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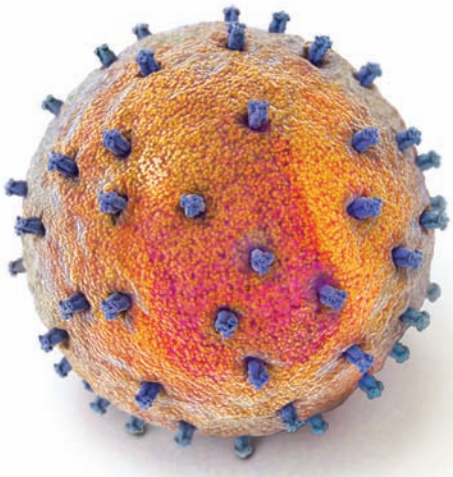

[SPOTLIGHT]

# It Eradicated Smallpox, But How?

**Researchers are collecting clues about the protection afforded by the smallpox vaccine, the gold standard of vaccines**

*By Andreas von Bubnoff*

One riddle currently confronting researchers is identifying the types of immune responses an AIDS vaccine would have to induce to protect against HIV. They are far from alone on this type of quest. For many vaccines, the immune responses that are actually responsible for providing protection against a pathogen, referred to as the immune correlates of protection, elude researchers even after the vaccine has been in use for decades (see *VAX* November and December 2006 *Primers* on *Understanding Immune Correlates of Protection Part I and II*).

Once researchers find a vaccine that works, there is little interest in figuring out why. But there are benefits to understanding how an effective vaccine affords protection. "We should really know how the things that work, work," says Shane Crotty, an associate professor for vaccine discovery at the La Jolla Institute of Allergy and Immunology.

Take smallpox, a disfiguring and often deadly disease caused by variola virus. A vaccine, called Dryvax, that protects against this virus led to the eradication of the disease in the late 1970s. Crotty refers to the smallpox vaccine as the gold standard since it is the only vaccine that has ever led to the eradication of a disease. Yet, for several reasons, the immune correlates of protection for the smallpox vaccine are still unknown. When smallpox was eradicated, many of the modern methods used by researchers to measure immune responses weren't yet available (see *VAX* February 2009 *Primer* on *Understanding How Immune Responses to AIDS Vaccine Candidates are Measured*). At that time, researchers could not measure T-cell responses, says Mark Slifka, associate professor at Oregon Health & Science University. Most of the data on how the smallpox vaccine works were collected from observational studies, and because there are no naturally occurring smallpox infections anymore, it would be impossible to do a randomized clinical trial of a smallpox vaccine today to study the immune correlates of protection.

However, there is now a renewed interest in trying to understand how the smallpox vaccine works. This is driven, in part, by the need to develop a new vaccine, with fewer side effects, which could be used to guard against a potential bioterrorism attack, Crotty says. Dryvax can cause serious side effects in people with compromised immune systems, including people with AIDS, according to D. Huw Davies, a project scientist at the University of California in Irvine. Another recently approved smallpox vaccine called ACAM2000 is a safer version of Dryvax, but it too causes side effects in immunocompromised people, Davies says. Researchers are therefore working on developing another smallpox vaccine and this has led them to try to figure out just how Dryvax provides such strong and long-lasting protection.

While identifying the exact immune correlates of protection for the smallpox vaccine may never be possible, researchers are now starting to collect clues about the way it protects by studying vaccinated individuals and people who have survived an infection, as well as using animal models. So far they have found that the smallpox vaccine primarily works by inducing neutralizing antibodies against the virus. These Y-shaped molecules can bind to the virus and inactivate or neutralize it before it has the chance to infect its target cells (see *VAX* February 2007 *Primer* on *Understanding*

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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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*Neutralizing Antibodies*). Antibody responses are considered a critical component in the protection provided by most, if not all, vaccines that are currently in use. Researchers have observed that the antibody responses induced by the first smallpox vaccine are surprisingly variable and redundant. They are now trying to identify certain markers in the antibody response that they hope will help them to predict whether a safer, alternative vaccine will also be protective.

