Obstacles to an AIDS Vaccine

Human tests of AIDS vaccines proceed as researchers cope with setbacks from chimpanzee trials and grapple with fundamental questions about the virus

THE development of a safe and effective vaccine for AIDS is proving to be a formidable task. "I don't know how a virus could be any worse in terms of trying to design a vaccine," says Larry Arthur of the National Cancer Institute in Frederick, Maryland. The AIDS virus hides in cells, it mutates rapidly, and it survives despite many immune responses that would normally rid the body of an invading virus. But public pressure to develop a vaccine is intense and the scientific kudos for success would be enormous.

Within the past 18 months, researchers have begun human tests of four candidate AIDS vaccines. During the same time period, researchers published data showing that experimental vaccines have not protected chimpanzees from infection. Whether humans will respond as the animals did is still an open question. And as the clinical tests proceed, scientists are still searching for the ingredients that will constitute a safe and effective vaccine for AIDS.

A persistent dilemma confounds the research. People infected with the live AIDS virus develop antibodies that inactivate it in laboratory tests, yet they become sick and die anyway. This leaves researchers in the position of trying to design a vaccine without knowing what kinds of immune responses will protect a person from infection with the AIDS virus, now called human immunodeficiency virus type 1 (HIV-1).

The uncertainty has prompted some researchers to question whether a protein from HIV or the entire virus should be used in a vaccine. Others think that vaccines can be designed to treat people already infected with HIV and two such preparations are currently being tested in humans.

Nevertheless, a unifying strategy has been to make a vaccine that stimulates the production of antibodies against the envelope protein that surrounds HIV. Theoretically, these antibodies should prevent virus particles from infecting cells if a person is exposed to HIV. The approach worked with the polio, measles, and hepatitis B vaccines, but it is not working for AIDS, at least so far. HIV is elusive because it can be transmitted in infected cells as well as by free particles. Thus, researchers must design a vaccine that also triggers the immune system to destroy HIV-infected cells.

Even the laboratory tests for evaluating the effectiveness of antibodies produced in response to a vaccine may need to be revised and standardized. Current tests are based on the assumption that HIV infects T lymphocytes in vivo, which it does, but new information indicates that the virus may infect monocytes and macrophages first. This may mean that antibody tests using only T cells are inadequate. But the overwhelming problem here is that antibodies that can neutralize the virus in laboratory tests may not do so in a patient.

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The paradox calls into question what immune responses, if any, will protect a person from infection with HIV: some researchers suggest that antibodies alone will not be adequate and that cell-mediated immune responses may also be required. The latter are directed by T lymphocytes and may rid the body of virus-infected cells.

Thus, many researchers view the problems of developing an AIDS vaccine as unprecedented. "I think right now we are sort of at point zero," says Maurice Hilleman, of Merck Sharp & Dohme in West Point, Pennsylvania. Like most researchers, Hilleman thinks that an AIDS vaccine should protect an immunized person from infection with HIV or at least limit infection. He believes that it will have to stimulate both neutralizing antibodies and cell-mediated immunity, but is skeptical that either will kill infected cells that are not actively producing virus.

Perhaps the failure of experimental vaccines to stimulate both neutralizing antibodies and cell-mediated immunity in chimpanzees explains why the animals were not protected from infection, says Jorg Eichberg of the Southwest Foundation for Biomedical Research in San Antonio, Texas. He has collaborated in trials of four different candidate vaccines in chimpanzees, none of which was effective. Two were able to generate cell-mediated responses and one, a collaboration with Alfred Prince of the New York City Blood Center in New York and others, involved the use of strong neutralizing antibodies, pooled and concentrated from the plasma of HIV-infected people. Still, the animals became infected. "The hope I still have is to put it all together and get both kinds of immune responses in the same animal," says Eichberg. "Perhaps that will be protective, but I am not optimistic."

