The development of a safe, effective preventive vaccine for human immunodeficiency virus (HIV) infection remains an area of vigorous research. Several highly innovative vaccine candidates are being developed, and more than 13 vaccine candidates have been tested in human phase I or II trials. All have produced antibody and several have produced modest neutralizing titers, but to date no reproducible evidence has suggested prolonged, high-titer neutralization across a diversity of HIV strains. Furthermore, only the live recombinant vector approaches have produced some evidence of cytotoxic T-cell responses. The principal obstacle to progress is the lack of definitive information on what constitutes a protective immune response. There is no animal model for HIV-induced disease. Hence, the identification of the correlates of immunity and more useful animal models is among the highest priorities for HIV vaccine research. Large-scale efficacy trials raise daunting scientific, ethical, and resource issues. Nonetheless, preparation in such trials is underway in order to be in a position to evaluate the most promising vaccine candidate.


Dr. Daniel F. Hoth (formerly, Division of AIDS, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH], Bethesda, Maryland): The worldwide human immunodeficiency virus (HIV) epidemic continues to grow. In the United States, it is estimated that one new HIV infection develops every 9 or 10 minutes (1). The Centers for Disease Control and Prevention (CDC) estimates that some 1 million HIV infections have developed in the United States. In addition, by the end of 1993, 361,164 cases of the acquired immunodeficiency syndrome (AIDS) and 220,736 AIDS-related deaths had been reported in the United States (CDC Quarterly Surveillance Report hotline, available by calling 404-332-4570). The CDC projects that by the end of 1994 there will be 415,000 to 535,000 cumulative AIDS cases and 320,000 to 385,000 AIDS-related deaths.

Even more devastating are projections by the World Health Organization’s (WHO) Global Programme on AIDS (2), which estimates that as of 1992, at least 8 to 10 million adults were infected with HIV and about 1 million children were born with it. The WHO conservatively projects that by the year 2000, 30 to 40 million people will develop HIV infection, more than 90% of them in developing countries. Epidemiologists from the Harvard School of Public Health (3) project even higher numbers than those of the WHO, especially for Southeast Asia and Africa.

The need for intervention is clear. The public health strategy for preventing and controlling HIV infection depends on changing human behavior to reduce illicit drug injection, needle sharing (4), and the number of unprotected sexual encounters and sexual partners (5); protecting the blood supply; expanding the diagnosis and treatment of other sexually transmitted diseases (6); achieving optimal prenatal care for pregnant HIV-infected women; and improving the availability of and compliance with the types of antiviral chemotherapy that reduce seminal viral burden and probably infectiousness (7). In impoverished developing nations, preventive measures remain largely unavailable, unaffordable, or unpopular (7). Changes in human sexual behavior are possible but may require intense intervention that may not be practical or affordable in all risk settings (8). An affordable, available HIV vaccine remains the single most important long-term goal of prevention research (9-11) to supplement expanded education on HIV infection and sexually transmitted diseases, condom marketing, sexually transmitted disease control, prevention and treatment of injection drug use, and development of new barriers to HIV acquisition (12-14).

The human immunodeficiency virus is unlike other viruses that have been successfully prevented by vaccination. Several factors favor control of HIV by vaccination: The virus is limited to a human host with no known animal reservoir; it is not highly infectious; and natural infection generates both antibody and T-cell responses. However, HIV also has many characteristics that make vaccine development difficult: subclinical cases and a carrier state, a long-term infection process, a nonspecific acute clinical disease, significant antigenic variation, viral DNA integration into the host genome, transmission through infected cells, and destruction or alteration of immunoregulatory cell function. For many patients, the first recognizable clinical manifestations of HIV are opportunistic infections that are secondary to immunodeficiency and that may develop years after initial infection. Despite these daunting scientific challenges, significant progress has been made in preclinical and clinical research in the worldwide effort to develop an effective HIV vaccine. We present a brief review for medical readers unfamiliar with HIV vaccinology.

Preclinical Vaccine Research

Dr. Dani P. Bolognesi (Center for AIDS Research, Duke University, Durham, North Carolina): Contempo-
rary viral vaccines usually work by imitating natural immune responses to pathogens to eventually clear the infection (15). Most licensed viral vaccines are based on either attenuated forms of the virus or whole inactivated preparations of the agents. A notable exception is the recombinant hepatitis B vaccine, but this also was designed to mimic a natural protective immune response (16).