The principles learned from a vaccine that protects against smallpox are unlikely to apply directly to development of an AIDS vaccine, but if anything, dissecting the lifelong protection afforded by the smallpox vaccine illustrates the critical role that antibodies play in vaccine-mediated protection.

## Searching for the correlates

"It's been thought for quite some time that the smallpox vaccine does work on the basis of neutralizing antibodies, but it was really just [a few] years ago that that was directly shown," says Crotty, referring to a study completed in 2005 that provided evidence in animal experiments that antibodies are required for protection by the smallpox vaccine. "That experiment nailed it," Crotty says.

In that study, researchers vaccinated monkeys with the human smallpox vaccine and then inhibited either their antibody or cellular immune responses to determine which of these was required for protection against the monkey version of the smallpox virus. They found that inhibiting the antibody response eliminated the protective effect of the vaccine.

While cellular immune responses, namely T cells, play a role in protection against smallpox, says Slifka, an antibody response may almost be completely sufficient for protection against infection with the virus that causes smallpox. He is now studying the antibody and cellular immune responses in a cohort of smallpox survivors and people who received a smallpox vaccination to see if the vaccine induces an immune response similar to that in natural infection.

Davies and Crotty have found that antibody responses to the smallpox vaccine are surprisingly variable among vaccinated people. The antibody responses also appear to be redundant, suggesting that there is not a single mechanism or one magic antibody that is required for protection against smallpox. It seems that as long as the antibodies that are induced by the vaccine cover the surface of the virus, they are able to neutralize it, and thereby protect against infection. "[It's like] throwing a net over the virus," says Crotty.

## Predicting protection

Researchers are also using animal experiments to identify markers in the antibody response that might help them predict protection for new smallpox vaccine candidates. They will use these markers to evaluate samples from a Phase I clinical trial of a new smallpox vaccine that utilizes a modified vaccinia Ankara (MVA) virus as a vector to see if the MVA-based candidate can protect as well as Dryvax and therefore offer a safer alternative to this existing vaccine. AIDS vaccine researchers are also exploring MVA-based vaccine candidates.

Now that the immune response elicited by the smallpox vaccine has been rather clearly described, Crotty says, the next big question is how it provides such long-lasting protection. "Why is it that you can give one immunization with this vaccine and you get a fantastic protective antibody response and it lasts for life?" he asks.

## Lessons for AIDS vaccines?

There are many differences between the smallpox virus and HIV, including their size. HIV has only a single protein covering its surface to which most antibodies would bind, and is made up of nine genes. Comparatively, the smallpox virus is very large, with about 200 genes and dozens of surface proteins. However, HIV is a more complicated pathogen to combat because of its nearly unrivaled ability to change or mutate to avoid the immune responses mounted against it.

Given these differences, understanding how the smallpox vaccine works may not be the best example to guide development of an AIDS vaccine. "We have been applying the rules of conventional vaccinology to HIV since it emerged in 1983," says Davies, "but this has largely failed us." While antibodies are likely important for protection to both smallpox and HIV, something very different from conventional vaccines needs to be developed against the rapidly evolving HIV, Davies adds.

Still, there are some general lessons. If there is anything to be learned from understanding the smallpox vaccine, "it's that neutralizing antibodies are so key for protection," says Crotty. "It's yet another piece of information that suggests that you probably need to be

Why is it that you can give **one immunization** with this vaccine and you get a **fantastic protective antibody response** and it **lasts for life?** *– Shane Crotty*

able to make neutralizing antibodies."

