers in attendance, the largest crowd in the nine-year history of the conference. "It's not time for being pessimistic. This should be a conference of hope," said Michel Sidibé, executive director of the Joint United Nations Programme on HIV/AIDS, who spoke at the opening session of the conference.

This sense of hope was fueled in part by recent results from clinical trials. Less than a month earlier, the initial results of the RV144 trial—a Phase IIb trial in Thailand of two vaccine candidates administered sequentially in what is referred to as a prime-boost regimen—provided the first evidence of possible protection against HIV infection through vaccination. "We have the first signal, modest as it may be, of efficacy. Now that I see this very small signal, I believe an HIV vaccine is feasible," said Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases (NIAID).

Several advances in pre-clinical research were also showcased in Paris, including promising news about newly discovered antibodies against HIV, which also contributed to the newfound optimism among researchers. Alan Bernstein, executive director of the Global HIV Vaccine Enterprise, called the quest to develop an AIDS vaccine "a robust, active field of research that is moving ahead very rapidly."

This is quite a transformation from two years ago when the field was grappling with the sobering results from the STEP trial—a Phase IIb trial of MRKAd5, a vaccine candidate developed by Merck. The STEP trial showed not only that MRKAd5 did not reduce the risk of HIV infection or the amount of virus in individuals who became infected despite vaccination, but that there was actually a trend toward an increased risk of HIV infection among certain sub-groups of vaccinated volunteers. While researchers will now focus on trying to understand why the vaccine candidates tested in RV144 may have provided some protection against HIV infection, researchers affiliated with the STEP trial are still trying to unravel the reasons why this vaccine candidate failed.

Data from RV144 unveiled

In September, researchers from the US Military HIV Research Program (MHRP) and the Ministry of Public Health in Thailand reported that the prime-boost regimen tested in RV144 had modestly reduced the risk of HIV infection, but had no impact on the amount of virus circulating in individuals who became HIV infected despite vaccination. In Paris, additional data from RV144 was presented to a standing-room only crowd in a special session that was added last minute to the meeting agenda. Supachai Rerks-Ngarm, principal investigator of RV144, explained three analyses of the trial results, which were also published online in the *New England Journal of Medicine* at the conclusion of the special session.

The first analysis, known as the intent-to-treat analysis (ITT), was based on the entire trial population of 16,402 volunteers. Individuals who were already HIV infected were excluded from RV144. However, after the six-month period during which the six shots of either vaccine or placebo were administered, investigators discovered that seven volunteers (five in the vaccine group and two in the placebo group) had actually been HIV infected at the start of the study, their infections just

ALSO IN THIS ISSUE

weren't detected. When these seven individuals were included in the statistical analysis, the estimated efficacy of the vaccine candidates was 26.4%—a result that was not statistically significant (see *Primer*, this issue, for more about the statistical analyses of RV144).

A second analysis, known as the modified-intent-to-treat analysis (mITT), excluded the seven individuals who were infected at the start of the study. By this analysis, the efficacy of the vaccine candidates was estimated to be 31.2%, a statistically significant finding. These were the results initially reported in September. Rerks-Ngarm called the mITT the "most preferred analysis because it is less likely to introduce bias into the results."

A third analysis, known as per protocol (PP) analysis, excluded 3,853 volunteers who did not receive all of the injections on schedule, as well as those individuals who became HIV infected during the six-month injection period. Based on this much smaller number of people who adhered to the study protocol exactly, the estimated efficacy of the vaccine candidates was 26.2%, which was not a statistically significant result.

"All three analyses showed the same trend and one, which included the most data and the least bias, was statistically significant," said Nelson Michael, director of MHRP. Leading up to the AIDS vaccine conference, there was some controversy surrounding the decision made by trial investigators to release only the mITT analysis when they first announced the results in September. But in Michael's opinion, the mITT analysis was the most relevant for this trial. Researchers tend to favor data from the ITT or mITT analysis because it more accurately reflects how well the vaccine would work outside of the setting of a clinical trial. "It's important to understand how a vaccine performs under more typical conditions," added Michael.

Fauci said that regardless of the analysis, the findings from RV144 appear to be biologically significant and warrant further study. The focus now is on how these vaccine candidates may have provided some degree of protection against HIV infection.

Four scientific advisory groups have already been set up to decide which tests to run on the samples collected from volunteers during the trial, as well as to consider which companion studies can be conducted in animal models. The focus of these groups will be trying to tease out the specific immune responses induced by these vaccine candidates that led to protection, which are referred to as the immune correlates of protection. The establishment of immune correlates would be a huge advance for the field, but will likely not come easily.