Because there are no established correlates of protection and no established natural protective immunity to HIV infection, the use of attenuated or whole inactivated virus vaccines is less certain as a strategy. Moreover, these approaches raise obvious safety concerns. Further, HIV presents unprecedented obstacles for vaccine developers because it can be transmitted as a cell-associated virus across mucosal surfaces, it can hide from immune attack by establishing latent infections, and it can escape from existing immunity by generating several antigenic variants. Sequestration of the virus into immunoprivileged sites such as the central nervous system or certain lymphoid organs also appears to be an important feature of pathogenesis (17).

Attenuated and whole inactivated vaccine products, along with many recombinant DNA, protein, and peptide approaches, have been studied in various animal models of HIV infection, including HIV itself and related viruses such as simian immunodeficiency virus or feline immunodeficiency virus. The results present a complex picture. Some studies with attenuated and whole virus vaccines have shown promise in simian immunodeficiency virus and feline immunodeficiency virus models, whereas recombinant approaches have been far less effective. However, when studied in the chimpanzee model of HIV infection, recombinant subunit vaccines involving parts of the HIV envelope have effectively protected animals from infection, whereas whole inactivated virus preparations were ineffective (18). Although more direct comparisons of each of the vaccine strategies should bring this picture into sharper focus, there are several possible reasons for the disparate results.

The most important of these are differences among the animal models. Infection with feline immunodeficiency virus and simian immunodeficiency virus leads to disease in cats or monkeys, respectively, whereas HIV infection of chimpanzees does not. This difference may be due, at least in part, to the "vigor" with which the infection is established and thereby the potency of the vaccine required for protection. It would appear that human HIV infection associated with a long disease-free interval may represent a middle ground between the rapid disease induced by some feline immunodeficiency virus and simian immunodeficiency virus strains and the indolent HIV infection of chimpanzees. It has been suggested that infection of monkeys with simian immunodeficiency virus strains that induce a slower onset of disease may be more relevant than any model now widely used in HIV research. It is interesting to note that recombinant approaches have proved effective in at least one experiment that used the slower simian immunodeficiency virus model (19).

The reasons for the failure of whole inactivated virus vaccine to protect chimpanzees (attenuated vaccines have not been widely tested) are not entirely clear (20). One possible explanation relates to the role of human cellular antigens associated with killed virus preparations used for vaccination and virus challenge stocks. Studies have recently shown that such antigens play a major role in protection (21). Similar antigens may play a lesser role in chimpanzee models, but this has not been tested directly (22).

When a short-term model for simian immunodeficiency virus infection and disease is used, the attenuated virus vaccine has provided the most impressive protection (23). Previous infection (about 2 years earlier) with a genetically modified virus that replicates poorly in monkeys and does not cause disease allowed animals to resist challenge with a relatively large dose of highly pathogenic simian immunodeficiency virus (23). It should be possible to take full advantage of this model to derive insights on correlates of protection. Because this approach involves infection with a retrovirus, studies should include potential nonimmune correlates, such as production of inhibitory cytokines or other factors as well as the phenomenon of in vivo viral interference, to achieve the broadest scope in a search for protection correlates.

Vaccine manufacturers have relied on two general factors in selecting candidates for development: safety considerations and results in chimpanzees who received candidate vaccines that were based on HIV itself. Consequently, manufacturers have largely shied away from inactivated or live attenuated vaccines and have relied on studies in chimpanzees in which vaccines based on various configurations of the HIV envelope could protect against infection. These were feasibility studies only, however, and did not examine the duration and breadth of protection or the efficacy of vaccines against various modes of virus transmission.

Several features of the HIV envelope are considered potentially troublesome. Studies in vitro indicate that the native envelope can induce T-cell anergy and even death by apoptosis if certain conditions are met (24, 25). Regions of molecular mimicry with normal-cell surface antigens, notably major histocompatibility complex (MHC) gene products, could induce autoantibodies (26). Such properties suggest that the HIV envelope might be immunosuppressive and possibly pathogenic. Fortunately, considerable experience with more than 1600 persons not infected with HIV who received envelope-based vaccines has not shown evidence of immunosuppression. Nonetheless, the appearance of autoantibodies, antibodies that might enhance infection, or more subtle T-cell defects should continually be carefully monitored in vaccine trials.

The absence of guiding principles for immune correlates of protection notwithstanding, challenges that remain for vaccine developers would seem to lie in establishing approaches that can induce long-term immunologic memory in each of several immune compartments (for example, humoral, cellular, and secretory). To date, most approaches have favored inducing neutralizing antibodies rather than emphasizing how to induce cellular or secretory immunity. More information on the latter is clearly desirable. How a single immunogen that effectively induces all of these responses could be designed is difficult to envisage, but combinations of vaccines tailored to a specific compartment should be useful.

