Strategies for an AIDS Vaccine

In their attempts to develop a vaccine against AIDS, many scientists focus on raising antibodies to a protein from the AIDS virus, a strategy that some researchers think may require modification

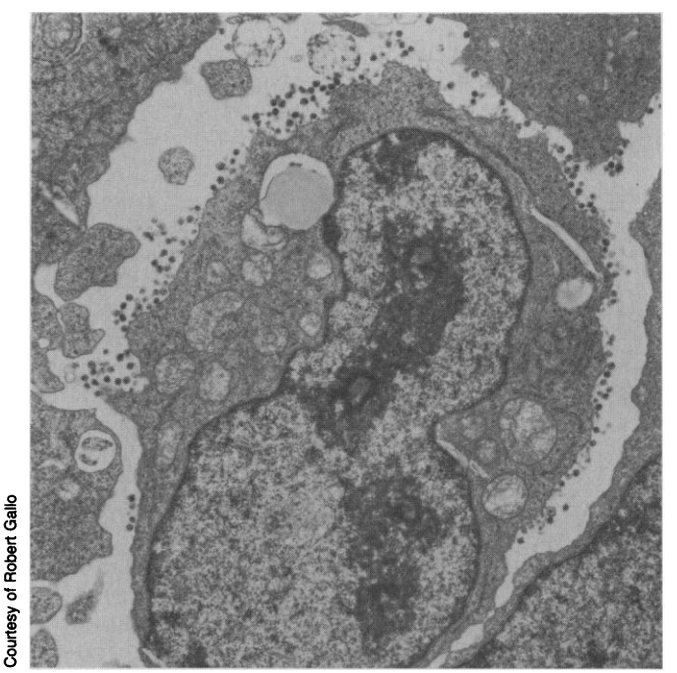
An estimated 1 to 1.5 million people in the United States alone are infected with the virus that causes acquired immune deficiency syndrome (AIDS), a number that continues to grow. The public is eager for an AIDS vaccine and scientists are working furiously to produce one, but it will not happen overnight. "We're at the beginning of this. We're at stage one and it's a multistep process," says Dani Bolognesi of Duke University.

Because of a number of factors peculiar to the AIDS virus itself and the disease context as a whole, the process toward developing a vaccine faces unusually difficult challenges in both technical and social realms.

One of the stumbling blocks is that scientists hesitate to use the traditional approach to vaccine production involving whole virus preparations. Vaccines for rabies, polio, and measles, for example, contain the whole virus, used in a weakened or killed form. But many researchers shy away from this strategy for an AIDS vaccine, because they fear it may cause the disease they are trying to prevent or make the natural disease worse.

Therefore, many researchers focus on developing an AIDS vaccine by using discrete protein subunits of the virus, which are less likely to cause disease. Because of its position on the exterior of the virus, the envelope is likely to be very immunogenic and is also an accessible target for attack by antibodies in an immunized animal. To date, such an approach has been successful with hepatitis B and influenza viral vaccines.

One possible drawback to a subunit approach for an AIDS vaccine is that a single viral protein, such as the envelope, may fail to stimulate an adequate antibody response because the immune system "sees" the appropriate antigens less effectively than in the whole, intact virus. A second potential problem is that a combination of antigens, rather than a single protein or peptide, may be required to elicit a protective immune response. A third concern is that a subunit vaccine might be strain-specific and protect against only one strain of the rapidly mutating virus rather than against multiple strains. Researchers have collected several hundred isolates of the AIDS virus, and it is likely that most AIDS patients will have been exposed to more than one strain.



Courtesy of Robert Gallo

AIDS virus particles *(small dark dots) erupting from the surface of an infected cell in tissue culture.*

Even if scientists develop a vaccine that conveys protection in humans, it is not at all clear that biotechnology companies will feel secure that they will be able to make it a commercial success. The reason is partly the real prospect of lawsuits in the event of adverse reactions (*Science*, 5 September, p. 1035), and partly the uncertainty that anyone other than those in the high-risk groups will seek such a vaccine, which might make the potential market uneconomically small.

Currently, several laboratories are in the process of developing potential subunit vaccines for AIDS that are based on various experimental approaches. Each strategy has advantages and disadvantages and most potential vaccines either are being or shortly will be tested in animals. Some scientists believe that the perceived sense of urgency in the research community may be pushing certain aspects of the work ahead prematurely (see box).

