HIV Vaccines: Prospects and Challenges

by David Baltimore and Carole Heilman

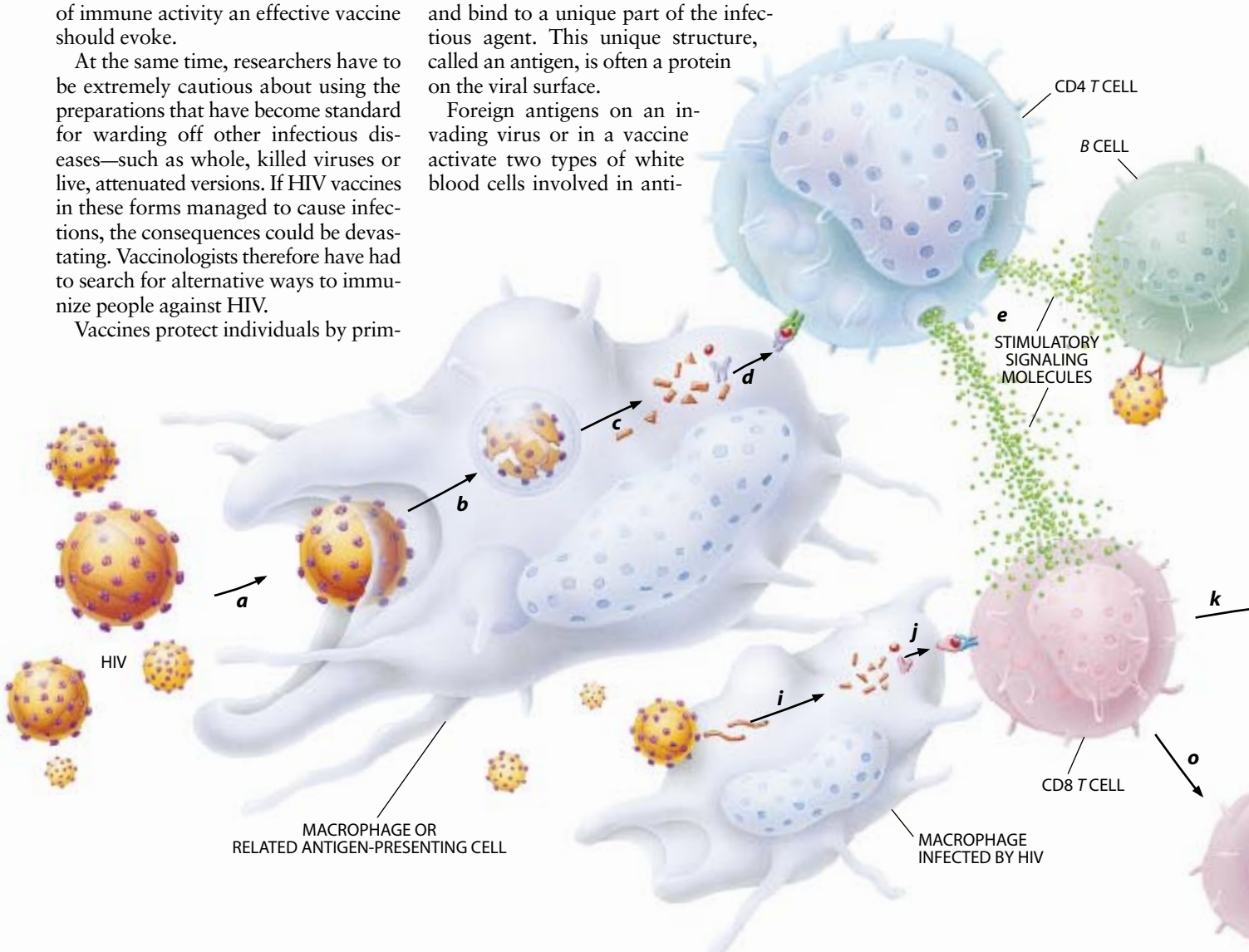
Scientists know more about HIV—the human immunodeficiency virus that causes AIDS—than any other virus. Yet designing a vaccine able to protect against it remains as much of a challenge today as when the virus was discovered. Part of the problem is that, unlike the body's response to most acute viral infections, the natural immune response does not destroy HIV. This failure makes it difficult for investigators to know what type of immune activity an effective vaccine should evoke.