Ultimately, it may be that the only effective means of
Table 1. Types of HIV Vaccines and Their Current Clinical Status*

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Testing Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated vaccines</td>
<td>None</td>
</tr>
<tr>
<td>Inactivated whole-virus vaccines</td>
<td>None</td>
</tr>
<tr>
<td>Recombinant subunit proteins</td>
<td></td>
</tr>
<tr>
<td>HIV envelope proteins</td>
<td></td>
</tr>
<tr>
<td>gp160—baculovirus vectorialum</td>
<td>Phase II trials complete</td>
</tr>
<tr>
<td>gp160 vaccinia—infected Vero cells</td>
<td>Phase 1 trials</td>
</tr>
<tr>
<td>gp120 CHO cells (IIB and MN strains)—alum adjuvant</td>
<td>Phase II trials</td>
</tr>
<tr>
<td>QS 21 adjuvant</td>
<td>Phase 1 trials</td>
</tr>
<tr>
<td>gp130—CHO cells, SF-2 strain in MF-59 adjuvant</td>
<td>Phase II trials</td>
</tr>
<tr>
<td>gp120—yeast, SF-2 strain-MF-59, and MF-59 MTP adjuvant</td>
<td>Phase II trials</td>
</tr>
<tr>
<td>Fusogenic envelope peptides</td>
<td>Phase 1 trials</td>
</tr>
<tr>
<td>gp120—“cocktails”</td>
<td>Phase 1 trials</td>
</tr>
<tr>
<td>gp24—T4 particles</td>
<td>Phase 1 trials</td>
</tr>
<tr>
<td>Envelope-striped particles— incomplete Freund’s adjuvant</td>
<td>Phase II trials testing only seropositive persons</td>
</tr>
<tr>
<td>Modified live-virus vectors†</td>
<td>Phase II trials</td>
</tr>
<tr>
<td>Vaccinia/gp160 recombinant</td>
<td>Phase I trials</td>
</tr>
<tr>
<td>Vaccinia (vaccagp10) construct</td>
<td>Phase I trials</td>
</tr>
<tr>
<td>Avipoxvirus (gp160)</td>
<td>Phase I trials</td>
</tr>
<tr>
<td>Combination</td>
<td></td>
</tr>
<tr>
<td>vac/env-gp160 and recombinant gp160 or gp120 boost</td>
<td>Phase I trials</td>
</tr>
</tbody>
</table>

* CHO = Chinese hamster ovary; HIV = human immunodeficiency virus.
† Vectors under investigation for use as modified live-virus vaccines include vaccinia, Avipoxvirus, adenovirus, Rhinovirus, BCG, Salmonella, hepatitis B virus, and adenov-associated virus.

Preventing HIV infection are live attenuated virus vaccines or whole inactivated virus vaccines; efforts to make these approaches unequivocally safe should be put in place. In the meantime, preclinical strategies should be exploited to help us gain insights into correlates of protection—information that may allow us to use the safest possible means to achieve protection.

Phase I and Phase II Clinical Trials

Dr. Lawrence Corey (Virology Division, University of Washington, Seattle, Washington): One of the key issues in developing a strategy for HIV prevention is evaluating our current knowledge about the mechanisms of transmission and about protection from infection. It was understood from the outset that transmission could occur with cells infected with HIV (27, 28). It has also become evident that cell-free virus is present in plasma and genital secretions (29–32). Therefore, vaccine developers have sought immunogens or vaccine regimens that elicit both antibodies to bind infectious virions and host cellular immune responses to reduce the number of viral-infected cells.

Our knowledge of host protective immune responses to HIV-1 is limited. In fact, many researchers have asserted the view that effective protective host responses do not exist. Neutralizing antibodies are directed almost exclusively at the HIV envelope protein (33–37), which has several conserved and unique neutralizing domains. Neutralization to strain-specific isolates is detected soon after infection, and the neutralizing antibody response broadens over time (38, 39). However, the correlation between development of high titers of neutralizing antibodies and disease progression is limited at best (27, 39–41). Neutralization-resistant mutants appear to emerge weeks to months after infection, and it appears that they are probably inevitable in all patients (42–44). It is perhaps most disappointing to note that little direct correlation exists between containment of viral infection during acute HIV-1 infection and the development of neutralizing antibodies (45–48). The marked reduction in plasma viremia that occurs with acute infection develops before antiviral antibodies are noted, whether measured to V3 peptides, gp120, or neutralization of the autologous isolate (48). However, investigators at the National Cancer Institute have recently shown a correlation between high neutralizing activity and clinical progression in HIV-infected children (49), and other studies suggest similar findings. Thus, although high titers of neutralizing antibodies may be a reasonable marker of protective immunity to HIV-1, they are unlikely to be the sole protective correlate. Because of this, a successful HIV-1 vaccine should induce reasonable titer of neutralizing antibodies to the circulating phenotypes of viruses in the population.