Investigators have just started the process of analyzing the immune responses induced by the candidate vaccines. "We have a very unclear pathway ahead to figure out exactly what correlates with this effect," said Michael.

In his presentation at the conference, Michael pointed out two intriguing questions that have already emerged from the RV144 data. One is whether the modest protective effect of the vaccine candidates was limited to individuals who were at low risk of HIV infection. In the trial, the efficacy of the vaccine candidates seemed to be higher among individuals who reported being at low-risk of HIV infection as compared to those who said they were at high risk or who had engaged in what is considered a high-risk activity (sharing a needle, having sex with an HIV-infected partner, working as a commercial sex worker, or having multiple sex partners, among others). Another provocative question is whether the protective effect of the vaccine candidates waned with time. Data from the trial suggests that the efficacy of the vaccine candidates may have decreased over the first year following vaccination.

However, the trial was not designed specifically to answer either of these questions. Still, these observations will likely be studied in coming months. "These hypotheses merit further investigation and we are assembling experts to interpret the results and to maximize the knowledge gained through this study," said Michael.

Data still emerging from STEP

If the STEP trial is any indication, it may take some time before researchers are able to fully unravel the findings of RV144. Investigators working on the STEP trial are still collecting data from volunteers and generating hypotheses about the effects of MRKAd5 two years after immunizations were stopped early because the vaccine was found to be ineffective.

Initially, investigators observed a trend toward more male volunteers in the vaccine group becoming HIV infected if they were uncircumcised, and if they had pre-existing antibody immunity from natural exposure to the strain of the common cold virus (adenovirus serotype 5, Ad5) that was used in the vaccine candidate as a vector to deliver non-infectious HIV fragments to the immune system.

Between October 2007 and January of this year, 48 additional volunteers in the STEP trial have become HIV infected through natural exposure to the virus. And although investigators still see an increased risk of HIV infection among uncircumcised men who received MRKAd5 as compared to those who received placebo, the trend toward more infections among those with pre-existing Ad5 immunity is no longer evident, according to Susan Buchbinder, principal investigator of the STEP trial. "Whatever effect we were seeing [with Ad5] appears to have gone away," she said.

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MANAGING EDITOR
Kristen Jill Kresge

SENIOR SCIENCE WRITER
Andreas von Bubnoff, PhD

SCIENCE WRITER
Regina McEnery

PRODUCTION MANAGER
Nicole Sender

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Whether this indicates that the increased risk of HIV infection associated with Ad5 immunity occurred early and then waned over time or that Ad5 immunity actually did not have any effect on risk of HIV infection is still unclear, added Buchbinder. However, she cautioned that all of this data must be interpreted carefully because it was collected after volunteers were told whether they received the vaccine candidate or placebo, a process called unblinding, which could affect risk behaviors taken by the volunteers.

Investigators initially observed a decline in self-reported risk behavior among vaccinees with Ad5 immunity following unblinding, but the level of risk behaviors have increased again with time. "We're not able to more thoroughly reduce risk in these study participants even when we tell them that there is a potential increased risk of [HIV] acquisition," said Buchbinder.

New antibodies

While RV144 dominated much of the news at the conference, scientists were also encouraged by the discovery of several potent new antibodies—Y-shaped proteins that bind to HIV and stop the virus from infecting cells. For the first time in a decade, researchers have discovered new antibodies against HIV from HIV-infected individuals that will offer clues about how to design improved AIDS vaccine candidates.

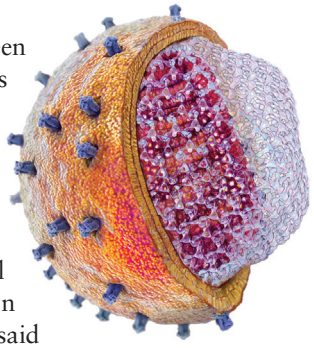
Most, if not all, existing vaccines work because they train the body's immune system to produce antibodies. The influenza vaccine, for example, stimulates the production of flu-specific antibodies. When a vaccinated person is naturally exposed to the influenza virus, these antibodies bind to the virus and inactivate or neutralize it, thereby protecting the individual from getting sick with the flu. But inducing antibodies through vaccination that could neutralize or disable the diverse subtypes or clades of HIV in circulation, so-called broadly neutralizing antibodies, has been challenging.

