Development and Testing of AIDS Vaccines

WAYNE C. KOFF* AND DANIEL F. HOTH

Recent advances in delineating the molecular biology of human immunodeficiency virus type 1 (HIV-1) have led to innovative approaches to development of a vaccine for acquired immunodeficiency syndrome (AIDS). However, the lack of understanding of mechanisms of protective immunity against HIV-1, the magnitude of genetic variation of the virus, and the lack of effective animal models for HIV-1 infection and AIDS have impeded progress. The testing of AIDS vaccines also presents challenges. These include liability concerns over vaccine-related injuries; identification of suitable populations for phase 3 efficacy studies; balancing the ethical obligation to counsel research subjects to avoid high-risk behavior with the necessity to obtain vaccine efficacy data; and the effect of vaccine-induced seroconversion on the recruiting and welfare of trial volunteers. Several candidate AIDS vaccines are nevertheless currently under development, and some are undergoing phase 1 clinical trials. Rapid progress will depend on continued scientific advancement in conjunction with maximum use of resources, open information and reagent exchange, and a spirit of international collaboration.

SINCE HIV-1 WAS IDENTIFIED AS THE ETIOLOGIC AGENT OF AIDS (*1*) considerable effort has been directed toward the development of a safe and effective vaccine. Currently, a broad spectrum of strategies, from the use of small synthetic peptides to whole inactivated viruses, are being considered as potential vaccines (*2*). An ideal AIDS vaccine would need to mimic or improve upon the immunological stimuli elicited by natural HIV-1 infection, cause minimal adverse reactions, be highly stable, and be relatively easy to produce and administer. The scientific and public policy challenges surrounding the development and testing of AIDS vaccines are formidable and will require active participation by academic, commercial, and government research laboratories, as well as innovative strategies for public-private sector interaction. In this review, we delineate the major issues in the development and evaluation of AIDS vaccines and identify areas where greater research and understanding are required to expedite progress.

Mechanisms of Immunity

Significant advances have been made in understanding the molecular biology and genome organization of HIV-1 (*3*). However, the basic information on the mechanisms of immunity that is necessary to predict whether immunization against HIV-1 is possible and to determine which host responses must be stimulated to elicit protection from subsequent HIV-1 challenge has not been defined. Therein lies the first major obstacle to AIDS vaccine development.

Enveloped viruses, including HIV-1, elicit both humoral and cell-mediated immune responses (*4*). In other retrovirus systems, most notably feline leukemia virus (FeLV), the presence of neutralizing antibody correlates well with protection against severe infections (*5*). Moreover, the ability of rhesus macaques to survive infection with simian immunodeficiency virus (SIV) is directly related to the magnitude of the humoral response (*6*). However, the role of humoral immunity in the host defense against HIV-1 infection is unknown. Studies in several laboratories have shown that HIV-1 neutralizing antibodies are present in the serum of HIV-1–infected individuals, yet the mean titers of these antibodies are markedly lower than those observed in other human retrovirus infections (for example, human T lymphotropic viruses I and II) under comparable assay conditions (*7*). Although neutralizing titers in HIV-1–infected individuals are generally low and do not effectively halt the progressive nature of AIDS, it cannot be concluded that elicitation of high titers of neutralizing antibodies by vaccination would be ineffective because vaccine-induced antibodies may be functionally different and more effective than those induced by persistent HIV-1 infection. For example, vaccine-induced antibodies of a particular isotype may confer more protection than antibodies of other isotypes, as has been demonstrated in murine systems for antibodies of the immunoglobulin G2a (IgG2a) isotype (*8*). Moreover, in human systems, generation of antibodies of the IgG1 and IgG3 isotypes can activate complement-mediated and antibody-dependent cellular cytotoxicity better than antibodies of other isotypes (*4, 8*). Thus, elicitation of specific types of high-titered neutralizing antibodies may need to be factored into the equation of producing effective protective immunity against HIV-1 infection. In addition, the role of mucosal immunity in defense against HIV-1 infection is not clearly understood. Since sexual transmission of the virus may be associated with the interaction of HIV-1 with cells of the mucosal linings, secretory immunity may be involved as an early defense mechanism, and the role of IgA responses against HIV-1 infection needs further investigation.