At the same time, researchers have to be extremely cautious about using the preparations that have become standard for warding off other infectious diseases—such as whole, killed viruses or live, attenuated versions. If HIV vaccines in these forms managed to cause infections, the consequences could be devastating. Vaccinologists therefore have had to search for alternative ways to immunize people against HIV.

Vaccines protect individuals by priming the immune system to recognize disease-causing organisms when they are encountered. In the case of HIV, a successful vaccine should be able to eliminate incoming virus and destroy quickly any cells that become infected.

Most vaccines activate what is called the humoral arm of the immune system, stimulating formation of protective antibodies: molecules that mark free virus (which circulates outside cells) for destruction. The antibodies recognize and bind to a unique part of the infectious agent. This unique structure, called an antigen, is often a protein on the viral surface.

Foreign antigens on an invading virus or in a vaccine activate two types of white blood cells involved in antibody manufacture. After contacting antigens, cells known as *B* lymphocytes mature and produce antibodies. In addition, helper, or CD4, *T* lymphocytes direct *B* cells to manufacture more antibodies or to take the form of memory *B* cells. The memory cells do not produce antibodies immediately but respond vigorously to subsequent exposures. Following vaccination, the long-term production of small amounts of antibody and the persistence of memory

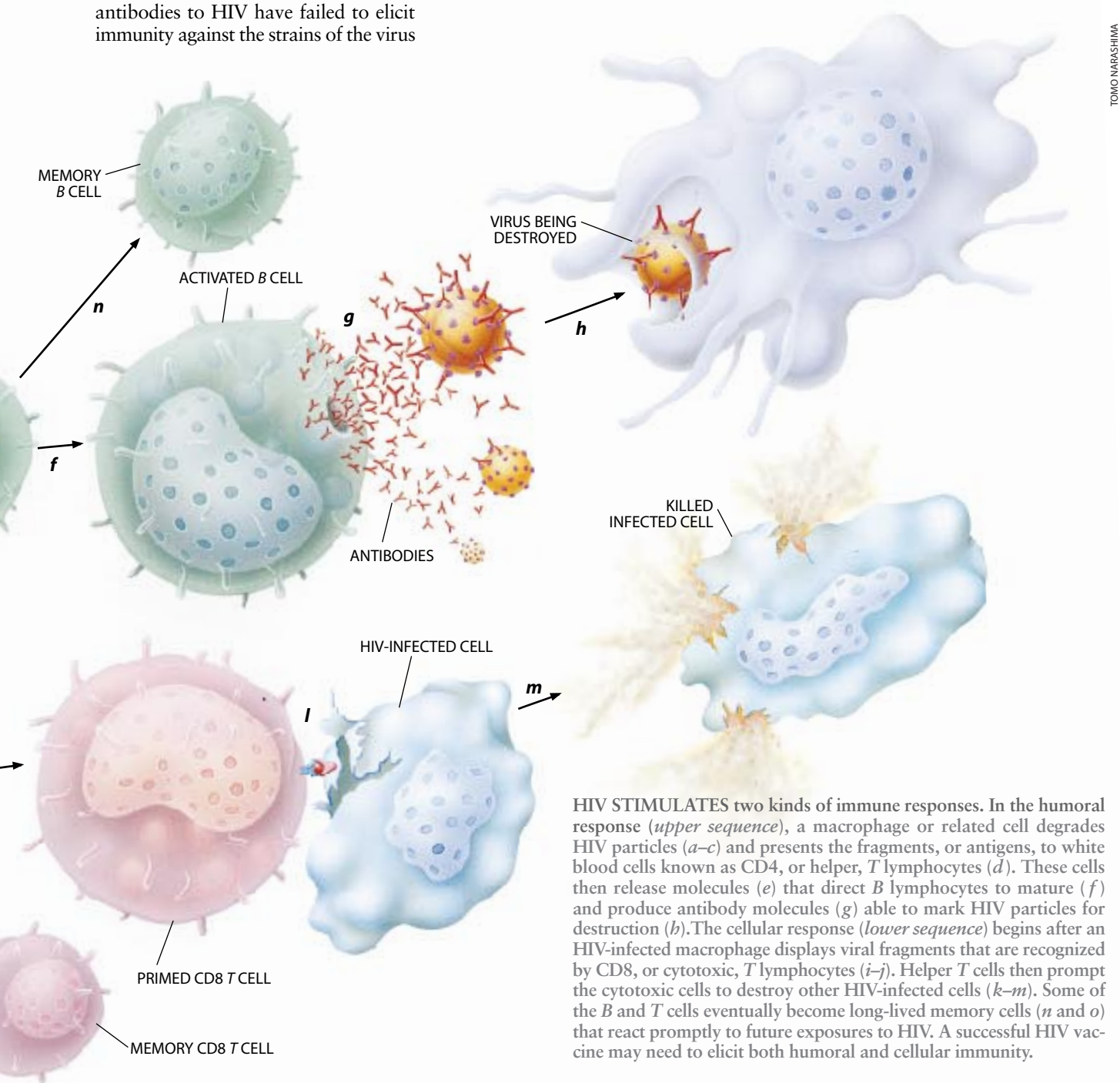


Unlike vaccines for many viruses, those for HIV may have to go beyond generating antibodies. Devising approaches that will fully activate the immune system is far from simple

cells allow the body to mount a rapid defense if ever it encounters the virus.

