Are two really better than one? Researchers are testing to see if two different vaccine candidates can together induce improved immune responses against HIV.

We all use things every day even though we have little appreciation of how they work. Cars, cell phones, and computers come to mind. But usually someone somewhere knows exactly how they function. This isn’t the case with many of the vaccines that are routinely administered to children and adults throughout the world. For many of these licensed vaccines scientists don’t know exactly how they work.

This is also true for AIDS vaccine candidates that are in various stages of clinical testing. Researchers are yet to find a candidate that protects people from HIV infection, although many different approaches are currently being explored. Some candidates in clinical trials use HIV proteins, viral vectors (see VAX September 2004 Primer on Understanding Viral Vectors), or DNA to deliver fragments of HIV to the immune system with the intention of inducing an immune response against the virus. Each of these induces an immune response to some extent but to improve upon these responses researchers are now testing different approaches in combination—a strategy known as prime-boost—to see if delivering different candidates in sequence can augment immune responses against HIV.

Although researchers don’t know exactly how the prime-boost strategy works, the rationale for it is simple. The first administration (the prime) generates a collection of immune cells that recognize HIV, and these cells then allow for a quicker and stronger immune response to the second vaccination (the boost). And this prime-boost approach seems to work. “Essentially, most vaccination strategies are primes and boosts,” says Larry Corey who leads the HIV Vaccine Trials Network (HVTN) in Seattle. However, usually the same exact vaccine is given multiple times, as is common practice with vaccines against chickenpox and measles.

But using different vaccines for the prime and boost is now the regimen of choice for many AIDS vaccine candidates that are in clinical trials. The hope is that the combination will lead to increased immunogenicity (see Primer, page 4) and could also result in a broader immune response because each vaccine component might stimulate a different type of immune cell. Hildegund Ertl of the Wistar Institute, a research institution in Philadelphia, thinks a successful AIDS vaccine is likely going to consist of two different candidates administered in a prime-boost regimen. “That’s where I’d put my money right now,” she says.

Deciding which vectors to use for prime and boost still involves a lot of guesswork. Peggy Johnston of the Division of AIDS at the National Institute of Allergy and Infectious Diseases (NIAID) refers to this approach as “thoughtful empiricism.” “Try it and see what happens, but there is some thought behind it,” she says.

Several different combinations have been tested and still more are planned—about half of the 30 or so ongoing AIDS vaccine trials use such combinations. Even so, there is surprisingly little known about how prime-boost works or the reason that some combinations work better than others. Finding the right combination often comes down to trial and error.

One of the first questions researchers tackled was which candidate to use as the prime and which for the boost. This was primarily determined by experimentation, says Tomas Hanke of the University of Oxford, UK, who did some of the earliest work with DNA and MVA-based vaccine candidates. “We wanted to try their combinations, first without really thinking why we should use this one first as opposed to the other,” he says.

Trial and error

Many of the prime-boost regimens now undergoing evaluation combine a DNA-based vaccine with a viral vector such as adenovirus or modified vaccinia Ankara (MVA) to deliver fragments of HIV to the immune system.
work in humans. Some of the first DNA/MVA regimens, for example, worked well in mice and monkeys but not so well in humans. Clinical trials with some of the early DNA/MVA prime-boost combinations developed by Hanke and Andrew McMichael, also of Oxford, showed that few of the volunteers had substantial immune responses to HIV.

David Ho of the Aaron Diamond AIDS Research Center in New York is currently testing a different DNA/MVA prime-boost regimen in clinical trials. In pre-clinical studies in mice and rabbits the combination works about 10 times better than when either candidate is administered by itself, and Phase I trials have already shown that each individual component is both safe and immunogenic. Ho will soon test this DNA/MVA combination in Phase II trials and is optimistic.

Other groups are working on prime-boost regimens that combine different viral vectors, rather than using DNA-based candidates. Dan Barouch at Harvard University is testing different combinations of adenovirus-based candidates in non-human primates. He has found that certain combinations of different strains or serotypes of adenovirus are much more immunogenic than others. Researchers at the Vaccine Research Center (VRC), part of NIAID, are now testing a prime-boost regimen with two different adenovirus candidates—adenovirus serotype 5 (Ad5) and Ad35—in a Phase I trial (see VAX June 2007 Global News).

Early results

Although most of the evidence supporting use of a prime-boost administration of two different vaccine candidates comes from pre-clinical studies, some regimens have already proven safe in humans and appear to be immunogenic in Phase I trials. Giuseppe Pantaleo of the University Hospital in Lausanne, Switzerland, is one of the coordinators of a Phase I trial in Europe that uses a combination of a DNA-based candidate and a poxvirus-vector candidate known as NYVAC. This combination induced much better immune responses than when the poxvirus candidate was used alone. A Phase II trial with these candidates has already started enrolling volunteers.