Cell-mediated immune responses play a prominent role in the recovery from viral infections and may also be implicated in protection immunity (*9*). Sensitized T cells can selectively lyse autologous virus-infected cells, including HIV-1–infected cells (*10*). Antigenic stimulation of monocytes and T cells causes the release of monokines and lymphokines, respectively. These substances can activate natural killer cells and macrophages that can then kill HIV-1–infected cells in vitro (*11*). In other enveloped virus systems these activated cells can protect against in vivo challenge (*12*). Although the natural mechanisms of HIV transmission (for example, by cell-

W. C. Koff is chief of the Vaccine Research and Development Branch, AIDS Program, National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD 20892. D. F. Hoth is director of the AIDS Program, NIAID, Bethesda, MD 20892.

*To whom correspondence should be addressed.

Table 1. Types of approaches to AIDS vaccines.

Live attenuated HIV
Whole inactivated HIV
Live recombinant viruses
Synthetic peptides
Natural products
Recombinant DNA products
Anti-idiotypes
Passive immunization

free or cell-associated virus) are not clearly understood, transmission by cell-to-cell contact within the infected patient may play an important role. Thus, a vaccine capable of priming T cells for lymphokine production to enhance cell-mediated immune responses may be required to interrupt virus transmission by cell-to-cell contact. In addition, induction of cross-reactive cytotoxic T lymphocytes (CTL) may protect against viruses with extensive genetic variation at glycoprotein regions that are sites associated with antibody binding (*13*). These CTL may thus be important to the repertoire of immunologic responses elicited by an HIV-1 vaccine. Recently, HIV-1–specific CTL responses were identified against *pol* gene products, which are more highly conserved than other HIV-1 genes among different isolates (*14*). Finally, until the host response against HIV-1 infection is more clearly understood, it must be assumed that production of an effective AIDS vaccine will require induction of both humoral and cell-mediated immune responses.

Types of Vaccines

Some of the approaches being used to develop a vaccine for AIDS are listed in Table 1. The efficacy of vaccines containing live attenuated viruses to induce both humoral and cellular immunity is well established for a variety of viral systems (*4*). Moreover, whole virus vaccines are advantageous from the standpoint of presenting viral antigens to the host in a form equivalent to that of natural infection. Live attenuated vaccines have drawbacks, however, in that persons with immunodeficiency may be prone to severe vaccine-induced reactions. This may be particularly relevant with HIV-1, since persons in developing nations often have malnutrition-induced immunodeficiency (*15*). Potentially, deletion mutants of HIV-1 could be developed to create a noncytopathic mutant HIV-1 that theoretically could grow and express viral antigens and induce host immune responses against HIV-1 without causing disease (*16*). However, retroviruses have a high rate of genetic mutation, and genetic information from these viruses can become integrated into the host cell DNA, can potentially persist for the life of the host, and may be associated with malignant transformation (*2, 17*). Thus, it is not likely from a safety perspective that live attentuated HIV-1 vaccines will be acceptable for human beings in the foreseeable future.

Whole inactivated viral vaccines have been used with relatively high degrees of success to prevent polio and influenza. Several strategies for whole virus inactivation are being investigated, including gamma radiation and psoralen-ultraviolet light (*18*). The goal is to destroy the functional capability of the viral nucleic acid, while maintaining the conformational integrity of the viral proteins so that the virus retains the capacity to stimulate virus-specific immune responses. This approach was used successfully to vaccinate juvenile rhesus monkeys against simian type D retrovirus–induced immunodeficiency (*19*). However, kittens immunized with inactivated FeLV demonstrated enhanced disease upon challenge with live virus when compared to unimmunized controls (*20*). Vaccine-induced enhancement of disease has also been observed with lentiviruses (*21*) and underscores the importance of understanding the host response to viral infection as it relates to vaccine development. In addition, antibody-mediated enhancement of virus infection, in which viral antibody predisposes the host to a more severe infection, occurs predominantly in virus-host systems where cells of monocyte lineage are sites of viral infection (*22*). Antibody-mediated enhancement of infection may thus potentially be an important factor in strategies for AIDS vaccine development, since HIV-1 readily infects cells of monocyte-macrophage lineage (*23*).