No vaccines have been designed specifically to stimulate the other arm of the immune system, known as the cellular component. But many AIDS researchers are working on just that aim because, thus far, vaccines designed to generate antibodies to HIV have failed to elicit immunity against the strains of the virus commonly found in infected patients.

In cellular immunity, activated white blood cells called cytotoxic *T* lymphocytes (CD8 *T* cells) multiply and cruise through the bloodstream and tissues, searching for and eliminating virus-infected cells. Some also become memory cells, ready to leap into action after a later exposure to a pathogen. Unlike antibodies, cytotoxic *T* lymphocytes recognize infected cells, rather than the infectious agent itself. Like *B* cells in the humoral arm of the immune system, however, cytotoxic *T* cells are activated in part by signals from helper *T* cells. In



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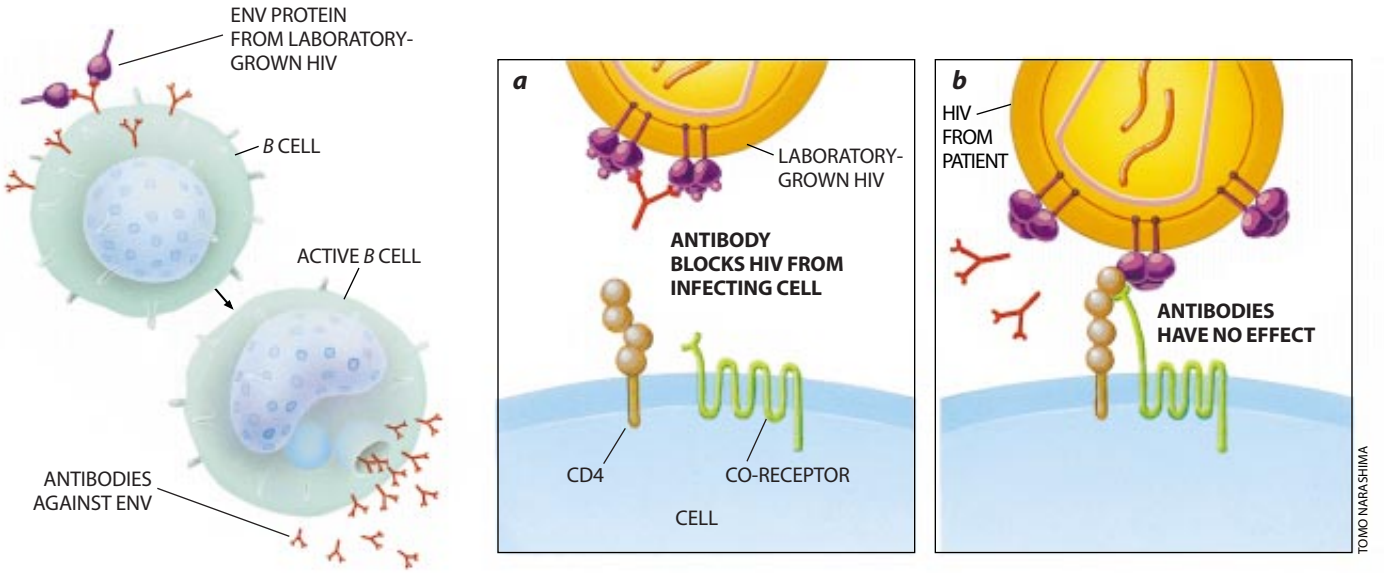
HIV STIMULATES two kinds of immune responses. In the humoral response (*upper sequence*), a macrophage or related cell degrades HIV particles (*a–c*) and presents the fragments, or antigens, to white blood cells known as CD4, or helper, *T* lymphocytes (*d*). These cells then release molecules (*e*) that direct *B* lymphocytes to mature (*f*) and produce antibody molecules (*g*) able to mark HIV particles for destruction (*h*).The cellular response (*lower sequence*) begins after an HIV-infected macrophage displays viral fragments that are recognized by CD8, or cytotoxic, *T* lymphocytes (*i–j*). Helper *T* cells then prompt the cytotoxic cells to destroy other HIV-infected cells (*k–m*). Some of the *B* and *T* cells eventually become long-lived memory cells (*n* and *o*) that react promptly to future exposures to HIV. A successful HIV vaccine may need to elicit both humoral and cellular immunity.