Another regimen that appears promising uses DNA as a prime and Ad5 as a boost. According to Corey, results from Phase II studies with the DNA/Ad5 vaccine candidates developed at the VRC suggest that more than 70% of the recipients develop immune responses to HIV. The regimen will soon be tested in a Phase IIb test-of-concept trial called PAVE 100 (see VAX September 2005 Primer on Understanding Test of Concept Trials). This trial will take place at multiple trial sites affiliated with HVTN, the United States Military HIV Research Program (USMHRP), and IAVI.

**Ideally you would have a single product. The only reason why we are doing prime-boost is that we don’t.**

Sarah Schlesinger

These preliminary clinical trial results are encouraging, but not everyone has observed that prime-boost combinations with different candidates work better in humans than using the same vaccine repeatedly. “We haven’t found anything that shows that prime-boost adds a synergetic effect in people, and we have tested probably more things than anybody else,” says John Shiver of Merck. The company is currently conducting two Phase IIb trials in North and South America, the Caribbean, Australia, and South Africa that use repeated injections of their Ad5 vaccine candidate.

Mysterious mechanism

Given that, in many cases, prime-boost appears to induce stronger immune responses, the question remains how. “Exactly why it’s better, I don’t think anybody knows,” says Rockefeller University’s Sarah Schlesinger, who collaborates with Ho. Part of the mystery may be because it’s difficult to directly measure the effect of the prime, she suggests.

Pantaleo thinks the enhanced immune responses occur because each candidate does something very different—perhaps targeting different types of immune cells. And there is some evidence suggesting that using two different candidates in a prime-boost strategy does induce more varied types of cellular responses to HIV than using the same vaccine more than once.

For many of these prime-boost combinations it is very difficult to know the precise mechanism of how they induce an improved immune response, especially since researchers know very little about why the individual components are immunogenic. “We don’t know a lot about the mechanism of how DNA is immunogenic,” says Gary Nabel, director of the VRC.

**Apples and oranges**

Another challenge facing researchers is that it is difficult to understand which combinations of vaccine candidates work better than others because comparing the results from different studies isn’t straightforward. Research groups often use slightly different viral vectors or different fragments of HIV (antigens) within the viral vector or DNA-based candidate. This makes the comparison between trials that appear to be using similar candidates more complicated. “I think there is a false assumption that a DNA is a DNA and an MVA is an MVA,” Johnston says. “That’s just not true.”

These slight variations could, in part, account for the widely-disparate results from studies that use similar prime-boost combinations of vaccine candidates. For this reason Nabel and others are promoting use of a standardized genetic insert containing the same fragments of HIV, which can be included in different DNA or viral vectors. This could help eliminate one of the variables between related vaccine candidates and help researchers decipher exactly which prime-boost com-
A combination is the most effective. Nabel says the HVTN just started a series of trials using different viral vectors that are all carrying this standardized genetic insert.

Another concern is that if a prime-boost combination of two different vaccine candidates is found to be superior, administering it will likely be more complicated and costlier than if it were just a single component. There is currently no licensed vaccine against any other disease that consists of two different vaccine components. “Ideally you would have a single product,” says Schlesinger. “The only reason we are doing prime-boost is that we don’t.”

This could drastically improve the speed and ease of enrolling volunteers.

In an AIDS vaccine trial previously conducted by USMHRP in Uganda, 58% of potential volunteers were unable to participate because their laboratory results were outside of the established reference ranges. When a second trial was conducted by USMHRP at the same site using the newly-established reference range for that population, researchers only excluded 23%.

Local reference ranges will also help researchers differentiate naturally-occurring laboratory abnormalities from any possible side-effects caused by the vaccine candidate or other intervention being tested. Africans often have different results for many standard laboratory tests due to their exposure to a greater number of parasites and pathogens, which affects the functioning of the immune system.

AVAC receives grant to advocate for HIV prevention research

The AIDS Vaccine Advocacy Coalition (AVAC) recently received a five-year, US$14 million grant from the Bill & Melinda Gates Foundation to support the organization’s international advocacy efforts. This new funding will expand AVAC’s focus beyond AIDS vaccines to include the broader field of HIV prevention research. AVAC now plans to step up efforts to advocate for several interventions that are currently being tested in clinical trials, including microbicides and pre-exposure prophylaxis (PrEP), which involves the use of antiretrovirals to prevent HIV infection.

There are currently several ongoing Phase III efficacy trials that are separately testing both microbicides and PrEP, and AVAC plans to work with the communities that are involved in and affected by this research to help prepare them for the results of these trials. The organization, which is based in New York City, will also work to ensure that any benefits of this research become available globally.
How do researchers measure the immune responses induced by AIDS vaccine candidates?