Live recombinant viruses are a more recent entry into strategies for vaccine development and capitalize on the revolution in molecular biology. Live vaccinia virus, for example, can be used as a carrier for genes encoding for antigens from unrelated viruses, and the genetically engineered recombinant vaccinia virus can then be used as a candidate vaccine (*24*). Such live recombinant HIV-1 vaccines offer the advantage of greater immunogenicity than inactivated vaccines, since viral replication results in the production of significant amounts of HIV-1 antigens. Moreover, since the recombinant viruses do not contain genes associated with HIV-1 replication, safety concerns regarding genetic integration and persistence are nullified. This approach has been tested by the production of a recombinant vaccinia virus expressing the envelope glycoproteins of HIV strain LAV-1. Inoculation of the vaccinia-HIV vaccine into chimpanzees produced HIV-1–specific humoral and cellular immune responses (*25*). However, disseminated vaccinia after smallpox vaccination has been reported in a military recruit infected with HIV-1 disease (*26*), and vaccinia viruses may cause a range of severe complications in the immunocompromised host (*27*). Thus, even if ultimately effective in preventing HIV-1 infection, this approach may not be appropriate in populations (for example, in certain areas of Africa) where high rates of HIV-1 seropositivity or other immunodeficiency diseases already exist. The possibility of using other DNA viruses such as adenovirus in place of vaccinia is being investigated (*28*).

Synthetic peptide antigens can be designed to include both neutralizing and T cell epitopes, to exclude immunosuppressive epitopes, and to be free of any contaminating viral nucleic acid (*29*). Antigenic determinants for antibody induction can be predicted by amino acid sequence modeling, examination of hydrophilicity plots, analysis of secondary structure, and use of overlapping peptides for epitope mapping (*30*). Synthetic peptides have already been used to identify HIV-1 gp160 neutralizing epitopes (*31*), and studies with picornaviruses have shown that synthetic peptides may be more effective immunogens than whole viral proteins in inducing neutralizing antibodies (*32*). However, tertiary structures defined by x-ray crystallography have indicated the importance of protein conformation in the elicitation of antibody responses and, in general, synthetic peptides may not efficiently reproduce the conformational epitopes required for induction of B cell immunity. Moreover, synthetic peptides may require adjuvants or carrier proteins to enhance their immunogenicity, and thus this vaccine approach may be impeded by the lack of clinically approved vaccine adjuvants other than alum. However, T cell epitopes can induce cell-mediated immune responses without the requirement for tertiary structural homology with the whole protein and may also act as adjuvants for stimulating B cell responses (*33*). The observation that T cell antigenic sites may be determined on the basis of amphipathicity has stimulated research into the identification of HIV-1 T cell epitopes that may be used in future HIV synthetic peptide vaccine approaches (*34*).

Genetically engineered purified viral antigens are considered the most promising approach to AIDS vaccine development. Recombinant DNA technology has already led to the production of viral

Table 2. HIV-1 gene products as potential immunogens for AIDS vaccines.

HIV gene	Gene products	Function
env	gp160	Precursor of envelope glycoprotein
	gp120	CD4 receptor binding
	gp41	Transmembrane anchorage
gag	p55	Precursor of *gag* proteins
	p24	Major core protein
	p17	?
	p15	?
gag-pol	p14	Protease frameshift protein
pol	p66/51	Reverse transcriptase
	p31	Endonuclease
sor	p23	Regulatory
3'-orf	p27	Regulatory
tat	p14	Transactivation
art/trs	p18	Antirepressor transactivator
R	p15	?

subunit vaccines, such as the recombinant hepatitis B vaccine (*35*). In contrast, production of native viral antigens, which requires large quantities of virus or virus-infected cells, has high research and development costs. Nonetheless, native proteins serve as baselines from which to compare the immunogenicity of genetically engineered viral subunits.

Several expression systems are being used for the production of recombinant HIV-1 antigens; these systems include insect viruses, *Escherichia coli*, yeast, and mammalian cells (*36*). The host expression system plays a prominent role in the posttranslational modification of expressed proteins, and one of the factors potentially capable of modulating the immunogenicity of recombinant proteins is the glycosylation pattern derived from the host expression system. With HIV-1, envelope glycoproteins have a high percentage of carbohydrates that might interfere with the induction of host defense mechanisms in a similar fashion to other retroviruses (*37*). However, in studies with recombinant deglycosylated gp120 subunits expressed in *E. coli*, neutralizing antibody titers were equivalent to those of the native protein, suggesting that glycosylation might not be required for induction of HIV-1 neutralizing antibodies (*38*). The role of glycosylation in the host response to HIV-1 infection needs closer examination, since the relative importance of neutralizing antibody and cell-mediated immune responses in generating protective immunity against HIV-1 remains undefined. In this regard, the carbohydrate structure of HIV-1 glycoproteins has been implicated in both the binding of the glycoproteins to virus receptors on T lymphocytes and virus-induced cell fusion (*39*), suggesting that glycosylation remains a factor to be considered in designing recombinant or synthetic proteins as vaccines.