Almost all HIV-infected persons tested early in the course of the disease have evidence of cytotoxic lymphocytes to the virus (48, 50). Cytotoxic lymphocyte activity has been shown for many structural and nonstructural gene products (50–52). In fact, it appears that in most series, cytotoxic lymphocyte response to gag, polymerase, and nef gene products is more plentiful than the response to envelope gene products, thereby raising the issue of whether concentration on envelope-based vaccines is wholly appropriate (53). Cytotoxic lymphocyte activity in the HIV envelope appears to vary in cell class and MHC restriction (54, 55). CD8+ T-cell lymphocytes from infected persons repress HIV growth in vitro, and the cytotoxic lymphocyte precursor cell frequency and activity decrease before clinical deterioration, although how much pathogenesis is caused by a decrease in CD8+ activity and how much is caused by a decrease in CD4+ cells themselves is unclear (27, 56). Cytotoxic lymphocytes specific for HIV-1 have been derived from seronegative donors by in vitro stimulation, which indicates the potential for eliciting such responses with an HIV immunogen (57).
At least 13 HIV-1 vaccines are currently being studied in human phase I trials (Table 1). All prophylactic vaccine trials to date have used subunits of HIV-1, and most have involved HIV-1 envelope proteins. Human immunodeficiency virus subunit peptides and vaccines using envelope and gag gene products entered clinical trials in 1993. Subunit HIV vaccines are safe and feasible to manufacture and may be able to present a protein or an epitope in a manner that is even more immunogenic than natural infection (58).

Currently, 2 gp160 vaccines, 2 gp120 products, 1 envelope yeast-derived protein, and 1 vaccinia gp160 recombinant vaccine have entered clinical trials in the United States, and a gag particle product has been studied in the United Kingdom. These vaccines are all safe and immunogenic. The first HIV subunit vaccine to enter human clinical trials was gp160, which is made in a baculovirus vector. This product has been extensively studied in a series of dose-escalating trials consisting of protein in amounts ranging from 40 mg to 1280 mg per dose in a series of 3- and 4-dose regimens (59). Antibodies to HIV-1 denatured proteins and viral-infected cells increased in frequency and titer in a dose-dependent manner. However, even with a vaccine regimen of 640 mg of protein given at 0, 1, 6, 12, and 18 months, antibody responses increase and then rapidly decrease; neutralizing antibodies are present in only 30% of volunteers (60). In addition, neutralizing titers are usually 100-fold lower than those seen in naturally infected persons, no fusion inhibition antibodies are present, and antibodies are restricted to the homologous virus types. (Fusion inhibition antibodies inhibit syncytia induced by a homologous fusogenic HIV strain.) Moreover, these titers are lower than those achieved with mammalian cell-derived gp120 vaccines, perhaps because the abnormal glycosylation of this gp160 protein limits conformationally derived epitopes to the HIV-1 IIIB strain. The question of whether a similarly produced gp160 product from an MN-like strain produces similar results is currently being studied.

Another gp160 immunogen is a purified vaccinia recombinant virus that expresses gp160 in Vero cells (61). This product binds CD4 more readily and is reported to be more "naturally" glycosylated than is the baculovirus protein. This vaccine is still in early development, but it appears to be safe and elicits antibodies to gp160 by Western blotting. However, at such low doses, neutralizing antibodies are not detected; until higher doses are administered, the overall immunogenicity of this product cannot be definitively evaluated.

Two gp120 subunit vaccines have been studied. Both are purified from mammalian (Chinese hamster ovary) cells. One is to a IIIB strain in an alum adjuvant and the other is to an SF-2 strain in a squalene adjuvant called MF59. Both gp120-derived proteins appear to elicit neutralizing antibodies. In a recent trial, 100- and 300-mg doses of the IIIB gp120 alum product were given in a 0-, 1-, and 6-month dosage regimen (62). Of the 10 volunteers who received 300 mg/dose, 90% had neutralizing antibodies at a titer of 1:53; fusion inhibition antibodies were seen in 70% (62). A similar immunogen has been derived from an MN prototype strain and is being studied with 300 mg and 600 mg of protein per dose. In addition, the first bivalent vaccine trials combining the IIIB and MN strains are under way.

