

aids vaccine approaches in development

PATRICIA KAHN

WHEN TODAY'S common viral vaccines were first developed, their makers didn't have many choices about how to get the job done. They usually didn't know a lot about how the *virus* caused disease, nor (by today's standards) much about the virus or its life cycle. The technologies they had to draw on were also very limited. So most vaccines were made either by killing virus or by weakening it so it couldn't cause disease, then using the resulting particles to *immunize*.

For HIV, these traditional approaches are essentially off the table. Using live virus, even if it's weakened, is too risky, while killed vaccines haven't shown much promise so far. Luckily, AIDS vaccine designers now have a treasure chest of *genetic engineering* tools that allow them to pluck out any portion of the virus—so they can make vaccines that use only parts of HIV, which is a safer strategy. And they can join their selected pieces of HIV genes to gene segments from other sources that might contribute useful properties to a vaccine.

getting started

So where do researchers begin when they set out to make an AIDS vaccine?

One of the first questions they ask is which arm of the immune system it should target, since the answer determines what types of designs to consider.

To make a vaccine that targets *humoral immunity* (the best hope for blocking HIV infection), the basic idea is to use the HIV *envelope protein (Env)*, which protrudes from the surface of free virus particles. There it is “seen” by the immune system and recognized as foreign, triggering specialized white blood cells called *B-cells* to make *antibodies* against specific regions of Env. Some of these antibodies (depending on exactly which regions they recognize) will be *neutralizing antibodies (NAb)s*, and these are the ones vaccine developers especially want to induce. That’s because if NAb)s encounter HIV from a real infection later on and can recognize its particular Env, they would (in theory) lock onto the virus particles and prevent them from infecting cells.

Neutralizing antibodies offer the best hope for blocking infection with HIV.

But so far, there’s been only failure, since HIV has evolved sophisticated ways of evading the NAb response (more on this below). So researchers are now working on new strategies to make forms of the envelope that can outsmart the immune system.

Cellular immunity kicks in once HIV succeeds in infecting host cells. HIV *proteins* are then made inside the cell and incorporated into the *membrane* surrounding the cell, where they are recognized as *antigens* (that is, as foreign) by the immune system—alerting the *killer T-cells* to the presence of infected cells. In other words, for vaccines to induce cellular responses, it’s not enough to expose the immune system to free-floating HIV particles or antigens; instead, the antigens need to be “displayed” on the cell surface.

Although making vaccines that target cellular responses is a relatively new idea, nearly all the AIDS vaccine candidates now in *clinical trials* fall into this category. And a number of them are turning out to have at least some ability to do

the job—although we don't yet know if this will translate into protection. Most researchers think that the effect of this type of vaccine (if it works at all) won't be to block HIV infection but to control *viral replication*, which in turn could slow or prevent the onset of AIDS. (See chapter 8 for more discussion of how these vaccines may work.)

Both humoral and cellular responses also contribute to *mucosal immunity*—immune defenses in tissues that line the body cavities, including the genital tract, anus and gut. How much of a role they play in protection against HIV, and how to best induce them, are areas that still get very little attention in the field.

Let's now take a closer look at some of the vaccine designs in the pipeline.

approaches targeting cellular immunity

THE CRUX of any strategy for stimulating T-cell responses is how it delivers the HIV genetic material (or in some cases small protein fragments called *peptides*) into the cells of a vaccinated person. The main strategies being tested are:

› *Naked DNA vaccines*

These vaccines contain pieces of HIV genetic material (*DNA*) joined to pieces of harmless bacterial DNA (called *plasmids*). When this type of vaccine was first developed around 1989, many researchers thought it would revolutionize vaccine development: Not only did DNA vaccines induce strong *immune responses* in mice, but they are simple and inexpensive to produce, and could eventually be stable without refrigeration—a great advantage for getting vaccines to remote settings. But candidates developed for many diseases (including HIV)

Table 2.2
Types of vaccines in clinical trials (February 2005)¹

Vaccine Type	Number in trials
Viral vector	16
DNA	9
Protein	5
Peptides	4

¹ Sources: International AIDS Vaccine Initiative (IAVI) trials database; the Pipeline Project (see resources and appendix 3).

haven't yet lived up to this promise in humans, since they usually stimulate only weak responses.

Because of their potential advantages, the HIV field hasn't given up on DNA vaccines, but is looking for ways to augment them. One possibility is to pair them with a second vaccine, an approach that's being tested in several clinical trials—most of them using a DNA vaccine to “*prime*” the immune system,

The prime-boost approach—pairing 2 vaccines—may be more potent than either vaccine alone.

followed by a “*boost*” with a second vaccine (usually a *viral vector*-based product; see below) a few weeks later. In monkeys, these combinations are often more potent than either vaccine alone, although in humans, several *Phase I* trials have given disappointing results. Other trials are testing whether DNA

vaccines are more *immunogenic* when given together with substances that boost the immune system (one of the body's natural boosters, called *cytokines* or other immune-stimulating compounds called *adjuvants*).

› *Live vector vaccines*

Most *live vector vaccines* use harmless viruses engineered to ferry foreign genes (like HIV genes) into cells. Each viral vector has its advantages and disadvantages, based on properties such as how much foreign DNA it can carry, the types of host cells it infects best, how long it persists in the host and how easy or difficult it is to grow in large amounts.