A major issue in the production of viral subunits by recombinant DNA technology is which HIV-1 gene products should be considered for vaccine development (Table 2). The major focus is on the *env* gene products, gp160, gp120, and gp41. These glycoproteins are attached to the viral lipid envelope and are therefore exposed to the host immune defense mechanisms. Neutralizing epitopes that induce protective immunity against feline and murine retroviruses are located on the viral envelope glycoproteins (*2, 5*), and neutralizing and T cell epitopes of HIV-1 have been demonstrated on the *env* gene products (*34, 38, 40*). However, gene products of viral proteins located within the whole virion may be expressed on the cell surface of infected cells and thus may be an integral factor in the induction of host immune responses. For example, *gag* gene products occur on the cell surface in other retroviral systems (*41*), and CTL often recognize virus core proteins (*14, 42*). Thus, although emphasis continues to be on the *env* gene products of HIV-1, other HIV-1 gene products should continue to be screened and evaluated for evidence of immunogenicity. Moreover, an effective subunit vaccine for HIV-1 may require combinations of viral proteins to stimulate protective immunity.

Anti-idiotype antibodies represent another approach to AIDS vaccine development. Idiotypic determinants identify the variable region of antibodies, and these determinants may be distinguished through the generation of anti-idiotype antibodies (*43*). The potential use of anti-idiotype antibodies as candidate vaccines has been demonstrated against a variety of infectious agents (*44*). The CD4 molecule, a glycoprotein on the surface of the T helper-inducer subset of T lymphocytes, is the primary receptor for HIV binding (*45*). Investigations have therefore focused on ways of inhibiting the HIV-1–CD4 interactions, including the use of anti-idiotype antibodies. Chanh *et al.* (*46*) have shown that an internal image anti-idiotype response against monoclonal antibodies to CD4 can inhibit HIV-1 binding to CD4 on target cells. Moreover, an anti-idiotype response generated in mice to an antibody to CD4 neutralized three genetically divergent HIV-1 isolates and one HIV-2 isolate in vitro (*47*). However, the anti-idiotype approach may face other obstacles, including the potential for immunopathologic consequences of multiple injections with heterologous antibody, the potential multiple recognition sites of HIV-1 on CD4, the fact that CD4 plays a central role in the orchestration of T helper-inducer immunological responses, and the possibility that inoculation with antibodies to CD4 may induce immunosuppression (*48*). Efforts to overcome these obstacles may follow the successful strategy of protecting chimpanzees against hepatitis B virus challenge by active immunization with anti-idiotype antibodies against hepatitis B surface antigen (*49*).

Finally, passive immunization with high titers of neutralizing antibody may have a role in prevention of HIV-1 infection in selected populations such as newborn infants of HIV-1 seropositive mothers. This approach has not yet been demonstrated as effective in any animal model for AIDS, but since passive immunization can be an effective method of prevention in other viral systems (*50*), continued investigation is warranted.

Genetic Variation of HIV

Nucleotide sequence analyses of HIV genomes have revealed extensive intertypic and intratypic genetic variation among isolates of these viruses (*17*), which is not surprising, since the mutation rates of other lentiviruses are known to be high (*51*). Moreover, lentiviruses have the capacity to undergo several cycles of viral variation associated with antibody production, as demonstrated by sequential infections of equine infectious anemia virus (EIAV) (*51*). Hahn *et al.* showed that HIV-1 genetic variation can occur in a given HIV-1–infected person and that this variation appears to be distinct from multiple infections (*52*). The most extensive variation occurs in *env* gene, the gene that is a current major focus of AIDS vaccine efforts. An effective AIDS vaccine may therefore need to induce both type-specific and group-specific neutralizing antibody or cell-mediated immune responses. While conserved domains on *env* gene products and group-specific epitopes on HIV-1 variants continue to be identified (*53*), it is unclear whether incorporation of these epitopes into candidate AIDS vaccines will be sufficient to overcome the problem of genetic variation in the virus. The inclusion of cytotoxic T cell epitopes, which may be more cross-reactive than type-specific antibodies, is another approach to circumvent the genetic heterogeneity problem, yet the role of cell-mediated immune responses in protection against HIV-1 remains undefined. Finally, HIV-2 has been isolated in West Africa and

found to cause disease in humans that is virtually indistinguishable from HIV-1–induced AIDS (*54*). Although the core antigens of HIV-1 and HIV-2 may share some common epitopes, the envelope glycoproteins from these viruses are quite distinct (*54, 55*). The elucidation of multiple serotypes for HIVs with the potential for causing AIDS magnifies the complexities associated with AIDS vaccine development.