New results just reported by Daniel Zagury of the Pierre and Marie Curie Institute in Paris and his co-workers in France, Zaire, and the United States may address this issue. They show that multiple vaccine preparations based on the HIV envelope and a vaccinia virus that is genetically engineered to make it, can elicit neutralizing antibodies and cell-mediated responses against two isolates of HIV. In addition to the primary vaccine, Zagury also injected himself with his own T cells, after they had been infected with the recombinant virus and chemically fixed, and with two boosting doses of envelope protein. But the protocol was tested in only one person—Zagury himself—who does not know whether he is protected from infection by live HIV. Zagury and collaborator Robert Gallo of the National Cancer Institute (NCI) say the procedure is far too complex for large-scale studies.

Several other research teams are dissecting the molecular architecture of the outer part of the HIV envelope, a 120,000-dalton glycoprotein (gp120), to determine which part or parts of it should be included in a vaccine preparation. Many molecules of gp120 coat the surface of HIV and the protein has several functions critical to the life cycle of the virus.

One problem with the approach may be that crucial parts of the protein are hidden. "Many of the regions of gp120 are not accessible to the immune system," says William Haseltine of the Dana-Farber Cancer Institute in Boston, Massachusetts. "They are either covered up by sugars or in recesses

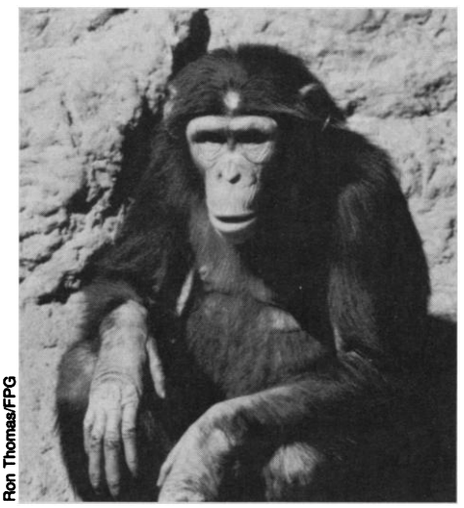
of the molecule." So antibodies directed against those regions would not bind and disrupt their functions, he says. "But if you make an antibody that pokes its finger into the CD4 binding region, or that disrupts the subunits, or interferes with cell fusion, then you could prevent infection of cells."

Other groups also focus on ways to generate antibodies that block the functions of gp120. Larry Lasky of Genentech in South San Francisco and his colleagues at Harvard Medical School in Boston and the Chester Beatty Laboratories in London, for instance, have new data suggesting that antibodies against either end of the gp120 molecule or against its sugar residues may prevent HIV binding to cells. But Lasky cautions that the attraction between gp120 and its CD4 receptor is a strong one that antibodies may not be able to disrupt. Working independently, Scott Putney and his collaborators at Repligen in Cambridge, Massachusetts, NCI, and Duke University, have recently shown that RP-135, a fragment of gp120, is important for viral neutralization. Antibodies against the fragment prevent HIV-infected cells from fusing with uninfected cells, but do not block viral binding.

Using RP-135 as a vaccine immunogen presents a specific problem, however. The region of the gp120 gene that codes for it mutates very rapidly. A more general problem is that the *env* gene that codes for gp120 contains many such hypervariable regions. In terms of vaccine development, this means that antibodies against any variable region of gp120 will probably not protect against infection by other viral isolates.

Perhaps, as Putney suggests, the problem can be addressed by using a "cocktail" of RP-135 fragments from different viral isolates in a vaccine. Or perhaps a conserved part of gp120 can be exploited, as David Ho of the University of California at Los Angeles School of Medicine, Mark Gurney of the University of Chicago in Illinois, and their co-workers propose. They find that antibodies against a central region of gp120 can neutralize multiple isolates of HIV.

A novel approach is to base a vaccine on the more constant p17 core protein of the AIDS virus. Typically, core proteins are inaccessible to antibody attack because they are sequestered in the virus particle, but Allan Goldstein of George Washington University Medical Center in Washington, D.C., and others claim that parts of p17 may be on the surface as well. Goldstein, Prem Sarin of NCI, and their co-workers in collaboration with Viral Technologies, Inc., in Washington have developed an experimental vaccine based on HGP-30, a synthetic peptide from p17, and have just received permission from the British Riverside Health Authority to begin testing it in England. Brian Gazzard of St. Stephen's Hospital in Fulham will head the trials, which are planned to begin this summer in HIV-infected and -uninfected volunteers. The researchers have not received approval from the Food and Drug Administration to test the product in the United States.