Researchers measure the efficacy of preventive AIDS vaccine candidates in Phase III clinical trials. A candidate is effective if it protects recipients from HIV infection or, in the case of a partially-effective vaccine, if it either slows or prevents disease progression in individuals who subsequently become infected through exposure to the virus (see VAX May 2007 Primer on Understanding Partially-Effective AIDS Vaccines).

In the earlier stages of clinical evaluation, during both Phase I and II trials, researchers are primarily measuring the safety of the vaccine candidates and the extent to which they induce immune responses against HIV, an idea referred to as immunogenicity. Together this information helps researchers prioritize candidates for further evaluation.

Detecting antibodies

There are two main types of immune responses to HIV that are routinely assessed. The first is the presence of HIV-specific neutralizing antibodies that are capable of latching on to the virus and disabling it (see VAX February 2007 Primer on Understanding Neutralizing Antibodies). Tests such as the enzyme-linked immunosorbent assay, or ELISA, are used to detect and quantify HIV-specific antibodies that are induced in response to an AIDS vaccine candidate. An ELISA is performed by exposing a blood plasma sample from a vaccinated individual to HIV antigens—the pieces of HIV that are in the vaccine—on a plastic plate. Any antibodies that are present will bind to the HIV antigen. The bound antibodies can then be separated from any other antibodies that were in the blood and the quantity of HIV-specific antibodies can be measured. The ELISA is also commonly used to determine if a person is HIV infected (see VAX November 2005 Primer on Understanding HIV Testing). More specific assays can also measure the ability of HIV-specific antibodies to successfully neutralize the virus.

Cell counters

The other category of immune responses is cellular immunity, which includes two specific types of immune cells known as CD4+ and CD8+ T cells. The majority of vaccine candidates that are currently in clinical trials primarily induce cellular immune responses and there are several different assays that are used to measure both the quantity and quality of these responses.

Seeing spots

An ELISPOT assay is most commonly used to measure the immunogenicity of AIDS vaccine candidates. It works by detecting CD4+ and CD8+ T cells that are producing cytokines, which are a group of proteins secreted by immune cells in response to a virus or bacteria. Cytokines are sometimes referred to as the messengers of the immune system and they can also inhibit a virus from replicating. The interaction of an HIV antigen (from a vaccine candidate) with an immune cell can result in the secretion of many different cytokines and researchers can detect the release of these proteins by using an ELISPOT assay. Usually researchers use an ELISPOT to detect the presence of a specific cytokine called interferon gamma (or IFN-γ) that is secreted by both CD4+ and CD8+ T cells as a defense mechanism against viruses.

During clinical trials ELISPOT assays are run in immunology laboratories on blood samples collected from volunteers that have received the AIDS vaccine candidate being tested. From these samples researchers isolate the white blood cells—called peripheral blood mononuclear cells or PBMCs—that are critical to the immune system. These cells are then added on to a plastic plate that is coated with antibodies. When the PBMCs are stimulated with HIV antigens they release different cytokines, including IFN-γ, that attach to the antibodies already on the plate. Other antibodies that are tagged with a chemical that produces a strong color are then added, so that wherever there is an immune cell that is producing cytokine a dark spot will appear. The presence of spots shows that there are CD4+ and CD8+ T cells that are responding to the HIV antigen included in the vaccine candidate.

By counting the spots, researchers can see how many cells are releasing IFN-γ, for example. This is referred to as the number of spot-forming cells. Although ELISPOTS are most often looking at secretion of IFN-γ, they can also look at many other cytokines that are released by immune cells. If the number of spot-forming cells for a vaccinated volunteer is above a threshold set by researchers before the start of the trial, then that volunteer is considered to have responded to the vaccine candidate. For many AIDS vaccine trials in developing countries, ELISPOT assays are run in immunology laboratories that are associated with the clinical trial site.

Correlation

ELISPOT assays are just one way to measure the activation of the immune system by a vaccine candidate. The difficulty with interpreting the results of these assays is that researchers don’t yet know if the production of IFN-γ by immune cells, or any other cytokine, correlates with even partial protection against HIV infection. The precise immune responses that correlate with protection against HIV have not yet been identified (see VAX November and December 2006 Primers on Understanding Immune Correlates of Protection). The results of the ELISPOT assay serve only as an indication of immune function. Researchers are currently studying already-licensed vaccines for other diseases that induce cellular immune responses to provide clues about whether or not the ELISPOT assay provides an accurate measure of immunogenicity.

Although imperfect, the results from ELISPOT assays help researchers compare the immunogenicity of different vaccine candidates and therefore decide which should undergo further clinical evaluation. Another laboratory test called flow cytometry analyzes the ability of immune cells to produce several cytokines at once. Researchers are also starting to use assays that test the ability of immune cells to directly suppress the virus. All of these tests help researchers further classify the immunogenicity of different candidates.