Animal Models

A major impediment to the development of a safe and effective vaccine against HIV-1 infection is the lack of a readily available animal model to evaluate whether primary HIV-1 infection and the development of AIDS can be prevented by immunization with candidate AIDS vaccines. Chimpanzees appear to be the only animal system in which experimental infection with HIV is readily successful (*56*). After inoculation with HIV-1, chimpanzees seroconvert and generate HIV-1–specific T cell responses, and virus can be isolated from peripheral mononuclear cells. However, HIV-1 infection has thus far failed to produce an AIDS-like disease in chimpanzees. Moreover, the number of chimpanzees available for AIDS vaccine testing in the United States is currently between 500 and 600, making extensive analyses of experimental vaccines difficult in this model. Chimpanzees previously immunized with an experimental AIDS vaccine and subsequently challenged with HIV-1 were not protected: virus was recovered from circulating peripheral blood cells (*25*). These animals did develop vaccine-induced humoral and cell-mediated immune responses against HIV-1. However, the nature of the viral challenge, that is, dose of virus, route of virus inoculation, cell-free virus versus cell-associated virus inoculum, and homologous versus heterologous HIV-1 challenge have not been clearly defined. These experiments magnify the need for standardized reagents, assay systems, and challenge protocols for AIDS vaccine studies. Despite its limitations, the chimpanzee model remains the only animal model of HIV-1 infection, and it may become a more valuable model for vaccine efficacy testing provided that adequate numbers of chimpanzees are made available.

Rhesus macaques are susceptible to infection by SIV, and infection in this primate model results in an AIDS-like disease characterized by a wasting syndrome and opportunistic infections. Like HIV-1 infection, SIV infection is associated with depletion of the CD4 subset of T lymphocytes, and the immunologic responses are similar to those induced by HIV-1 in humans (*57*). Thus, the SIV model may be suitable for determinations of vaccine efficacy if either prevention of infection or of disease is used as an endpoint. It might therefore be practical to develop potential HIV-1 vaccine candidates simultaneously with the development of similar vaccines in the SIV system to test the safety and efficacy of the experimental approach. Moreover, determinations of the role of neutralizing antibody and cellular immune responses to lentivirus-induced immunodeficiency could be characterized in this model. Since commercial vaccine manufacturers may not be inclined to develop SIV vaccines because of economic disincentives, this work could be accomplished by the academic and government biomedical research sectors. It is not known, however, whether genetic and biological differences between HIV-1 and SIV will limit the value of the SIV model in vaccine development.

Other animal models from which information potentially important to AIDS vaccine development may be obtained include the ungulate lentiviruses and feline retroviruses. The ungulate lentiviruses, including visna, caprine arthritis encephalitis virus (CAEV), and EIAV, and the recently described bovine immunodeficiency virus are difficult to grow in vitro. Animal studies with these viruses have not been widely undertaken because of the difficulty in obtaining large numbers of sheep, goats, horses, and cows for research purposes (*58*). However, studies on the prolonged incubation periods, growth in macrophages, persistent viremia, weak neutralizing antibody responses, and other virus-host responses of this subfamily of retroviruses may contribute significantly to understanding HIV-1 infection. In addition, the lack of efficacy of lentivirus vaccines in animal models, some of which induced detrimental responses, may provide insights into the potential difficulties in developing effective AIDS vaccines. For example, goats vaccinated with inactivated CAEV developed more severe arthritis after infectious CAEV challenge than control goats vaccinated with placebo; and postinfection immunization with purified visna virus increased the severity of lesions (*21*).

Both FeLV (*59*) and the recently isolated feline T lymphotropic virus (*60*) offer the possibility of evaluating candidate vaccine approaches in cat model systems. Although FeLV is a type C retrovirus, it causes an immunosuppressive disorder with certain similarities to AIDS, and it has been studied extensively with regard to vaccine development. This model system may be valuable in assessing the efficacy of candidate vaccine adjuvants (*61*). The feline T lymphotropic virus may potentially be valuable as a model for AIDS, but the current lack of reagents available for this virus is impeding the pace of development of this system.