The concept for vaccine development from viral subunits may seem simple, but the process is not. Researchers are now struggling to identify which viral protein, or piece of protein, or combination of proteins, will make the best antigen and elicit a protective immune response. This is a large and complex research effort, which is bringing many laboratories together in close collaboration.

Researchers are using several different methods within the subunit approach for an AIDS vaccine. One involves genetic engineering of some cell type—mammalian cells, bacteria, or yeast—to induce the expression of AIDS viral protein. In a related technique, the gene for the AIDS virus envelope protein is inserted into a second, non-AIDS, virus, which then becomes a vehicle for producing the required protein. A third method involves the chemical synthesis of small peptide segments of an AIDS protein. A fourth is to isolate and purify protein from whole virus. And a fifth separate technique is to produce an anti-idiotype vaccine, which may offer certain unique advantages.

Using the first approach, Laurence Lasky, Phillip Bermann, and their colleagues at Genentech in South San Francisco recently

induced a mammalian cell line to synthesize AIDS virus envelope protein. Unlike bacteria, mammalian cells can synthesize viral proteins complete with their normal sugar residues. The Genentech recombinant glycoprotein, gp130, is slightly larger than the native AIDS virus gp120 envelope glycoprotein, and it induces antibody formation in test animals.

Jerry Groopman and his colleagues at New Deaconess Hospital in Boston showed that some of the antibodies to gp130 from Genentech's test animals prevent replication of the AIDS virus in a cell culture system. Nearly all researchers agree that this in vitro test of an antibody's neutralization strength is one that should be performed early in the course of vaccine development.

A key requirement for a useful vaccine is that it should stimulate antibody production against as wide a spectrum of virus strains as possible. Robin Weiss and his colleagues at the Chester Beatty Institute of Cancer Research in London have just shown that antibodies from the Genentech test animals neutralize to some extent two American and one British isolate of the AIDS virus. The antibodies do not prevent replication of a Haitian, African, or Californian isolate, making them somewhat strain-specific.

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Flossie Wong-Staal *of the National Cancer Institute. "If you can induce the antibody response before infection by the natural [AIDS] virus, you might be protected."*

William Haseltine *of the Dana-Farber Cancer Institute. "The envelope glycoprotein is a good target for a vaccine, but not the way it is naturally presented."*

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Genentech scientists are reluctant to discuss their current experiments, but it is fairly clear that they have injected chimpanzees with gp130 to see if these animals will develop neutralizing antibodies to the AIDS virus. Although the AIDS virus infects and multiplies in chimpanzees, these animals usually do not develop clinical symptoms. Regardless, they are still the best animal model for AIDS. If the chimpanzees immunized with Genentech's protein produce neutralizing antibodies, they will then be challenged with the natural virus to see if they are protected from infection.

Using a different system from Genentech's, but also a recombinant approach, Robert Gallo, Flossie Wong-Staal, and Marjorie Robert-Guroff, all of the National Cancer Institute (NCI), and a visiting scientist, Kai Krohn of the University of Tampere in Finland, in collaboration with Scott Putney and other scientists from RepliGen in Cambridge, Massachusetts, have preliminary evidence that envelope proteins lacking the natural sugar residues may be effective vaccine immunogens.

"Since last Christmas, we have been making several parts of gp120 in *E. coli* bacteria," says Putney. "Then, working with the NCI group as well as with Bolognesi and Tom Matthews at Duke University, we found that when these peptides are injected into goats, they induce antibodies that neutralize the AIDS virus. But the proteins that *E. coli* make have no sugars. It means that sugars are not necessary for a neutralizing response."

Whether the envelope proteins produced by *E. coli* will generate an immune response that protects against infection by the natural AIDS virus remains to be determined. Gallo and Putney say they would like to begin immunizing chimpanzees with the recombinant peptide to find out.

Working independently, two groups of scientists have tried a second approach toward developing a subunit vaccine. Bernard Moss and Sekhar Chakrabarti, at the National Institute of Allergy and Infectious Diseases (NIAID), in collaboration with Gallo's NCI group, and Shiu-Lok Hu and Steve Kosowski of Oncogen in Seattle, Washington, in collaboration with Joel Dalrymple of U.S. Army Medical Research Institute for Infectious Diseases in Frederick, Maryland, genetically engineered the vaccinia virus to produce AIDS virus envelope protein. "We put the entire envelope gene from the AIDS virus into vaccinia, the virus used in the smallpox vaccine," says Moss.