PURE ENV PROTEIN, isolated from virus grown in the laboratory, has been studied as a vaccine. The protein successfully induced *B* lymphocytes to make antibodies that recognized the Env protein (*left panel*). Further, the antibodies prevented laboratory-grown HIV from infecting cultured cells (*a*), perhaps by blocking binding to cell-surface receptors or by enhancing elimination of the virus. Disappointingly, though, those antibodies have not been able to bar infection by virus isolated directly from patients (*b*).

the long run, the most effective HIV vaccines may well be the ones that stimulate both the humoral and cellular arms of the immune system, generating antibodies and activated cytotoxic *T* cells.

Efforts to design an HIV vaccine that maximizes production of antibodies or stimulation of cytotoxic *T* cells have been hampered by a lack of basic knowledge about how the immune system functions. Until investigators can learn how to induce the body to generate and maintain memory cells and cytotoxic *T* cells, those attempting to develop HIV vaccines will have to rely on a certain amount of trial and error, hoping to hit on an approach that will work.

The Antibody Approach

Vaccines that stimulate the production of protective antibodies have proved successful for combating diseases such as poliomyelitis, measles and influenza. At present, the most extensively tested HIV vaccine candidates contain some part of the envelope protein (Env), the molecule that coats the surface of the virus. Because the virus uses Env as a kind of key for gaining entry to human cells, generating antibodies that attach to the business end of this protein should prevent HIV from binding to and infecting cells.

The Env protein, also called gp160, is actually an association of two units: gp120, a sugar-shrouded protein that juts out of the virus membrane and interacts with receptors on the surface of human *T* lymphocytes, and gp41, the small protein that anchors gp120 to the membrane. Both gp120 and gp160 have been tested as HIV vaccine candidates in human volunteers.

In tests, the proteins elicited the production of antibodies, a result that raised hopes that they might form the basis for an effective HIV vaccine. Further, the resulting antibodies effectively neutralized live HIV in a test tube, blocking its ability to infect cultured human lymphocytes.

Unfortunately, the antibodies only recognized strains of HIV that were similar to those used to generate the vaccines. The gp120 and gp160 proteins in the preparations were made from HIV strains that had been cultivated in the laboratory. The antibodies elicited against proteins from such lab-adapted virus strains were ineffective at neutralizing HIV strains isolated directly from infected patients; the isolates were quite able to infect cultured cells.

Why did the antibodies fail to neutralize the HIV obtained directly from patients? The structure of the Env protein in laboratory-grown strains appears to be somewhat looser than that of the surface protein in patient isolates; those in isolates are folded tightly. Antibodies to laboratory strains of HIV may recognize parts of the Env protein that are not normally exposed in viruses from patients, probably because the recognition sites are buried within the more folded form of the protein. Antibodies to laboratory-grown virus, then, would not "see" their targets on HIV isolated from patients.