Although the lack of an ideal animal model for AIDS represents a major barrier to AIDS vaccine development, the model systems that are available can provide significant virological, immunological, safety, and efficacy information directly related to the development of AIDS vaccines.

Vaccine Adjuvants

The efficiency of the approaches being used to develop AIDS vaccines may be limited by the lack of immunostimulatory adjuvants to enhance humoral and cell-mediated immune responses. At present, the only adjuvants approved for human use in most countries are aluminum hydroxide and aluminum phosphate. Although these adjuvants are effective immunostimulants for some vaccines, other adjuvants, such as Freund's complete adjuvant, are more efficient in stimulating both humoral and cellular immune responses but are unacceptable because of safety considerations (*62*). Several other adjuvants are therefore being evaluated. These include liposomes, muramyl dipeptide derivatives, immune stimulating complexes (ISCOMS), Freund's incomplete adjuvant, synthetic polymers, nonpyrogenic subunits of lipopolysaccharide, and antigenic modification (*62, 63*). These strategies for development encompass the varied mechanisms of actions for adjuvants, including immunogen localization and delivery, and direct effects on lymphocytes and macrophages. For an HIV-1 vaccine to be successful, it may need to be a superior immunogen when compared with the natural HIV-1 infection, and development of effective adjuvants may thus be central to AIDS vaccine development.

Future Directions for Preclinical AIDS Vaccine Development

X-ray crystallographic and nuclear magnetic resonance data of HIV-1 proteins should provide three-dimensional structural analyses of antigenic sites (*29, 64*), which may facilitate the prediction of peptide regions that might be effective in synthetic vaccines. Discontinuous epitopes, that is, linearly separated amino acid sequences brought together by protein folding to form an epitope, may be

important immunogenic determinants and can theoretically be reproduced in the form of "mimotopes" to mimic the epitope functional activity while bearing little direct sequence relation to it (*65*). The mimotope approach requires monoclonal antibodies that define the epitopes, and monoclonal antibodies to HIV-1 proteins continue to be developed to identify neutralizing epitopes. The yeast transposon Ty codes for a series of proteins that can assemble into virus-like particles Ty:VLP (*66*). Hybrid HIV-Ty:VLP have recently been expressed in yeast, stimulate the production of antisera against the HIV-1 components, and offer a potential for presenting HIV-1 antigens to the immune system in a multivalent and particulate form (*67*). In efforts to stimulate cell-mediated immune responses against HIV-1, immunization with fixed, autologous cells expressing HIV-1 antigens is being examined (*68*). Vaccination as a means of immunotherapy, to inhibit the progression from the asymptomatic HIV-1 seropositive state to AIDS, has also been proposed (*69*). These examples show that basic research in many areas may contribute to the identification of new approaches to AIDS vaccine development. However, even if an effective AIDS vaccine were developed today and prepared for clinical testing, several clinical research, social, legal, and economic issues could potentially impede the vaccine from being licensed, marketed, and distributed to the public.

Design of Clinical Trials

Before being approved for clinical testing, a candidate AIDS vaccine must fulfill a series of requirements from the Food and Drug Administration to ensure that it conforms to standards for purity, composition, and stability. In addition, studies in animal models must demonstrate that the candidate vaccine is safe and immunogenic (*70*). Considerable debate in the scientific community has revolved around the subject of whether a candidate AIDS vaccine must show protective immunity in an animal model system before initiation of phase 1 safety and immunogenicity trials in humans. Currently phase 1 clinical trials of candidate AIDS vaccines are proceeding before the demonstration of protection immunity in animal model systems. Reasons for this procedure include the following: lack of effective standardized challenge criteria for evaluating protective immunity in animals, lack of an ideal animal model system for HIV-1 infection and AIDS, the urgency of the AIDS pandemic, and the potential capacity for obtaining valuable scientific information regarding the human immunological response to HIV-1 antigens under carefully controlled conditions.