Recombinant vaccinia viruses produce envelope proteins that are very similar to the naturally occurring glycosylated proteins from the AIDS virus when vaccinia is grown in cultured cell lines. But despite this chemical similarity, the vaccinia proteins fail to induce very high levels of antibody production in mice. Moss believes that higher antibody levels are going to be necessary for neutralization and says he is now "modifying the envelope gene that is inserted into the vaccinia genome to make the protein product more immunogenic."

Although many scientists believe that a vaccine for AIDS will be based at least partly on the envelope glycoprotein of the virus, they do not agree what the chemical composition of the immunogen should be. Lasky and his Genentech co-workers try to mimic the naturally occurring envelope protein as much as possible. Gallo, Putney, and their collaborators think an envelope protein without its natural sugar residues may do the trick. Max Essex of the Harvard School

The Challenge of Testing Potential AIDS Vaccines

Given that chimpanzees are the only animals other than man that can be infected with the AIDS virus, it is inevitable that many researchers will want chimpanzees for testing possible AIDS vaccines. Some scientists are already injecting chimpanzees with what they hope will be a future vaccine for AIDS in people, but others look at the limited supply of chimpanzees with a sense of panic and concern. They fear that existing preliminary tests for potential vaccines are inadequate and that screening in chimpanzees may be both wasteful and premature.

But the chimpanzee shortage is only part of the problem. Because scientists are still learning what components of the immune response are necessary for protection against AIDS, it is not clear what the appropriate preclinical tests for an AIDS vaccine are. And when a potential vaccine is finally ready for clinical trials in humans, another series of questions arises.

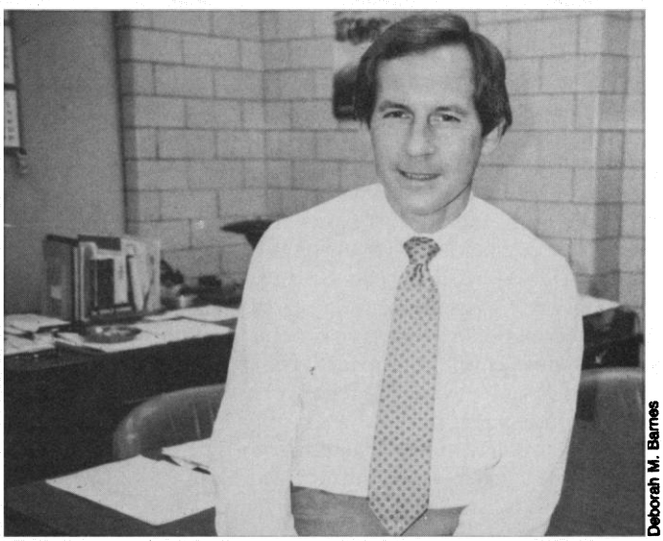
Researchers who gathered at a recent workshop on AIDS vaccine development* raised the following issues, some of which are already being addressed:

■ **In vitro tests**. A potential vaccine should be able to induce reasonably high levels of specific antibodies in test animals (including mice, guinea pigs, and rabbits) that can block replication of the AIDS virus in cultured cells. Unfortunately, there is no existing test for measuring exactly how much virus is present and for quantitating the effectiveness of vaccine-induced antibodies. The most commonly used in vitro tests for virus neutralization are indirect, one of which measures enzyme activity to indicate that the virus is replicating. All scientists agree that a direct in vitro test to quantitate the amount of virus is critical.

■ **Animal models**. Animals other than chimpanzees are now used for two major purposes in AIDS vaccine research: to see if a potential vaccine generates a good antibody response, and to study the natural history of retrovirus-induced diseases that resemble AIDS in some respects. The critical issue with the latter concerns how closely these animal models for AIDS—in mice, cats, goats, sheep, and horses—parallel the human disease and whether studying them is relevant in terms of vaccine development.

Perhaps the nonchimpanzee model that most closely resembles human AIDS is infection of monkeys with STLV-III, simian T-lymphotropic virus type III. STLV-III naturally infects African Green monkeys without causing disease, but in captive rhesus macaque monkeys, it produces an immune deficiency disease similar in many respects to human AIDS. Max Essex, Phyllis Kanki, and their colleagues at the Harvard School of Public Health are currently trying to sort out why the same virus affects two monkey species so differently, with the hope that it will lead to an understanding of which immune responses are necessary to protect man from the similar AIDS virus.