Researchers are currently developing vaccines based on surface proteins prepared from patient isolates. Such preparations may present Env in the conformation found in patients. Yet even these vaccines may not work. The Env protein on such isolates may be very densely packed and highly camouflaged by sugars. As a result, *B* cells may be unable to find many antigens and so may produce relatively few kinds of antibodies. Such an outcome would be consistent with the finding that people who are infected with HIV generally produce a limited repertoire of antibodies that react with the surface of HIV.

When Env binds to a cell, the protein changes its shape somewhat. A vaccine that duplicates the conformation adopted by gp120 as it attaches to receptors on the cell surface may succeed best at raising antibodies able to block HIV from infecting human cells.

Individuals who are infected with HIV but remain healthy and keep viral replication in check may offer some hope for guiding the design of an effective HIV vaccine. Some of these long-term survivors make a very small amount of antibody, which, when isolated, can neutralize HIV from patient isolates. Further, those antibodies can neutralize viruses from many different patient isolates—a necessity for an AIDS vaccine that will be effective against a broad spectrum of HIV strains. Unfortunately, even these antibodies may not be the whole answer. Tests of cells in culture indicate that the antibodies must be present at surprisingly high concentrations to block HIV entry into cells effectively.

Pure protein vaccines may not be the

best way to stimulate antibody production: in isolation, gp120 does not appear to have a precise conformation, and gp160 clumps into an ineffective aggregate. To get around these difficulties, researchers are currently testing two different vaccine strategies designed to present the Env proteins in a more natural conformation.

One plan of attack involves using whole, killed virus particles. This disabled form of HIV, incapable of multiplying, might present the immune system with more natural forms of Env proteins. With a better target, *B* cells might produce a better quality and a higher quantity of protective antibody.

Making a killed-virus vaccine requires a rigorous inactivation procedure, because residual virus and even residual viral genetic material could potentially be dangerous. Harsh treatment makes the vaccine less effective, however; the inactivation process can cause HIV to shed its weakly attached gp120. Many researchers have therefore been moving away from this design, although the gp120 stability problem may ultimately be solvable.

Env proteins can also be presented to the immune system embedded in "pseudovirions," artificial structures that resemble virus particles. These empty lipid shells could be made to carry nothing but gp160. Pseudovirions would be safer than whole, killed virus, because they lack the genes that could propagate HIV infection. Unfortunately, pseudovirions are very difficult to manufacture and produce in a stable form. Researchers hope, however, to have sturdier versions ready for safety trials in humans shortly.

Recruiting Cytotoxic *T* Cells

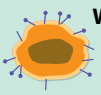
Different vaccine strategies are required to generate activated cytotoxic *T* lymphocytes. Although surface proteins or even whole, killed virus particles can elicit antibody production, they are poor stimulants of cellular immunity. Cytotoxic *T* cells recognize short pieces of foreign protein that appear on the surface of an infected cell. Infected immune cells generate these antigenic peptides as they digest samplings of viral proteins—surface proteins such as Env as well as the internal proteins that drive viral reproduction and assembly. A carrier protein then escorts the protein fragments to the cell membrane, where they are displayed on the outside of the cell.

For an HIV vaccine to stimulate cell-based immunity, it must direct selected cells to synthesize and display one or more peptides from the proteins normally made by the virus. These cells would trick the body into mounting an immune response against all cells displaying the viral peptides, including ones truly invaded by HIV.

The Sabin vaccine against polio, which consists of a live poliovirus, turns out to evoke cytotoxic *T* cell activity against polio-infected cells, yet it does not cause polio, because the virus has been weakened in the laboratory by certain genetic mutations. So far, though, no mutations have been identified that will transform HIV into a vaccine that will be completely safe.