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Chimpanzee. *Studies show that experimental vaccines have not protected against infection.*

While some researchers seek ways to avoid the problem of viral variation, others question its significance. "A doctor will tell you that AIDS is AIDS; it doesn't matter what isolate of the virus a patient is infected with," says Haseltine. "I think the problems for HIV vaccination are formidable, but they are not due to viral variation." Gallo and Flossie Wong-Staal, also of NCI, disagree, noting that genetic variations of HIV, even within one isolate, may result in different biological properties that affect the ability of the virus to cause disease or escape antibody attack. Meanwhile, Gerald Myers of Los Alamos National Laboratory in New Mexico argues that, should experimental vaccines be tested in Africa, preparations based on American and European isolates of HIV-1 may not protect Africans from the isolates to which they are exposed.

Despite these potential problems, two different vaccine preparations in addition to Zagury's, which are based on a form of the HIV envelope, are currently being tested in humans. All contain viruses that have been genetically engineered to make HIV gp160, a larger molecule that includes gp120. These vaccines are in the first phase of testing, which measures the safety of the vaccine and immune responses to it. It is still too early to tell if any of them will protect a person from HIV infection.

Anthony Fauci and Clifford Lane of the National Institute of Allergy and Infectious Diseases (NIAID) and Franklin Volvovitz of MicroGeneSys in West Haven, Connecticut, and their collaborators are testing a vaccine that contains genetically engineered baculovirus, an insect virus. The initial NIH-based trial began last year with FDA approval and has been expanded to include six centers outside NIH. Several volunteers have developed antibodies against gp160 and no one has experienced adverse side effects.

Another experimental vaccine, which has failed to protect chimpanzees against HIV infection, according to a recent report by Shiu-Lok Hu and George Todaro of Oncogen in Seattle, Washington, and their colleagues, is being tested in humans anyway. "We think it is important to get human data in an FDA-approved trial," says Todaro. Lawrence Corey and Ann Collier of the University of Washington in Seattle are heading the clinical tests, which include about 30 volunteers.

Some researchers think that any vaccine based on only one protein from HIV will probably not elicit a protective immune response. "Perhaps we can use a replicating virus that does not cause disease," suggests Malcolm Martin of NIAID. While such an approach may induce strong immune responses, Martin acknowledges that it may not be an acceptable alternative for HIV because of the danger of causing disease.

One way to determine which immune responses are likely to protect against HIV infection is to study the natural history of the disease in people. New data along these lines suggest that certain cell-mediated responses may keep the AIDS virus in check after it has infected a person.

Another approach is to examine why vaccinated and subsequently infected chimpanzees often have stronger immune responses to HIV than do animals receiving vaccine alone. Antibody levels are higher in the infected animals and can neutralize several strains of HIV in laboratory tests—not just the strain used to make the vaccine, according to Peter Fischinger of the Public Health Service. This suggests that their gp120-based vaccine served as a priming stimulus and the live virus potentiated the initial immune response, says Larry Arthur of NCI who coauthored the study. In terms of testing vaccines, multiple doses and more time between doses may be required to elicit broad-based immune responses in chimpanzees and in humans.

Dani Bolognesi of Duke University Medical Center in Durham, North Carolina, emphasizes that a vaccine must also be presented or packaged in the right way to be effective. Researchers still have not found the best adjuvant, a chemical substance that

boosts the immune response to a viral protein antigen, he says. The problem is especially important for vaccines that are based on a single viral protein, because one protein typically does not stimulate strong immune responses if used alone.

Most vaccine researchers are looking for ways to induce immune responses that will protect an individual from infection. But some, including the groups led by Zagury, Goldstein, and Jonas Salk of the Salk Institute in La Jolla, California, are experimenting with vaccines in people already infected with HIV. Salk, Brian Henderson, and Alexandra Levine of the University of Southern California Medical School are currently testing a therapeutic vaccine that contains killed whole AIDS virus as an immunogenic agent. Fourteen HIV-infected people with signs of disease have received it and as yet, no dramatic effects, beneficial or adverse, have been observed in anyone.