Clinical trials of AIDS vaccines will be carried out in three phases. Phase 1 trials are designed to determine that the candidate vaccine preparation is safe and does not elicit unanticipated adverse reactions. Safety is determined on escalating doses of vaccine, and the trials are generally carried out on low numbers of healthy adult volunteers. Phase 1 trials also generate preliminary immunogenicity data and aim to identify safe and immunogenic doses that are more comprehensively evaluated in phase 2 trials. For phase 1 trials of AIDS vaccines, healthy adult volunteers at low risk for acquiring HIV infection will probably be used. These may include heterosexuals as well as homosexual men who are not practicing high-risk behavior. Phase 2 trials require larger numbers of volunteers, including persons at high risk for acquiring HIV-1 infection, and are designed to determine an optimal dosage regimen with respect to safety and immunogenicity. Hence, phase 2 trials may detect safety problems unique to the population at high risk for infection, or adverse reactions with low event rates. These trials provide the basis for the vaccine regimen that will be used in phase 3 trials. Phase 3 trials are designed to evaluate the capacity of the candidate vaccine to protect against disease and to provide further knowledge on its safety. Most vaccines against viral diseases protect against disease, but not necessarily against viral infection.

However, in the case of HIV-1 infection and AIDS, the lengthy incubation period coupled with increasing estimates on the percentage of HIV-1–infected persons who eventually progress to develop AIDS may necessitate evaluation of protection against infection rather than disease as the criterion for vaccine efficacy. The phase 3 trials are generally placebo controlled, randomized, and double-blind, and sample size is based on the incidence of infection in the study population and the confidence levels sought for significant protection from infection or disease (*36*).

One of the major concerns regarding the safety of candidate AIDS vaccines is the possibility of vaccine-induced immunosuppression. Recently, synthetic peptide fragments of HIV-1 gp41 were shown to be immunosuppressive in vitro (*71*). Clinical evaluation of these vaccines will therefore include a more comprehensive immunologic analysis than that of any vaccine previously tested. It may be difficult to determine whether alterations in immunologic parameters are due to the specific vaccine preparation being tested or are a function of immunization with any protein antigens. Therefore, the first phase 1 studies will probably include a comparative vaccine group (for example, hepatitis B), to delineate between AIDS vaccine–induced immunologic changes and transient immunologic changes resulting from immunizations in general.

Several factors make the clinical testing of AIDS vaccines more complex than any vaccine trials previously undertaken. Volunteers will be screened for evidence of prior HIV-1 infection; such evidence will serve as an exclusion criterion from the trials since safety, immunogenicity, and efficacy data could be compromised by a concurrent HIV-1 infection (*72*). Major issues affecting recruiting of volunteers for the clinical testing of AIDS vaccines will be confidentiality of all information about the subjects and the likelihood of vaccine-induced seroconversion. Confidentiality will have to be maintained for the duration of the trial because subjects may be identified as belonging to populations at risk for HIV-1 infection—a factor that potentially could lead to discriminating or other detrimental situations. Persons immunized with candidate AIDS vaccines who mount effective immune responses will appear positive by enzyme-linked immunosorbent assay (ELISA) for HIV-1 antibody. Although immunological responses to the present vaccine candidates can be differentiated from those of HIV-1 infection by protein immunoblot analysis, volunteers in the AIDS vaccine trials may be subject to social stigma and discrimination associated with appearing to be positive by HIV-1 ELISA (*73*). Moreover, future vaccine preparations that include combinations of antigens or whole virus may not be easily differentiated by protein immunoblots. Vaccine-induced seroconversion may lead to difficulties in donating blood, obtaining insurance, traveling internationally, or entering the military and foreign services. Vaccine-induced antibodies may be long-lived, and although volunteers in the AIDS vaccine trials will be given some form of documentation that certifies their participation in a study and their antibody status as being due to vaccination and not HIV-1 infection, the seroconversion issue may play a major role in the recruitment efforts and in the future welfare of vaccine trial participants.

Populations for Phase 3 AIDS Vaccine Trials

The study populations for phase 3 efficacy trials of candidate AIDS vaccines include groups at high risk for HIV-1 infection. These populations include homosexual men, partners and spouses of hemophiliacs, intravenous drug users, prostitutes, prisoners, mili-

tary personnel in regions of high HIV-1 incidence, newborns of HIV-1 seropositive mothers, and patients with sexually transmitted diseases. If an AIDS vaccine had been available for testing in the United States 2 to 3 years ago in phase 3 clinical trials, the preferential population for testing might have been homosexual men at high risk for HIV infection; however, the incidence of infection in this population (because of education efforts) has now decreased, which may limit their capacity to be used in vaccine trials (*74*). The incidence of HIV-1 infection in both intravenous drug users and prostitutes continues to rise, and these groups may be suitable populations for use in phase 3 trials, provided that approaches for long-term follow-up can be identified (*75*). Partners and spouses of HIV-1–infected hemophiliacs are at high risk of acquiring infection and would be expected to be highly motivated as vaccine volunteers, but the limited numbers of these persons diminish the prospect of evaluating vaccines in this population. Consideration may therefore be given to including more than one of these groups in a single phase 3 study. Because of the greater population size and costs associated with phase 3 trials, only a few vaccines are likely to be chosen for such studies.