Investigators are, however, developing other methods for inducing cells to produce and display HIV proteins. One approach, construction of a so-called live vector vaccine, takes advantage of

Vaccine Strategies under Study

Vaccine Constituents	Status	Advantages	Disadvantages
Vaccines Eliciting Anti-HIV Antibodies			
 **Viral surface proteins,** such as gp120	In phase I and II trials, which examine safety	Safe and simple to prepare	Vaccine-elicited antibodies have failed to recognize HIV from patients
 **Whole, killed HIV**	Not under study in humans	Should present HIV surface proteins in a relatively natural conformation; simple to prepare	Slight risk that preparations might include some active virus; inactivated virus might shed its proteins and become ineffective
 **Pseudovirions** (artificial viruses)	Close to phase I trials	Present HIV surface proteins in a relatively natural conformation	Difficult to produce and to ensure long-term stability
Vaccines Eliciting Cellular Responses			
 **Live vector viruses** (non-HIV viruses engineered to carry genes encoding HIV proteins)	In phase II trials	Makers can control amount and kinds of viral proteins produced	Complicated to prepare; current vaccines elicit modest immune response
 **Naked DNA** containing one or more HIV genes	In phase I trials	Simple and inexpensive to prepare	Some worry that integration of HIV genes into human cells could harm patients
 **HIV peptides** (protein fragments)	In phase I trials	Simple to prepare	Do not elicit strong immune response
Vaccines Eliciting Antibody and Cellular Responses			
 **Combinations of elements,** such as pure gp120 protein plus canarypox vector	In phase II trials	Should stimulate both arms of the immune response at once	Complicated to prepare
 **Live, attenuated HIV**	Not under study in humans; being assessed in nonhuman primates	Most closely mimics HIV; may interfere with infectious HIV's ability to replicate	Virus could potentially cause disease

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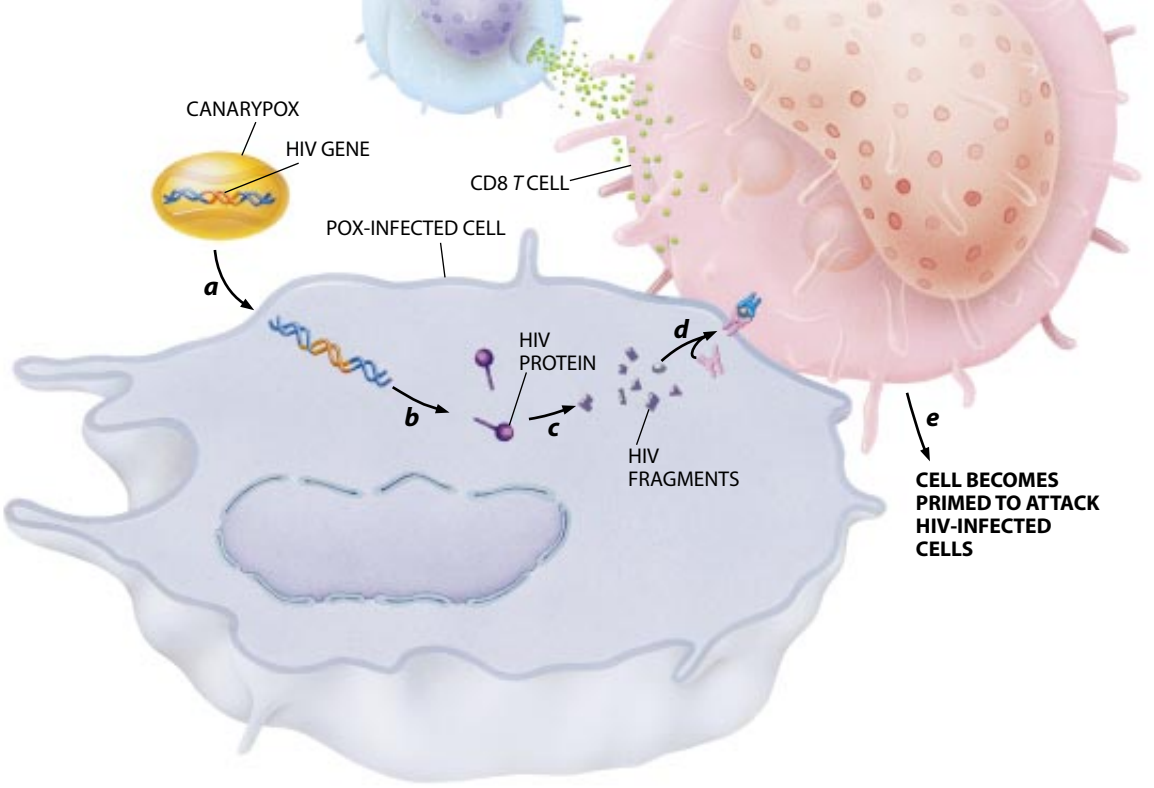