The AIDS vaccine candidates tested in clinical trials to date have been largely unsuccessful at inducing broadly neutralizing antibodies against HIV. To develop candidates that are more likely to generate an antibody response, researchers are now increasingly focusing their efforts on looking for new broadly neutralizing antibodies from HIV-infected individuals and studying the handful that have already been identified. The big challenge then is figuring out how to design immunogens—non-infectious fragments of HIV that are included in vaccine candidates—that can induce these antibodies in people (see *Primer*, this issue). ■

### *New www.iavireport.org*

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## GLOBAL NEWS *by Regina McEnery*

# Kenya AIDS Vaccine Initiative Marks 10-year Anniversary

It was 10 years ago that the Kenya AIDS Vaccine Initiative (KAVI) became involved in the search for an AIDS vaccine. But the seeds of this organization, which is headquartered at the University of Nairobi and was created by local researchers with funding from IAVI and the Medical Research Council's Human Immunology Unit at Oxford University, were planted much earlier. In the early 1980s, a number of Kenyan scientists—in partnership with researchers from the University of Manitoba—started to notice that a small percentage of commercial sex workers remained HIV uninfected over time despite repeat exposure to HIV (see *VAX* September 2008 *Spotlight* article, *Individual armor against HIV*).

Three leading Kenyan scientists involved in this research helped establish KAVI in 1999—Professor Omu Anzala, KAVI's Program Director; Professor Walter Jaoko, Deputy Program Director of KAVI; and the late Professor Job Bwayo, a co-founder of KAVI, who was tragically killed in 2007. "Until KAVI, vaccine research had never really been carried out in this country," says Anzala. "KAVI really raised a lot of community awareness to make people understand that vaccines just don't fall from heaven."

When KAVI was first established, some people were skeptical that an institution of this kind in Kenya would be able to meet the "level and standards" needed to conduct clinical trials, according to Anzala. But he says KAVI has not only met those standards, but raised the bar, both scientifically and ethically.

KAVI has been a productive partner in vaccine research and development, conducting four Phase I trials and a Phase IIa trial of a clade A HIV-DNA/modified vaccinia Ankara prime-boost candidate at Kenyatta National Hospital (KNH) in Nairobi. KAVI is also participating in an IAVI-sponsored project known as Protocol G, which seeks to find broadly neutralizing antibodies—Y-shaped molecules that can latch onto and neutralize HIV—from HIV-infected individuals.

To mark its 10-year anniversary, KAVI is hosting a scientific forum, "Emerging Vaccines: A Public Health Priority" on March 26. The forum will highlight efforts to develop vaccines against HIV/AIDS, tuberculosis, malaria, and human papilloma virus. Key guests include IAVI President Seth Berkley; Andrew McMichael, director of Oxford's Weatherall Institute of Molecular Medicine; and Adrian Hill, an investigator at Oxford's Jenner Institute. KAVI will also recognize the work of its community stakeholders on World AIDS Vaccine Day, which is observed annually on May 18.

While a primary goal is testing AIDS vaccine candidates, Anzala says KAVI also has the capacity to test preventive vaccines for malaria and tuberculosis, and he hopes KAVI can broaden its scope to include more basic research. Anzala says talks are underway to establish a mentorship program for young investigators to come work at KAVI. "The virus is here, the patients are here," says Anzala. "We must be able to also begin to engage in basic research."

▲ **The Kenya AIDS Vaccine Initiative (KAVI), launched 10 years ago, has been an important player in clinical research of AIDS vaccine candidates. Shown above is the late Professor Job Bwayo, a KAVI co-founder, speaking at an HIV Vaccine Awareness Day event in Nairobi in 2004.**

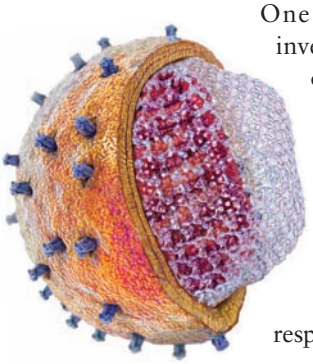
[PRIMER]

# Understanding How Inserts for Vaccine Candidates are Designed

**What strategies are being explored to design better inserts for inclusion in AIDS vaccine candidates?** ***By Regina McEnery***

Many vaccines contain an intentionally weakened or attenuated form of the pathogen the vaccine is designed to protect against. The vaccine against influenza (flu) is one example. It contains a live influenza virus but the vaccine is not able to cause any harm because researchers purposely disable parts of the virus. Even though it doesn't make people sick, the attenuated flu virus does trigger the immune system to make immune responses against it. Some of these immune responses are stored away in the body as memory cells. If the immune system detects that same flu virus in the future, these memory cells "remember" the virus and can act quickly to destroy it before an infection is established and illness occurs.