In a separate phase I trial, the SF-2-strain-derived gp120, grown in mammalian (Chinese hamster ovary) cells, was administered with MF-59 adjuvant as three 50-mg doses. Neutralizing antibody titers to SF-2 virus were seen in 80% to 90% of recipients, with titers ranging from 1:30 to 1:50 after the third dose (62). A fourth dose of vaccine has been shown to further boost neutralizing antibody titers (64). In another study, a nonglycosylated, yeast-derived SF-2 product from the same adjuvant was evaluated. This vaccine elicited neutralizing and fusion inhibition antibodies in 30% of recipients, suggesting that nonglycosylated, non-V3 loop aspects of the SF-2 strain participate in viral neutralization (65).

Many investigators have studied whether vaccine serum samples can neutralize wild virus derived from persons who have recently seroconverted to HIV-1 in the United States, but no subunit vaccine has yet consistently elicited neutralizing antibodies that can inhibit HIV-1 among persons receiving vaccines from strains currently circulating in North America (66). T-cell responses to HIV-1 envelope vaccines have received limited study. All consistently elicit lymphoproliferative activity to envelope proteins; neither the envelope immunogens nor the p24 vaccine in an alum adjuvant elicit a detectable CD8+ cytotoxic lymphocyte response to HIV-1 envelope proteins.

Most recently, several cases of naturally acquired HIV infection have developed among recipients of various envelope vaccines, including persons who received gp120 immunogens to SF-2 and MN. Almost all of these persons acquired HIV infection before receiving a full course of immunizations. The development of naturally acquired HIV infection in vaccine recipients enrolled in clinical trials who have been strictly counseled about the risks for HIV acquisition underscores the public health importance of developing effective interventions against acquiring this pandemic infection.

As Dr. Bolognesi has noted, live virus vaccines have traditionally induced the best mucosal and cellular immune responses. The modified subunit protein in a live virus vector that has been most extensively evaluated to date is a recombinant vaccinia virus expression gp160 (IIIB strain, HIVAC-1e). Two separate phase I trials have been done, one involving vaccinia-naive persons and one including both naive and previously immune persons. The recombinant vaccine was shown to be genetically stable and easy to administer. After inoculation, a gauze pad covered by dressing has been shown to contain the recombinant virus at the skin site and to provide excellent containment of the virus from the environment (67-69). The stability and ease of administration of the recombinant form indicate its potential economic and practical utility. However, all recipients and their household contacts were screened for immunodeficiency before vaccination, and persons at risk for transmitting the virus to household contacts were not included. Such precautions are a research luxury that will not be feasible in field settings in which a licensed vaccine product is used. An attenuated strain of vaccinia was used, but disseminated vaccinia is a known complication among HIV-infected persons (70); adequate antiviral chemotherapy is currently unavailable. Further work is thus needed to develop a
Table 2. Core and Optimal Guidelines for Entry of HIV Vaccines into Efficacy Trials in Noninfected Volunteers*

<table>
<thead>
<tr>
<th>Core Guidelines</th>
<th>Optimal Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evidence of safety in phase I clinical trials</td>
<td>Evidence of safety in phase I clinical trials</td>
</tr>
<tr>
<td>Demonstrated efficacy of a given vaccine construct in HIV-infected chimpanzees</td>
<td>Protects animals in challenge studies from HIV infection or disease induced by cell-associated as well as free virus</td>
</tr>
<tr>
<td>or SIV-infected monkeys†</td>
<td>Protects against a broad spectrum of heterologous isolates</td>
</tr>
<tr>
<td>Elicits long-lasting neutralizing antibody that broadly reacts against</td>
<td>Protects against intravascular and mucosal challenge</td>
</tr>
<tr>
<td>heterologous isolates in phase I clinical trials; a candidate vaccine</td>
<td>Induces long-lasting immunity so that volunteer is protected when virus is administered months to years after immunization</td>
</tr>
<tr>
<td>would be more attractive if long-term and broadly reactive cellular immunity were also induced†</td>
<td>Shows induction of the likely correlates of immunity in phase I clinical trials</td>
</tr>
<tr>
<td>Shows immunologic or genetic similarity to HIV isolates from the proposed efficacy trial site†</td>
<td>Shows immunologic or genetic similarity to HIV isolates from the proposed efficacy trial site†</td>
</tr>
</tbody>
</table>

† For a candidate vaccine to enter an efficacy trial, at least two of the three criteria should be met in addition to safety.

safer modified vaccinia vector and an effective antiviral treatment for vaccinia virus.