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RECOMBINANT CANARYPOX VACCINE is among those being studied as a way to elicit cell-based immunity against HIV. Such vaccines deliver HIV genes to human cells (*a*). The viral genes are translated into proteins (*b*), which are subsequently digested into fragments (*c*) and displayed on the cell surface (*d*). These fragments stimulate HIV-specific cytotoxic, or CD8, *T* lymphocytes, thereby priming them to kill any cells that may actually be infected with HIV (*e*).

the ability of different viruses to invade cells. Researchers insert selected HIV genes into a virus that is not harmful and then allow the benign virus, or vector, to deliver the DNA to cells in the body. Because genes are the blueprints for proteins, the infected cells will produce HIV proteins. These viral proteins are then chopped and shipped to the cell surface, where they can attract the attention of wandering cytotoxic *T* lymphocytes. The *T* cells, in turn, should multiply in response to the antigenic stimulation and stand ready to kill any cells that actually become infected with HIV.

The most extensively tested live vector vaccines are based on the canarypox virus. This nonpathogenic relative of the smallpox virus enters human cells but is incapable of assembling new viral particles. Researchers have engineered canarypox viruses to deliver the genes that direct the production of Env and gp120 and a variety of nonsurface HIV proteins, such as Gag (the core protein) and protease.

To date, the canarypox vaccines tested in humans have proved safe and have elicited modest cytotoxic *T* cell–based immune responses. To stimulate a more vigorous immune response, researchers are developing viruses that will produce greater quantities or varieties of HIV proteins inside infected cells. Administering multiple doses of these vaccines may help generate and maintain high numbers of activated cytotoxic *T* cells.

Other researchers are looking into administering viral peptides—fragments of viral proteins—to induce an immune response. Because antigenic peptides derived from viral proteins activate cytotoxic *T* lymphocytes, perhaps peptides would work as a vaccine. Unfortunately, peptides by themselves do not elicit a strong immune response, cellular or antibody-based, in humans. The peptides may be degraded before they reach the target cells, or they may not be presented efficiently by the cells that encounter them. Peptide vaccines may benefit from the development of better adjuvants, materials delivered along with a vaccine that induce the immune system to respond more strongly.

A rather novel approach to eliciting a cellular immune response involves injecting "naked" HIV DNA—genetic material with no proteins or lipids to deliver or protect it. At one time, scientists believed that naked DNA would be degraded too rapidly to be effective as a vaccine. In reality, the DNA does get into cells and can direct the production of viral proteins. In animal studies in mice and nonhuman primates, DNA vaccines have successfully generated cytotoxic *T* lymphocytes that recognize HIV proteins. In some but not all experiments, the DNA vaccine protected animals from subsequent infection with HIV. Further studies in animals and humans are evaluating the safety and effectiveness of this approach.

Combination Strategies

The most effective strategies—and the ones that are furthest along in human testing—incorporate elements that will stimulate both arms of the immune response. For example, a patient might receive a vaccine containing a canarypox virus carrying the *Env* gene to stimulate cellular immunity. Months later the same patient might receive pure gp120 to elicit the generation of antibodies. This combination strategy is called a prime boost, because the canarypox vector primes the cytotoxic *T* cells, and the gp120 protein then strengthens, or boosts, the immune response by eliciting antibody production.

Early trials have demonstrated that humans vaccinated using such a combination strategy develop both humoral and cellular immunity. But the antibodies generated have been against laboratory-adapted HIV strains, and the cytotoxic *T* cell response has not been strong. The next generation of combination vaccines will use canarypox viruses that carry

more HIV genes capable of producing greater quantities of viral protein, and the boost may contain gp120 proteins made from HIV isolated from patients. Such vaccines are being produced and may soon be ready for testing in humans.

Many researchers also continue to look into developing a live, attenuated HIV vaccine. Because such a vaccine would closely mimic active HIV, it should theoretically be effective at inducing cellular immunity, antibody-based immunity and perhaps other unknown modes of protection. By systematically deleting genes critical for HIV replication, scientists hope to develop a variant of the virus that can elicit a strong immune response without giving rise to AIDS.