Researchers have been trying a reverse engineering approach. "You have an antibody and you try to work backward to how you would elicit that," said Peter Kwong, chief of the structural biology section at the Vaccine Research Center (VRC) at NIAID. But until recently, researchers only had four antibodies, which were considered broadly neutralizing, to work with. And efforts to design vaccine candidates to induce these antibodies have been unsuccessful so far.

This led researchers to try to find other broadly neutralizing antibodies and recently, five new broadly neutralizing antibodies were discovered. Two of these antibodies, known as PG9 and PG16, were identified by IAVI scientists in collaboration with researchers from The Scripps Research Institute in La Jolla, California. Through an effort known as Protocol G, IAVI researchers collected blood samples from 1,800 HIV-infected individuals at clinical research centers around the world. Two biotechnology companies, Monogram Biosciences in San Francisco and Theraclone Sciences in Seattle, Washington, then played pivotal roles in isolating both PG9 and PG16 using novel technologies. Both antibodies were discovered from a sample collected from a single African donor. These are the first antibodies to be isolated from an individual infected with a subtype of HIV that circulates primarily in developing countries.

PG9 and PG16 are able to neutralize many laboratory strains of HIV, including some that cannot be neutralized by the four previously identified antibodies. PG9 and PG16 are also able to neutralize at relatively low concentrations of antibody, which means that a vaccine may not have to induce large amounts of these antibodies to confer protection. These findings were published in *Science* magazine in September and presented at the AIDS vaccine conference by Sanjay Phogat, a principal scientist at IAVI's AIDS Vaccine Design and Development Laboratory.

The PG9 and PG16 antibodies bind to a different site on the protein spikes that coat the surface of HIV than those previously described, providing a new target for AIDS vaccine researchers to exploit. This site offers an advantage because it is more accessible to antibodies. "It's fair to say that it [the site on the virus where PG9 and PG16 bind] is a new vaccine target," said Phogat. Researchers at IAVI will now turn their focus to this binding site and try to utilize it to design immunogens—the fragments of the virus that are included in vaccine candidates to invoke an immune response. "The aim is to design vaccine candidates that prompt the immune system to produce similar neutralizing antibodies," said Dennis Burton, a professor of immunology at Scripps and scientific director of the IAVI Neutralizing Antibody Center.



And now that this method for isolating antibodies has been identified, scientists predict that it may lead to other new discoveries. "We expect to identify additional antibodies and novel targets on HIV in the near future," said Burton.

Three additional broadly neutralizing antibodies, one of which is a variant of another, were also recently discovered by researchers at the VRC. Gary Nabel, director of the VRC, presented data on one of these antibodies, known as VRC01, at the conference. VRC01 binds to HIV at what is known as the CD4 binding site because it is also where the virus binds to human $CD4^+$ T cells, the primary target of the virus. Small quantities of VRC01 are capable of neutralizing more than 90% of 89 different tier 2 viruses, which are considered by researchers to be more difficult to neutralize. VRC01 can also neutralize many clade A, B, and C viruses in the laboratory at very low concentrations of antibody.

Nabel also reported that scientists at the VRC tested an immunogen—based on the site on HIV where VRC01 binds—in rabbits and found that it stimulated the production of antibodies. "These are not broadly neutralizing antibodies, but really this is the first time in animals that we've had the ability to immunize and elicit antibodies that will neutralize," said Nabel, who called these experiments "guides for vaccine development."

In his concluding address, Fauci highlighted the discovery of the new antibodies by IAVI and the VRC as key findings of the year. He also highlighted other work from Burton's group that shows that high levels of neutralizing antibodies may not be required to block HIV infection, as well as observations that suggest that the version of HIV that establishes an infection may be easier to combat than HIV circulating in a chronically infected individual. Together these findings go a long way toward inspiring optimism. "We are at the beginning of a new phase of HIV vaccine research," said Yves Levy, co-chair of the conference. ■

Regina McEnery contributed to this article.

[PRIMER]

Understanding the Statistical Analysis of Clinical Trial Results

What are some of the statistical methods that are used to interpret AIDS vaccine trial results? *By Regina McEnery and Kristen Jill Kresge*

AIDS vaccine candidates are evaluated for safety, their ability to induce immune responses against HIV, and ultimately their efficacy in randomized, controlled, double-blind clinical trials (see *VAX* Oct.-Nov. 2007 *Primer* on *Understanding Randomized, Controlled Clinical Trials*). Biostatisticians, who specialize in statistical analysis, play an important role in how these trials are designed, as well as how the results are analyzed and interpreted.