Comparing the antibody and T-cell responses to the vaccinia virus recombinant protein with those of the recombinant soluble proteins is interesting. The vaccinia gp160 recombinant virus appears to elicit more durable T-cell responses than does the baculovirus-derived gp160 antigen, but the humoral antibody responses to vac/env appear poorer than the soluble recombinant protein (69). Because neither vaccine alone seemed to be ideal, a trial combining the two was initiated by first administering the vac/env construct to prime T-cell responses and then boosting with a soluble recombinant protein. This combination vaccine regimen produced a marked increase in antibody and T-cell responses to HIV envelope, and the responses were greater than those elicited with either vaccine alone. Although neither vaccine produced neutralizing antibodies alone, both vaccinia-naïve recipients showed neutralizing responses with the combination. In addition, for the first time, a CD8+ MHC-restricted cytotoxic lymphocyte response to HIV-1 envelope was shown in vaccine recipients; in one patient, this response remained for longer than 10 months (71).

Lymphocytes taken from persons who received the combination vaccine were adoptively transferred to SCID/Hu PBL mice, which were subsequently challenged with HIV (100 TCID50 of the IIIB virus). Partial protection was achieved but waned over time (72). It is noteworthy that in the few patients studied, mice were protected by serum samples from persons both with and without neutralizing antibodies to HIV-1; this finding suggests that cell-mediated responses were likely protective immune responses in this model.

This approach of combining subunit vaccines may also be useful for other modified live-virus vaccines such as vaccinia or avian pox vectors. The combined vaccine approach uses the unique attributes of both the modified subunit and recombinant protein approach. The disadvantage is that two types of vaccines must be developed; this is both a commercial and regulatory issue for industry.

In summary, the first generation of HIV-1 vaccines has entered clinical trials, and more than 1600 people have received a vaccine (73). The vaccines have been carefully evaluated and are safe and reasonably immunogenic. Immunogens have now been produced that can elicit neutralizing antibodies in levels approaching those seen in natural infection. The duration of these antibodies is being scrutinized to see if boosting and novel adjuvants will be needed to enhance the duration of the responses. Little information has been gathered on human mucosal responses to vaccination. T-cell responses, as measured by lymphoproliferation, are present, but they vary by immunogen and laboratory. There have been no CD8+ responses with the HIV-1 recombinant envelope proteins used as the sole immunogens. However, a combination vaccine approach that uses a modified subunit vaccine vector followed by a recombinant protein has elicited in some persons CD8+ cytotoxic lymphocytes to HIV-1 envelope.

Efficacy Trials

Dr. Sten H. Vermund (Departments of Epidemiology, Medicine, and Pediatrics, University of Alabama at Birmingham): More than a dozen HIV vaccine candidates are now being studied in phase I and II clinical trials (73), but no phase III vaccine efficacy trial has begun. To effectively forge consensus, criteria for vaccine selection have been developed by a working group convened by the Director of the National Institute of Allergy and Infectious Diseases in 1991 and again in 1992. Optimal criteria for vaccine selection present an idealized goal, whereas core criteria are meant to provide guidelines for identifying when the product would be worth testing in an efficacy trial (Table 2). Even before a candidate vaccine has been selected, several issues must be considered for large vaccine trials.

1. Efficacy trials must be designed to investigate suitable primary (HIV infection) and secondary (surrogate and clinical) end points.
2. Route of viral exposure must be assessed in trials, and sexually transmitted disease status should be considered.
3. Estimates for site selection, plans for population identification and recruitment, and sample size must maximize study success.
4. Behavioral research can assess the frequency of high-risk activity, willingness to participate in trials, and efforts that trial participants might make to unblind themselves to HIV testing.

5. Several ethical, social, and political issues must be addressed.

1. Trial end points. The goal of an HIV vaccine is to prevent infection in hematopoietic tissues and cells with both cell-free and cell-associated HIV. The suitable primary endpoint for measuring protection from infection is assessing the infection event itself in a placebo-controlled, double-blinded clinical vaccine trial. Vaccine-induced antibodies must be distinguished from wild virus–induced immune responses, preferably with field-appropriate technologies such as epitope-specific enzyme-linked immunosorbent assay (ELISA) (74). Western blot confirmation would be needed, and virologic measurements would confirm that no infection had occurred. However, viral vaccines generally work to prevent disease without preventing subclinical infection (75). Given that a disease prevention outcome would be of immeasurable significance, efficacy trials must accommodate the study of secondary endpoints in case the primary one proves disappointing. An example of a secondary endpoint includes a decrease in the CD4+ T-lymphocytic count at the time of acute infection, which on average decreases 30% to 40% from baseline within 2 years after infection (76). Thus, selective reduction of the loss of CD4+ cells during acute HIV infection in vaccine recipients might provide an early indication of a positive effect. Sample size calculations will depend in large part on the magnitude of the "protective effect" of vaccines on CD4+ cell decrease with acute infection, but it may be feasible to assess this endpoint with sample sizes similar to those outlined below for primary infection endpoints. The viral burden after infection (77) for vaccine and placebo recipients should also be evaluated for its utility as a secondary endpoint. Although clinical endpoints will require long-term follow-up, they are needed in the first trials because the surrogate immunologic and virologic markers are not validated in vaccine recipients.