■ **Chimpanzee testing**. Chimpanzees are an endangered species, and there are 1600 or so in the United States. Of these, over 300 are being considered for AIDS research, according to George Galasso, who heads a Public Health Service animal model committee formed last year to handle the chimpanzee demand in AIDS research. To date, about 80 chimpanzees have been allocated for AIDS-related studies, about 25 of which are for vaccine screening. Anticipating a continuing demand, NIH leaders voted this spring to allocate $4.5 million of discretionary funds to the Division of Research Resources at NIH to organize breeding facilities for chimpanzees that will be used in AIDS research.



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Gerald Quinnan, *of the Food and Drug Administration, will review any potential AIDS vaccines.*

Any potential vaccine for AIDS will have to be tested in chimpanzees before it can be given to humans. Gerald Quinnan of the Food and Drug Administration says that effectively vaccinated chimpanzees should make high levels of antibodies that neutralize the AIDS virus in vitro, that the vaccine itself should have no adverse side effects, and that it should produce a "durable immunity" when chimpanzees are challenged with the real AIDS virus.

■ **Clinical testing in humans**. An AIDS vaccine will be designed for people who are not already infected with the real virus, and people participating in any clinical tests will need to be screened for evidence of prior infection. Quinnan says that the first volunteers should include "people who are at some risk of getting AIDS," although not at so high a risk that they might become infected with the real virus during the course of the study. These initial volunteers will probably be followed for years and evaluated for any toxic side effects to the vaccine as well as their immune responses to it. During this phase of testing, the doses and spacing between doses will also be worked out.

Human clinical trials raise two critical issues: which immune responses are necessary for protection against AIDS, and should an effective vaccine prevent infection or actual disease. Many scientists, including Anthony Fauci of the National Institute of Allergy and Infectious Diseases, think that an effective vaccine will need to induce cellular immunity as well as the production of neutralizing antibodies. Although testing for cell-mediated cytotoxicity is technically complex, Fauci thinks it should be evaluated in at least some of the people in clinical trials.

The best AIDS vaccine would prevent infection from the natural virus for the lifetime of the individual, but in the absence of the ideal, there is no general agreement whether prevention of disease would be considered an acceptable outcome in vaccinated individuals. ■ **D.M.B.**

*Workshop at NIH on the development of an AIDS vaccine, 28 and 29 July 1986, sponsored by the National Institute of Allergy and Infectious Diseases.

of Public Health considers that researchers need to spend more time trying to identify what specific region, or epitope, of the envelope triggers an appropriate immune response. And William Haseltine of the Dana-Farber Cancer Institute in Boston suggests that any vaccine that depends on the natural structure of the envelope protein is likely to fail.

"In my opinion, far too much has been made out of quick and obvious experiments for an AIDS vaccine. I am not saying they shouldn't be done, but the likelihood is that they won't work," says Essex, who regards potential vaccines based on naturally occurring glycoprotein envelope proteins as being in the "quick and obvious" category. He prefers to concentrate on the natural history of the AIDS virus and a close viral relative to understand why some people infected with the AIDS virus resist disease and why some AIDS-like viruses do not cause disease.

Essex believes that, by analyzing antibodies from AIDS patients, it might be possible to identify precisely which region of the natural envelope glycoprotein produces the best immune response during natural infection. This region of the protein might then be the best candidate for making a subunit protein vaccine.

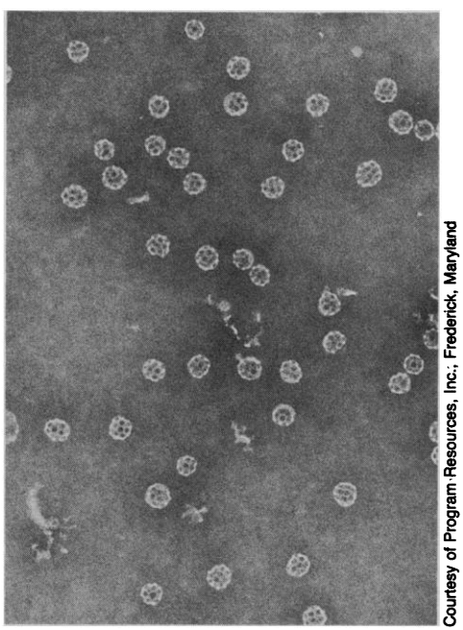
Haseltine shares Essex's suspicion that a vaccine based on an intact envelope protein resembling the natural one is likely to be ineffective. Haseltine's point is that people infected with the AIDS virus make neutralizing antibodies against envelope proteins, but they still get sick. Why, he reasons, should a vaccine that induces antibodies similar to those an AIDS patient makes protect someone against disease?