One of the other potential groups for consideration in AIDS vaccine trials are persons at high risk for acquiring HIV-1 infection in foreign countries, including military personnel stationed in areas of high HIV-1 incidence. Since the incidence of HIV-1 infection is high in central Africa and development of a successful AIDS vaccine would have wide application in this region, phase 3 trials may be carried out there (*76*). However, vaccine trials in Africa may be complicated by sociopolitical factors and by the genetic variation of HIV-1 strains in other parts of the world (*77*).

Ethical Issues

One of the dilemmas in carrying out HIV-1 vaccine trials is the equilibrium that must be reached between the ethical obligations of counseling research subjects about avoiding high-risk behavior for HIV-1 infection and the ability to obtain vaccine efficacy data. Thus, successful education of trial volunteers and a subsequent decrease in their high-risk behavior for HIV-1 infection could theoretically diminish the possibility of obtaining adequate efficacy data. These concerns impact on the design of the trials and particularly on sample size determinations. However, AIDS is a fatal disease, and a primary concern of all parties associated with the trials must be the health and welfare of those individuals volunteering to participate in the experimental evaluations. Once a subject has volunteered, been prescreened for previous exposure to HIV-1, and been accepted into an AIDS vaccine trial, the researchers carrying out the trial have an ethical obligation to inform, educate, and counsel the volunteer against high-risk behavior for HIV-1 infection.

The informed consent document can serve as an educational tool for volunteers in the trials; a thorough discussion of potential risks from participating in the trials will be outlined in this document. Since the preliminary phase 1 trials will be done in the absence of vaccine-induced protective immunity in any animal model system, this information must also be included in the informed consent document. Thus, ethical considerations reinforce the importance of institutional review boards and consultants on ethics in this process.

Liability Issues

Over the last 20 years, the number of vaccine manufacturers has continued to decline because of concerns over vaccine-related injury liability expenses, thereby potentially jeopardizing vaccine supply and immunization programs (*78*). Despite the enormous benefit to society that would result from a safe and effective AIDS vaccine, efforts to maximize resource allocation toward this goal continue to be hindered by the lack of resolution of the liability issues. Combinations of a highly litigious atmosphere surrounding product liability issues and the inability to actuarially define the risks associated with AIDS vaccines have stymied efforts at developing criteria that would allow for reasonable compensation for vaccine-related injury while simultaneously encouraging vaccine development. Potential situations for AIDS vaccine–induced injuries, for example, CD4-related immunosuppression (*48*), immunoenhancement of infection (*20, 22*), and neurological dysfunction (*79*), are compounded by the long incubation period between HIV infection and the development of AIDS (*80*) and magnify the need for resolution of the liability issues.

Recently, the state of California enacted legislation to encourage AIDS vaccine development (*81*). The statute generally protects the manufacturer of an AIDS vaccine approved by the Food and Drug Administration and distributed in California from strict liability for unavoidable risks due to a defect in product design or warnings. This statute also creates a vaccine compensation fund (financed by a surcharge on vaccine sales) for persons who are injured by the vaccine. However, the statute does not contain any provisions for liability for persons injured during clinical trials of AIDS vaccines.

There are no reported judicial decisions in the United States court records involving claims of injury associated with nontherapeutic randomized double-blind placebo-controlled clinical trials. However, the uniqueness and complexity of AIDS vaccine trials add greater potential for liability claims and reaffirm the necessity for well-designed, scientifically justified, and rigorously reviewed clinical trials.

Conclusions

The development and testing of AIDS vaccines face a series of difficult scientific and public policy challenges. The benefits to society of a safe and effective AIDS vaccine outweigh all of the scientific, economic, social, and political risks associated with AIDS vaccine development. Tremendous progress has been gained in the preclinical development of candidate AIDS vaccines, and efforts to address the significant challenges of AIDS vaccine testing are well under way. Acceleration of AIDS vaccine development will require international collaboration and cooperation. Maximization of resource allocation, technology transfer, and open information exchange, with the use of innovative approaches at public-private sector interactions, will be prerequisites for the success of this endeavor.

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