2. Route of exposure and effect on sexually transmitted diseases. It is possible that a vaccine will work better against one route of exposure than another. If a product induces systemic immunity but little or no mucosal immunity, it could conceivably protect against a low-dose percutaneous exposure but fail to protect against mucosal exposure. (The hepatitis B virus is reassuring here in that a systemic induction of immune response still protects against disease from a mucosally mediated viral infection [78].) Conversely, a given vaccine product may induce an immunologic blockade that prevents low-level exposures from sexual encounters but may not protect from high-dose inocula such as those from injection drug use or transfusion with contaminated blood and blood products. Hence, trials may have to be done with adequate recruitment among persons exposed to varying modes and levels of risk. Vaccines useful to mothers and infants may differ from those most useful for parenteral or sexual exposure. The NIAID is sponsoring preparatory studies at 17 sites in 15 locations and 9 countries to assess the feasibility of large-scale vaccine efficacy studies involving thousands of volunteers (79).

Table 3. Sample Size Calculations for a Hypothetical Two-Arm, Placebo-controlled HIV Vaccine Efficacy Trial*

<table>
<thead>
<tr>
<th>Length of Trial (years)</th>
<th>Sample Size, n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Annual HIV Incidence Rate</td>
</tr>
<tr>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>2</td>
<td>13700</td>
</tr>
<tr>
<td>2.5</td>
<td>9600</td>
</tr>
<tr>
<td>3</td>
<td>7400</td>
</tr>
</tbody>
</table>

* HIV = human immunodeficiency virus. For a two-arm trial with 90% power to detect 50% efficacy, efficacy achieved gradually over a 6-month immunization period. Loss to follow-up is assumed to be 10% per year. Adapted with permission from references 79 and 87 and from Rida WN (personal communication).

Sexually transmitted infections, particularly genital ulcers, are associated with a risk for HIV-1, possibly because of the facilitation of contact between HIV-1 and a CD4+ cell, which is more prevalent and accessible in disrupted mucosa (friable or bleeding) than in epithelially intact mucosa (80, 81). Viruses transmitted through an intact epithelial mucosal surface may differ in quality or quantity from infection through a disrupted epithelial surface infected with a sexually transmitted virus. Nested studies of sexually transmitted disease should be considered within HIV-1 vaccine trials to better evaluate their use as cofactors for failure of protection (82).

3. Site selection and sample size calculations. The relevance of "matching" circulating virus and specific vaccine constructs may be a dominant factor in selecting sites for vaccine trials. However, it is just the first of several criteria for site selection; others include community and government support, ability to recruit and retain an adequate number of cooperative volunteers, costs, seroincidence in the study population, adequate laboratory and shipping capacity, adequate clinical evaluation facilities and expertise, ability to manage study data to meet the auditing requirements of the U.S. Food and Drug Administration (and other regulatory agencies outside the United States), and ability to secure informed consent. Several thousand volunteers will probably be needed for a simple two-arm trial (Table 3). Testing many candidates or planning for stratum-specific analyses (for example, by transmission risk, sexually transmitted disease status, or host genetics) would require even larger sample sizes. Trials for HIV vaccines in high-risk populations of young homosexual men, injection drug users, professional sex workers, and others will be among the greatest public health research challenges faced to date.

Given the complexities and costs of HIV vaccine trials, the AIDS Research and Advisory Committee of the NIAID has recommended delaying expanded HIV vaccine trials of the recombinant subunit products now in phase II studies until other products can be compared directly or until more promising data are forthcoming (for example, successful cross-neutralization of circulating wild virus by serum samples from vaccine recipients). In the judgment of the Committee and with the concurrence of the NIAID Director, the scientific rationale for these trials is not yet sufficiently compelling to justify their initiation this time (83). An alternative view, held by most members of a NIAID-sponsored Vaccine Working Group, argued that...
trials in which many high-risk vaccine recipients were compared with placebo recipients would help determine if the envelope subunit approach shows promise. It is certain that our collective scientific ignorance of a correlate of protective immunity will fuel this debate for years to come.

4. Behavioral research. If the HIV-1 vaccine candidates are less than optimally effective, behavior changes within the context of the trial could have a major effect on the interpretability of the results. For example, a volunteer could seek HIV testing to find out whether his or her ELISA result had converted to positive and whether the Western blot showed new bands. The result might be the unblinding of the volunteer. If volunteers who discover that they have received the vaccine are falsely reassured and then engage in high-risk behavior at a greater rate than do placebo recipients, a less-than-optimal vaccine candidate (one with an efficacy of 50% to 60%) may be judged completely ineffective because of selective high-risk behavior among decoded vaccine recipients (Sheon A. Unpublished data; 84, 85). The unblinding of a few vaccine trial participants has already occurred in phase I trials.