Haseltine says that a vaccine must be able to block at least one of two reactions in order to be effective—viral binding to a cell and membrane fusion reactions in infected cells. "These reactions are mediated by the envelope glycoprotein," he says. "Therefore the envelope glycoprotein is a good target for a vaccine, but not the way it is naturally presented." Haseltine thinks it will be necessary to modify the envelope protein in a vaccine so that the immune response in a vaccinated person will be directed toward some part of the molecule that is not normally accessible as an antigenic stimulus.

Wong-Staal disagrees. "It's a matter of timing," she says. "The level of neutralizing antibody in a person infected with the AIDS virus does not go up until the virus has already established itself. But if you can induce the antibody response before infection by the natural virus, you might be protected."

Prem Sarin and his NCI colleagues and Allan Goldstein and Paul Naylor of George Washington University recently reported a third approach to a subunit vaccine for AIDS by using a core protein of the AIDS virus rather than its envelope protein as an immunogen. They showed that antibodies against a naturally occurring peptide from the thymus gland, thymosin α_1 weakly neutralize the AIDS virus in vitro. The peptide from the thymus is somewhat similar in its amino acid composition to a small peptide segment of a protein associated with the inner core of the AIDS virus. Sarin and his colleagues therefore speculate that the neutralizing activity of their antibody to anti-thymosin α_1 is due to the chemical similarity of these two peptides.

The rest of the scientific community, including Gallo, has been less than enthusiastic about Sarin and Goldstein's results. Nevertheless, Gallo now says that "as time goes by, their results cannot be ignored." Sarin is currently making a synthetic peptide based on the short segment (that is partly homologous to thymosin α_1) from the AIDS virus core protein, p17, and has preliminary evidence that antibodies raised in rabbits neutralize the AIDS virus in vitro. But the critical question of whether an animal immunized with any synthetic peptide will be protected from the natural AIDS virus remains completely unanswered.



Courtesy of Program Resources, Inc.; Frederick, Maryland

ISCOMS. *Rosettes of protein and lipid that can be manipulated to incorporate other proteins, such as the envelope protein from the AIDS virus.*

Peter Fischinger and Gerard Robey of NCI, Bolognesi at Duke, and other collaborators at Program Resources, Inc., in Frederick, Maryland, and Litton Bionetics, Inc., in Kensington, Maryland, are working with a fourth subunit approach to a vaccine. The group uses natural gp120 envelope glycoprotein made by cells infected with the AIDS virus as a potential vaccine antigen.

To date, the group has immunized goats, horses, and rhesus monkeys with native gp120, which stimulates the animals to make neutralizing antibodies. Robey now says that the horse antibodies neutralize the AIDS virus best and indicates that their strength "seems comparable to Genentech's antibodies against recombinant gp130 protein." But he acknowledges that "no one has really high neutralizing antibody titers," an aspect of the research he finds "distressing." Now the group is injecting chimpanzees with purified gp120, and plans to study the complete immune response in these animals before challenging them with the real virus.

Fischinger, Robey, and their colleagues, as well as Bror Morein of the Royal Veterinary College Biomedical Center in Uppsala, Sweden, and his co-workers, are also trying to display gp120 in a "natural" way on the surface of ISCOMS. ISCOMS are tiny artificial rosettes made of protein and lipid that can be chemically manipulated to include other proteins, such as the AIDS virus envelope. The idea is to use ISCOMS to mimic how natural virus particles present envelope protein and then see if the antibody response in test animals is stronger. But getting gp120 to incorporate into ISCOMS is technically difficult, a problem both groups are trying to work out.

Thus, there are both technical as well as biological difficulties surrounding the subunit approach to an AIDS vaccine. Perhaps the most overwhelming question is whether or not the subunit itself—the protein or peptide antigen in the vaccine—will induce the formation of antibodies that protect an immunized individual against the natural AIDS virus.

Hilary Koprowski and his colleagues at the Wistar Institute in Philadelphia are working on a fifth approach to an AIDS vaccine, one based on an anti-idiotype. In the first step, they use a protein from the AIDS virus and induce animals to make antibodies to it. (This is the point at which the other vaccine strategies begin testing.) But the Wistar technique involves two more generations of antibodies. They immunize mice with the first antibody, Ab1, to induce the animals to make a second antibody, Ab2, whose binding site resembles the original antigen from the AIDS virus. This second antibody is thus termed an anti-idiotype. Because an anti-idiotype vaccine contains no antigens from the actual virus, it should be safe.