One of the more promising vaccines in the pipeline uses a vector made from a weakened cold virus called *adenovirus*; this candidate is now being tested for *efficacy* in a “proof-of-concept” trial (see chapter 8). Another vector, based on a bird virus called *canarypox*, is used in several veterinary vaccines and was made into a series of AIDS vaccines that have been in clinical testing since 1994; one of them is now in a *Phase III* efficacy trial in southern Thailand, in a *prime-boost* combination with an envelope-based vaccine (see chapters 8 and 22). Yet another is based on a weakened virus called *MVA*, a relative of the virus used to make smallpox vaccine. Of the newer vectors, one of them (called *VEE*) infects important

“antigen-presenting” immune cells, while another (AAV) persists in the host for long times, which may lead to better immune responses.

Other types of vaccine vectors are in the pre-clinical stage of research. Several laboratories are developing weakened bacteria as vectors, and an AIDS vaccine made in yeast is also in the works. Scientists in South Africa are even working on plant vectors, which might be usable as oral vaccines.

› *Peptide (or lipopeptide) vaccines*

Peptide vaccines contain small fragments of HIV proteins, which are simpler and less expensive to make than whole proteins. There are almost endless numbers of possible variations. For example, peptide vaccines can include the most immunogenic snippets of any HIV protein, and/or peptides from different *strains* of HIV. Although earlier peptide-based candidates were dropped because they induced only weak responses, research groups in France have been developing candidates with peptides linked to fat molecules, or lipids (the hybrid is called a lipopeptide), which seem to enhance the peptides' *immunogenicity*. One lipopeptide vaccine is now in *Phase II* studies, and another is being tested as a boost for a canarypox-based vaccine prime. The pharmaceutical company Wyeth is also developing a peptide-based HIV vaccine.

Besides deciding what type of vaccine to make, researchers also need to choose which parts of HIV to include. It's a difficult decision, because they don't yet know which antigens matter most for protection. So for now, the choice is just an educated guess. Alongside the question of *which* genes or proteins (or parts of them) is the issue of *how many* to include. On one hand, it seems logical that more is better, since this avoids eliminating potentially useful antigens. But fewer antigens mean a simpler vaccine to make. And certain viral vectors are limited in how much foreign DNA they can incorporate.

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approaches that target neutralizing antibodies

Vaccines aimed at neutralizing HIV all start with the HIV envelope protein, which sticks off the surface of viral particles in spikes made of three individual envelope molecules (see illustrations in chapter 3). But HIV has evolved masterful strategies to hide parts of the envelope that could induce neutralizing antibodies, and to evade detection by those which are nevertheless made. That's turned the problem of how to design vaccines which induce effective NABs into one of the most important scientific hurdles facing the field—one that's only now starting to get the intense attention it needs, after years of frustration over the impasse.

In the early years of AIDS vaccine research, antibody-based vaccines were all anyone worked on—buoyed by the (then-recent) success of a hepatitis B vaccine made from its viral envelope. The strategy was to engineer cells in the laboratory so they produce a *subunit* of the envelope, then purify the subunit and use it as a vaccine. Unfortunately, it emerged that, although these subunits induced NABs to HIV strains grown in the lab, they couldn't neutralize viruses isolated directly from infected people. Still, one of these products went all the way through Phase III trials, but proved not to be protective (see chapters 22 and 23).

Despite all these difficulties, there's good reason to believe that this problem can be solved. In the late 1990's, researchers studying the blood of HIV-infected people found a few examples of just the kind of NABs needed—those which can neutralize a wide range of HIV strains, despite differences in their Env proteins. That led to an approach based on working backwards: analyzing the precise three-dimensional structure of these unusual antibodies, down to the position of individual atoms, to see exactly what part of the envelope protein they recognize. From there, the hope is that researchers can engineer envelope proteins which mimic these key structures. In the meantime, similar kinds of fine-structure studies are helping other researchers pursue different strategies—such as altering the envelope protein to unmask its critical neutralizing regions so

they are exposed to the immune system. Once candidates based on these “rational” approaches progress into clinical trials, Phase I studies will show whether they induce the broadly neutralizing antibodies vaccine designers hope they will—the property that most researchers think will give an HIV vaccine the best chance of preventing infection in the first place.

where are we headed

AS NEW CANDIDATES are finally being tested in Phase I trials, researchers are learning which ones induce the best immune responses and using this information to improve the design of the next candidates. As these precious clinical data accumulate, some of the guesswork in vaccine design will hopefully be eliminated. And with two efficacy trials in the works, finding a vaccine that works even a little would be a major advance, since it would give researchers a handle on finding out just what immune responses a vaccine needs to induce. But for now, the working assumption is that the ultimate AIDS vaccine will probably need to stimulate both humoral and cellular immunity, and possibly also mucosal responses—which will probably require a combination of different vaccines.

The ultimate AIDS vaccine will probably need to stimulate both neutralizing antibodies and cellular immunity.

resources

www.iavireport.org/trialsdb

Database of AIDS vaccine trials. Compiled by the *IAVI Report* (the newsletter of the International AIDS Vaccine Initiative), this is a searchable database of all preventive AIDS vaccine trials (ongoing and completed).

<http://chi.ucsf.edu/vaccines>

The Pipeline Project, a collaboration of the University of California San Francisco (UCSF) Center for HIV Information and the US HIV Vaccine Trials Network (HVTN). This website lists ongoing, planned and completed trials sponsored by the HVTN.

<http://clinicaltrials.gov>

More complete information on federally and privately supported trials from the US government’s clinical trials database.