For the first time, the recently completed RV144 trial in Thailand provided some indication that a combination of HIV vaccine candidates could provide some degree of efficacy (see *Spotlight* article, this issue). Although statistical analyses can be complex, understanding them is essential to proper interpretation of clinical trial results, including those from RV144.

Trial size

One statistical calculation that occurs before a trial begins is the sample size or the number of volunteers that need to be enrolled. Some of the volunteers enrolled receive the vaccine candidate(s), while others receive an inactive placebo. All volunteers in clinical trials receive risk-reduction counseling and available HIV prevention strategies, such as condoms, as a way to reduce their risk of infection. Still, some individuals in both vaccine and placebo groups will become HIV infected during the trial through natural exposure to the virus.

Having an accurate estimate of HIV incidence rates—the number of people who are newly infected with HIV per year—in the population that will be involved in the study is therefore useful in determining the trial size. If the overall incidence in the trial population is low, more volunteers are necessary. Biostatisticians determined that 16,000 volunteers would need to be enrolled in the RV144 trial because volunteers were recruited from the general population and not from specific populations known to be at an increased risk of HIV infection—such as injection-drug users or men who have sex with men.

Some trials are also designed to continue until a pre-determined number of HIV infections or endpoints occur. This doesn't require having as precise an estimate of HIV incidence: if the HIV incidence is low, the duration of the trial is longer. The precision with which the efficacy of the vaccine is determined is based on the number of HIV infections that occur during the study, not the total number of volunteers involved.

Efficacy and confidence intervals

The key to determining the efficacy of a vaccine is comparing the number of HIV infections that occurred in the vaccine and placebo groups. If more infections occur in volunteers who received placebo, as was the case in RV144, researchers can then estimate the efficacy of the vaccine candidates. In RV144, 74 infections occurred among volunteers in the placebo group, while 51 occurred among those who received the full prime-boost regimen. Based on this result, biostatisticians estimated that the efficacy of the vaccine candidates was 31.2%, which means that the vaccine recipients had a 31% lower risk of HIV infection than those who received placebo.

But 31.2% is just the best estimate of the vaccine efficacy. Biostatisticians also calculate something known as a confidence interval, which is a range of values around the best estimate of efficacy, all of which are contenders for the actual efficacy of the vaccine. Confidence intervals provide some perspective about how precise the estimated efficacy is—the wider the confidence interval, the less certain researchers are of the actual efficacy of the vaccine candidates. Take RV144 for example. In the originally reported results from this trial the confidence interval ranged from 1.2% to 52.1%. The efficacy of the prime-boost regimen could be anywhere in that range, yet the most likely efficacy is at the middle of that range, or 31.2%. Part of the reason that there was such a wide confidence interval for RV144 was because there were relatively few HIV infections overall that occurred during the trial.

Statistical significance

If there is a difference between the number of HIV infections that occurred in the vaccine and placebo groups, researchers ultimately want to know if this is because the vaccine actually worked, or if it happened merely by chance. There are several calculations biostatisticians use to try to determine this. One commonly used calculation is a p-value, and although it doesn't provide definitive information about whether the vaccine effect is real, it can provide evidence to suggest that the vaccine did have an effect. A p-value tells researchers how likely it would have been to get the result seen in the trial (74 infections in the placebo group and 51 in the vaccine group), or an even larger difference, if the vaccine had no effect. The less likely this is to occur, the lower the p-value, and the stronger the evidence is that the vaccine actually did have some effect.

Based on the 74-51 split in infections in RV144, statisticians calculated a p-value of 0.04. This means that if the vaccine had no effect whatsoever, there is a 4% chance that this split in infections, or an even larger one, would have occurred anyway. P-values are often misinterpreted. A p-value of 0.04 does not mean that there is only a 1 in 25 chance that the vaccine did not work at all, even though this is how it is commonly described.

It is a widely held convention to call any result with a p-value of less than 0.05 statistically significant. However, the 0.05 cut-off point was arbitrarily selected and so statisticians recommend not using this threshold as a hard and fast rule for judging whether the vaccine's efficacy is real. This is particularly true if the p-value is just on the cusp of statistical significance, as is the case in RV144. For example, trials with p-values of 0.06 or 0.04 provide virtually indistinguishable levels of evidence for whether the vaccine efficacy is real, even though one is statistically significant and the other is not. ■