The Wistar scientists then use Ab2 as an antigen to induce animals to make the third

generation of antibodies, Ab3, which should bind to the original AIDS virus protein. It is the Ab3 antibodies that the researchers hope will protect immunized people from the natural AIDS virus. Unlike other subunit approaches to vaccine, the anti-idiotype technique could be used effectively in young infants, who are unable to respond to the viral antigen. But to date no licensed vaccine for a human virus is based on an anti-idiotype.

The more traditional approach to making a vaccine, using a whole virus preparation as the immunogen, has obvious advantages because it gives the body a stimulus very similar to the immunogenic stimulus that occurs with natural infection, and may therefore be more likely to generate a strong and protective immunity. But using whole virus, even an inactivated preparation, still opens the possibility of disease.

"If you are going to start a vaccine program based on whole virus, it would be better to use a virus that is completely compromised," says Amanda Fisher. Fisher, Wong-Staal, Gallo, Mary Harper, Samuel Broder, and Hiroaki Mitsuya, all of NCI, in collaboration with Lee Ratner of Washington University in St. Louis and Lisa Marselle at Biotech Research Laboratories in Rockville, Maryland, have just reported a variant of the AIDS virus that infects cells but does not kill them efficiently. Thus, an inactivated version of this mutant might make a good vaccine immunogen because it would be much less likely to cause disease. This approach is still very much in the idea stage.

Essex recently described an AIDS-like virus, HTLV-IV, which infects people but does not seem to cause disease. Unlike the artificially engineered viral variant reported by the NCI group, HTLV-IV is a natural virus that infects people in West African countries. Essex, Phyllis Kanki, also of Harvard, Francis Barin of the University of Tours in France, and Souleyman M'Boup of the University of Dakar Medical School in Senegal are trying to understand why people infected with a virus so similar to the AIDS virus seem to remain healthy.

At about the same time, Luc Montagnier and his colleagues at the Pasteur Institute in Paris isolated an AIDS-like virus, LAV-2, from people who were sick with AIDS-like symptoms. Whether or not HTLV-IV and LAV-2 are the same virus is still unclear. If they are, it needs to be explained why some people become ill and others do not. If the viruses are different, characterizing them further may give researchers some idea of what parts of the virus are necessary to cause disease.

The AIDS virus poses certain special problems for demonstrating that an AIDS vaccine will be effective in people, one of which relates to how the virus is transmitted. When someone becomes infected with the AIDS virus—which most frequently occurs through sexual contact with an already infected person, by sharing a contaminated needle during intravenous drug use, or through contaminated blood or blood products—it is not clear in which form the virus is transmitted. At least some is probably passed as free virus particles, but many researchers think that virus-infected cells are also involved in person-to-person or blood transmission.

This, then, is the problem. Assume that an uninfected but vaccinated person has sex with someone who carries the AIDS virus. Will that person be protected against cells—probably infected lymphocytes or macrophages in semen or blood—that carry the AIDS virus in a dormant state? Will a vaccine be able to stimulate other kinds of cellular immunity, particularly cell-mediated cytotoxicity reactions, that may be required for killing virus-infected cells after they begin to express viral proteins? No one knows for sure, and it may not be possible to address these issues until a vaccine is ready for testing in humans.

Anthony Fauci of NIAID advocates continuing careful study of how the body naturally responds to infection by the AIDS virus with the hope that it will lead to clearer strategies for vaccine development. "We need to delineate, as precisely as possible, what the nature of an effective immune response is against this virus," he says.

The virus that causes AIDS is a retrovirus, and it must make DNA copies of its own RNA genome before it can reproduce itself inside a host cell. It can exist for a long time in a dormant DNA state until some stimulus triggers it to reproduce inside the cell. At this point the cell becomes a virus factory and ultimately dies. "We know very little about man's protection against a retrovirus," says Fauci. So far, although vaccines against retroviruses have been successfully developed for certain animal diseases, none has been made for humans.

Once a vaccine—of whatever nature—is developed, there follows a long process of testing, with two goals in mind: namely, demonstrating that the vaccine is both effective and safe in humans. At best, no vaccine is likely to be available for general use until some time in the 1990's. ■

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Anthony Fauci, *director of the National Institute of Allergy and Infectious Diseases. "We need to delineate, as precisely as possible, what the nature of an effective immune response is against this [AIDS] virus."*

Deborah M. Barnes

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