To discourage a false sense of security among vaccine recipients, proper education on the uncertainty of vaccine benefit and theoretical concerns of vaccine risk, such as enhancement of infection or disease progression, must be part of the enrollment and informed consent process (86). If incidence were to decrease substantially among the trial participants because of prevention messages, sample sizes or trial duration would have to increase to accommodate lower-than-expected incidence (Table 3) (87).

5. Ethical, social, and political issues. Ethical and sociopolitical concerns are compelling topics that can be only briefly reviewed here. Ethical considerations must be considered well before the trial begins. Given the socially disenfranchised status of many persons at high risk for HIV infection, exploitation of volunteers must not occur or be perceived to be occurring within participating communities. Informed consent may be difficult in the context of poverty and illiteracy. Because adolescents are at high risk in some environments (88, 89), they are suitable candidates for trial enrollment; however, they must not be manipulated, and an appropriate parental or guardian role must be established (90). Prisoners with a history of illicit drug use or high-risk sex may be available for vaccination while incarcerated and may revert to high-risk behavior when they are released from prison. They could benefit from enrollment in the vaccine trial, but the potential for coercion must be minimized (91). Active drug users must be recruited only when sober so that they can fully understand the nature of the study.

Persons at high risk may be especially concerned that their trial participation does not “label” them and lead to discrimination in the form of difficulty in securing employment or life and health insurance. Such consequences could result from having a positive HIV screening test result or even from participating in an efficacy trial that targets persons “at risk” for HIV infection. Phase I and II trials sponsored by the NIAID have provided an identity card for trial participants, a confirmatory toll-free telephone number that a volunteer can provide to a prospective employer or insurance company, and an explicit agreement with a wide cross-section of the insurance industry not to equate seropositivity with infection in the vaccine recipient without first securing follow-up specialized testing (Fast PE and Lawrence DN. Personal communications; 74, 92). As of August 1994, these protections have been applied to 1600 vaccinees. When thousands of high-risk volunteers are recruited, new agreements must be negotiated to maximize fair access to insurance for them.

A concern for both consumers and manufacturers is the liability for any untoward effects of a given vaccine. Within the research setting, safety cannot be guaranteed, a fact that must be made clear in the informed consent process. When a vaccine is licensed and broadly distributed, industry will probably require some risk-sharing for liability. An analogous circumstance nearly crippled the U.S. production of childhood vaccines until legislation controlled liability by modulating its financial risk (93).

Social and political concerns are of paramount importance for the long-term success of vaccine trials. Volunteers may have many compelling social needs, including health and child care, housing, transportation, treatment of addictions, and HIV education. Insofar as recruitment and retention depend on a volunteer’s compliance, these issues must be considered if a trial is to succeed. Political demagoguery could exploit the scientific uncertainties and suspicions of government-sponsored exploitation (94) to undermine even an ethical, important study. Therefore, community organizations, leaders, and advocates must participate in the planning process to ensure long-term community support.

If answers to vaccine efficacy are to be forthcoming, the agencies funding the trial must be able to ensure sustained support. If first-generation HIV vaccines are unsuccessful or less than optimally successful, funding for more promising vaccines may be difficult to secure if key government legislators and policymakers do not have a sustained commitment to an effective, safe HIV-1 vaccine.

Conclusion

In summary, the HIV vaccine research effort is of compelling public health importance, and preparations are under way to test promising HIV-1 candidate vaccines in large-scale efficacy trials with volunteers at high risk for acquiring infection. Recently, the NIAID has delayed but not abandoned larger-scale vaccine trials (AIDS Research Advisory Committee, National Institute of Allergy and Infectious Diseases, June 1994). The complexities of vaccine development (95), preclinical and phase I and II human testing, and eventually, community-based phase III efficacy trials will require a broad partnership of committed parties in science, government, industry, and community to achieve success.

Request for Reprints: Daniel Hoth, MD, Cell Genesys, 322 Lakeside Drive, Foster City, CA 94404-1146.

Current Author Addresses: Dr. Hoth: Cell Genesys, 322 Lakeside Drive, Foster City, CA 94404.

Dr. Bolognesi: Center for AIDS Research, Duke University Medical Center, Durham, NC 27710.

Dr. Corey: University of Washington, 1200 12th Avenue South, Room 9307, Seattle, WA 98144.

Dr. Vermund: Departments of Epidemiology and Medicine, University of
References