Recently a group of physicians volunteered to participate in the first clinical trial of a live, attenuated HIV vaccine. Such a protocol would allow researchers to monitor the volunteers' immune responses and study the long-term safety of the vaccine. The physician volunteers believe the value of testing this approach outweighs the potential risks to their health. Their plan remains highly controversial, and we and many other researchers feel that attenuated HIV viruses should be more fully investigated in nonhuman primates before any movement into human trials.

Monkeys and AIDS

Vaccines based on a live, attenuated simian immunodeficiency virus (SIV)—a relative of HIV that infects monkeys—have been tested in macaques and other nonhuman primates. Monkeys infected with pathogenic strains of SIV will develop an AIDS-like syndrome. By studying this monkey model, scientists are able to test live, attenuated vaccines for their safety and their ability to protect animals when they are challenged by subsequent exposure to pathogenic strains of SIV. Several different attenuated SIV vaccines have proved remarkably effective at suppressing the growth of a wild-type virus.

The basis of this immunity in macaques is unclear: animals that are effectively protected from SIV challenge do not necessarily have high levels of neutralizing antibodies or activated cytotoxic *T* lymphocytes. The protective effects may be a consequence of some combination of antibody, helper *T* cell and cytotoxic *T* cell activity, or the effects may derive from other aspects of immunity. Further work is needed to determine exactly how the SIV vaccines manage to confer protection.

Although initial studies suggested a high degree of safety for the live, attenuated SIV, extended and expanded safety studies are beginning to show increased numbers of vaccinated animals progressing to AIDS-like syndromes, even in the absence of exposure to wild-type virus.

FRED WHITEHEAD *Animals Animals*

RHESUS MACAQUE is a type of monkey being examined in vaccine studies. Animals receiving a live, attenuated simian version of HIV have been able to limit subsequent infection by the natural monkey virus. But in a worrisome finding, the vaccine itself has eventually caused disease in some monkeys.

The studies are now starting to look at a greater number of animals, but the results suggest that live, attenuated vaccines may not provide full, long-term immunity and may even cause disease. The findings also imply that investigators should proceed with caution before testing such vaccines in humans.

Prognosis

If the immune system in HIV-infected individuals cannot wipe out the virus, why should a vaccine that activates the same immune responses be expected to block infection? Vaccines may give the body an immunological "head start" by priming the immune system to attack HIV as soon as it appears, rather than taking time to initiate a defense from scratch. By doing so, vaccine-induced immunity may succeed in containing the virus where the naturally infected body does not.

At present, however, there is no proof that vaccination against HIV is possible, because no protective vaccine candidate has yet moved into Phase III trials, which are large-scale tests designed to evaluate effectiveness in humans. In addition, the wide genetic variability of HIV may reduce the utility of any vaccine under development, because HIV strains isolated from patients in different parts of the world have distinctly different structures in their Env and, to a lesser extent, other proteins. Whether these differences, or additional ones we have yet to appreciate, will significantly hamper vaccine development remains uncertain.

But there is hope. As the pathogenesis of HIV infection has become better understood, investigators have realized that if the virus can be kept at low concentrations in the blood, an infected person may never progress to AIDS. This insight is encouraging because it suggests that even a partially effective vaccine could be valuable in limiting the amount of virus in patients, thus potentially reducing their infectiousness and the symptoms they suffer.

It is unlikely that we will develop a vaccine suitable for wide-scale use in humans within the next five years. Even if the prime-boost combination approach appears to stimulate cellular immunity and generate good broad-spectrum antibodies, large clinical trials will still be needed to demonstrate its value. Those trials alone will take several years. In the meantime, researchers will continue to pursue every approach that might help the immune system combat HIV. SA

The Authors

DAVID BALTIMORE and CAROLE HEILMAN work together on the National Institutes of Health's AIDS Vaccine Research Committee, a group charged with reassessing the priorities of the vaccine initiative and identifying new and innovative areas of vaccine research. Baltimore, chairman of the committee since its formation in 1996, is president of the California Institute of Technology. Heilman, a microbiologist, is deputy director of the Division of AIDS at the National Institute of Allergy and Infectious Diseases in Bethesda, Md.