Some vaccines use an inactivated or killed version of the pathogen they are designed to protect against to train the immune system. The vaccine against hepatitis A virus, for example, contains a whole, but killed virus. Unfortunately, the nature of HIV—including its ability to rapidly change or mutate—makes using a live-attenuated or killed version of HIV in a vaccine both impractical and potentially dangerous. Researchers are concerned that a live-attenuated version of HIV could conceivably mutate and regain its ability to cause disease. Using a killed version of HIV in a vaccine candidate is also impractical because it is difficult to prove that the virus is completely inactivated. This has led scientists to look for better and safer strategies for developing an AIDS vaccine.



One method under investigation is using only fragments of HIV's genetic material rather than the whole virus to try to trigger cellular (T-cell) and antibody (B-cell) responses against HIV (see *VAX* July 2008 *Special Issue*, *Understanding the Immune System and AIDS Vaccine Strategies*). By using only a small part of the virus's genetic material, scientists can be sure that the vaccine candidate cannot cause HIV infection. The fragments of HIV that are included in vaccine candidates are referred to as antigens. These antigens can be delivered into the body many different ways, including via a viral vector—a virus other than HIV that is intentionally attenuated so it can't cause disease (see *VAX* September 2004 *Primer* on *Understanding Viral Vectors*). The aim is to get the immune system to recognize the HIV antigen—just as it does other foreign substances—and generate an immune response against it. Antigens that can induce immune responses are referred to as immunogens.

One of the biggest questions is which HIV antigens will induce potent immune responses, including long-lived T and B memory cells that will be critical for vaccine-induced protection against HIV in the future. Scientists are employing a number of different strategies to try to design such immunogens.

## Clues from HIV-infected individuals

One strategy involves analyzing long-term nonprogressors (LTNPs)—HIV-infected individuals who can control the virus without the aid of antiretroviral therapy. Researchers have found that cellular immune responses are usually involved in control of HIV in these individuals. These cellular immune responses target specific regions of HIV known as epitopes. By studying which HIV epitopes the T cells of LTNPs are directed against, researchers hope to be able to identify the HIV fragments that might make the best antigens for inclusion in a vaccine candidate that would induce primarily T-cell responses.

## Building a mosaic antigen

Researchers are also attempting to design HIV antigens that could address the overwhelming diversity of HIV. Because HIV mutates so rapidly, there is tremendous variation among the different viruses circulating in a population, and even in a single individual. Scientists at the Los Alamos National Laboratory in the US oversee a massive database that catalogues the genetic characteristics or sequences of many of the currently circulating versions of HIV. From this database, they can identify the regions of HIV that are consistent or conserved across many different viruses. Researchers can then combine these conserved regions into a single antigen, which is referred to as a mosaic. Vaccine candidates with mosaic HIV antigens have so far only been tested in animal studies.

## Antigens to induce antibodies

Another strategy is also being used to design HIV antigens that can induce neutralizing antibodies against the virus (see *VAX* February 2007 *Primer* on *Understanding Neutralizing Antibodies*). Antibodies that can effectively neutralize many different forms of HIV are referred to as broadly neutralizing antibodies. Only a handful of broadly neutralizing antibodies have been identified to date.

Researchers are now using X-rays to carefully study precisely at which location on HIV some of these broadly neutralizing antibodies bind. A fragment of HIV from this binding site could be used as an antigen, which, if included in a vaccine candidate, would hopefully induce this broadly neutralizing antibody.

Not knowing which HIV antigens will induce the necessary immune responses is a major barrier to the development of an effective AIDS vaccine. While researchers are actively investigating ways to design better antigens to put into a vaccine candidate, they are also exploring different viral vectors and other techniques to deliver HIV immunogens into the body. ■