As researchers pursue different strategies for developing an AIDS vaccine, the lack of a good animal model in which to screen the vaccines becomes more serious. Only a limited number of chimpanzees are available for AIDS research, a problem addressed at a recent conference sponsored by the World Health Organization in Geneva. "There was a consensus that simian immunodeficiency virus (SIV) infection in macaques is an excellent model for testing both prototype vaccines and drugs," says Patricia Fultz, of the Yerkes Primate Center in Atlanta, Georgia. Monkeys infected with SIV experience immune suppression and become highly susceptible to various infections. In addition, the virus itself has about 40% sequence similarity to HIV-1.

Many research groups, in addition to those mentioned here, are working to create a vaccine for AIDS and some are attempting novel approaches. Scientists have known since they began to work on an AIDS vaccine that it would not be easy, but perhaps no one realized it would be so difficult. Hilleman notes that it took 13 years to develop a vaccine for hepatitis B. "It certainly is going to take as long to develop a vaccine for AIDS," he says. ■

DEBORAH M. BARNES

ADDITIONAL READING

J. Salk, "Prospects for the control of AIDS by immunizing seropositive individuals," *Nature* **327**, 473 (1987).

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J. W. Eichberg *et al.*, "T-cell responses to human immunodeficiency virus (HIV) and its recombinant antigens in HIV-infected chimpanzees," *J. Virol.* **61**, 3804 (1987).

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Mathematics at 100

A snowflake, whooping cough, the superconducting supercollider, water passing under a bridge, and the price of a gallon of gas—what do they have in common? They were all part of a gallop through "Mathematics in the Sciences," a National Academy of Sciences (NAS) forum held last week to commemorate the centennial of the American Mathematical Society. With so eclectic a fare on offer, everyone there—on stage and off—was uninitiated in something, but all were united in the "power and beauty" of mathematics, as David Gross of Princeton University put it. The forum was designed "to demonstrate the large-scale involvement of math in the working sciences," said Felix Browder of Rutgers University, "even though it goes against the prejudices of many—both scientists and mathematicians."

Gross blithely explained how experimental particle physics lay some 17 orders of magnitude distant from the "who knows what" point to which String Theory leads. Put on a log scale it becomes "only a factor of 40." The superconducting supercollider would close the gap some, but not much. Nevertheless, "without the SSC, particle physics will die." Leo Kadanoff of the University of Chicago gave a lesson in the meaning of *chutzpah*, by following up a display of Leonardo's drawings of turbulence in water with his own. Chaos was his topic of course, and he showed how fine is the line between stability and chaos, and how there is chaos in order and order in chaos.

Robert May, a theoretical ecologist at Princeton University, could not match Leonardo sketches, but showed slides of stained glass windows in a church in Sussex instead. Population dynamics was his topic—of birds, and insects, and pathogenic organisms. "Chaos explains sex, not the weather," he said, which struck a chord. In the natural world, deterministic events can give the illusion of chaos, and chaos the illusion of determinism, and you have to distinguish between them. "Math is a way of thinking clearly, no more, no less." Herbert Scarf of Yale University needed no introduction as an economist when he said that the model he was about to describe bore no relation to the real world, but would illustrate his point. It did. Systems as huge and apparently chaotic as the national economy can be modeled with deceptively simple, but elegant, math. And Benoit Mandelbrot of IBM's Thomas J. Watson Research Center, Yorktown Heights, New York, showed once again that the world is made of bits and pieces—fascinating bits and pieces that continue to astonish, no matter how many times you see the phenomenon: namely, fractals.

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Dirac once said, "I'm not interested in proofs, only what nature does." And, it seems, mathematicians are inexorably drawn to nature, not just describing what is to be found there, but in creating echoes of natural laws. Mathematicians do not just "think of things out of nothing," said Gross, citing a famous conversation; "they are discovering what is real and natural." It is for this reason that so often math—to a mathematician—seems beautiful. "If math is about structures that are part of the real world, it is not surprising it is a powerful tool, not surprising that what we find as beautiful are those things that match the real world. Our minds have evolved to find this pleasing." ■ **ROGER LEWIN**