Potential for Adverse Reactions from HIV Vaccines

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The potential safety problems in the development and introduction of a vaccine for the prevention of HIV, type 1 (HIV-1) infection are addressed in this chapter.[1] Ethical, social science, and legal issues are presented more fully in chapters 3 and 4.

This chapter begins with a brief review of the biological basis for development of a vaccine to prevent AIDS. Next, principles underlying the preparation of a protective vaccine are reviewed, including observations on the unprecedented hurdles posed by HIV infection compared with successful vaccines developed in the past. This chapter also discusses the biological basis for safety concerns and why the nature, frequency, and severity of adverse reactions with HIV vaccines cannot be predicted at this point. In addition to adverse events that may be associated with *biological* mechanisms of injury, important adverse *social* consequences, termed "social harms," are addressed here and in chapters 3 and 4.

This chapter has been written for a diverse target audience, including legislators, policymakers, lawyers, ethicists, social scientists, and the AIDS community, in addition to biological scientists. Experts in the several disciplines will recognize the abbreviated and simplified approach in some areas. A more

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[1] In this background paper, reference to HIV will refer to human immunodeficiency virus, type 1 (HIV-1), unless otherwise noted. HIV-1 has been found throughout the world. Human immunodeficiency virus, type 2 (HIV-2) is found in West Africa and is in the same retrovirus family as HIV-1. Infection with either HIV-1 or HIV-2 can lead to the development of AIDS.

technical discussion of the theory and proposed mechanisms of HIV vaccine risks are presented in appendix A.[2]

ROLE OF VACCINES IN THE CONTROL OF INFECTIOUS DISEASE

Options for the Control of Infectious Diseases

There are three major options for controlling HIV infection: 1) halt transfer of virus from person to person through education and behavioral changes; 2) treat HIV-infected individuals with therapeutic drugs after infection is recognized; 3) prevent disease through introduction of "prophylactic" HIV vaccines[3] that prevent the establishment of infection. The possible uses of an HIV vaccine are described in box 2-1. The magnitude of the medical, social, and political impact of the AIDS epidemic will, for the foreseeable future, require continued intensive efforts using all three options.

Measures to control HIV infection have met formidable difficulties, and infection is spreading uncontrollably around the globe. Prevention of viral transfer by limiting risk behavior and the extensive research directed at development of drug treatments have had limited success (2). Treatment of infected pregnant women with the antiviral drug zidovudine (AZT) has decreased transmission of HIV infection to newborns, a significant achievement.

Vaccines capable of preventing infectious diseases are generally regarded as the most successful instrument of cost-effective, humane health care. Vaccines are credited with the global elimination of smallpox and, more recently, elimination of poliomyelitis from the Americas (26)—(105). In addition, the childhood vaccines, measles, mumps, and rubella (MMR), Haemophilus influenza type B (HIB), and diphtheria, tetanus, and pertussis (DTP)—have markedly reduced the number of cases and deaths from infectious diseases. More widespread use of influenza, pneumococcus, and hepatitis B virus (HBV) vaccines, in addition to the availability of hepatitis A virus (HAV) and varicella (chickenpox) vaccines, will add significantly to reduction of morbidity and mortality from infectious diseases. The historical success of conventional vaccines in preventing and even eradicating disease has stimulated an extensive quest for a safe and effective preventive HIV vaccine. This chapter will review the progress toward development of an HIV vaccine through 1994.

How a Vaccine Works

HIV is the most intensively studied virus of all time. Details of its molecular structure, replication strategies,[4] host-cell interactions,[5] and pathology are known. Despite a decade of research and advances in biotechnology, a successful HIV vaccine lies at least several years ahead. Most currently licensed vaccines for infectious diseases were developed when much less was known about the target microbe and its infection. The reasons an HIV vaccine has been so difficult to prepare, the unique features of the virus that elude vaccine control, and the implications for possible safety problems from an HIV vaccine will are discussed below.

Stated in its simplest form, a viral vaccine consists of a microorganism (such as a virus or-

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[2] Selected review articles are noted in the references. However, references are also cited in the text insofar as they may be linked to design and outcome of clinical trials of HIV vaccines.

[3] This background paper will focus on prophylactic HIV vaccines, and not on therapeutic HIV vaccines. Prophylactic vaccines prevent infection or disease in uninfected individuals (so-called classic prophylaxis) or reduce their infectivity should the vaccinated individual subsequently become infected. Therapeutic vaccines prevent or reduce disease progression in infected individuals, or reduce disease transmission to persons who come in contact with infected individuals.

[4] The viral genome is reproduced in a process called *replication*.

[5] In microbiology, the *host* refers to the organism or cells that are being infected by the microorganism.

BOX 2-1: The Spectrum of Possible Strategies for Use of HIV Vaccines

HIV vaccines have been proposed for prevention of HIV Infection (classic prophylaxis) and for therapy of HIV infection (as a form of post-infection immunotherapy). HIV vaccines have also been advocated as a tool to reduce the infectivity of HIV-infected individuals (i.e., to reduce the risk of transmission of HIV from infected vaccinees to their contacts or offspring) These approaches have been reviewed previously (9, 13, 29, 80) and are briefly outlined below.

1 *Classic Prophylaxis,* The classic prophylactic vaccination strategy requires a high rate of vaccination in the general population at childhood or adolescence, yielding individual immunity as well as "herd immunity" (inhibited spread of Infection through the population) if a sufficient percentage of the general population is Immunized Examples of successful classic prophylactic vaccination strategies include the worldwide smallpox vaccination program and, in the Unites States, the mandatory childhood vaccination program

2 *Targeted prophylactic vaccination.* Another well-established strategy IS to prevent infection by targeting "at-risk" populations for vaccination An example of this prophylactic vaccination strategy is the targeting of tropical disease vaccines, such as yellow fever, to travelers

3 *Immediate post-exposure vaccination.* Falling between prophylaxis and treatment IS the concept of vaccination immediately after exposure to an infectious pathogen to prevent establishment of permanent infection. Rabies vaccine, in which anti-rabies immunoglobulins are administered immediately following exposure to rabies virus, IS a model for this vaccination strategy

An Immediate post-exposure HIV vaccine would be most useful in cases of accidental exposures to HIV, such as following a needle-stick injury. A clinical trial of such a vaccine, however, would be unlikely to yield significant results due to the low rate of HIV infection following needle sticks or other accidental exposures (72),

4. *Therapeutic vaccination.* Therapeutic vaccination to prevent disease progression in an Infected individual has been proposed for several pathogens and has a long history as a concept (13) However, there are few examples of the successful application of this vaccination strategy for any infectious disease, with a recent report of decreased genital herpes lesions following vaccination with herpes glycoprotein a noteworthy exception (93).

Until recently, there has been little evidence that envelope-based HIV vaccines (77) or whole inactivated HIV vaccines (81) have had therapeutic benefits in HIV-infected individuals. However, recent results from a Phase II trial of a whole Inactivated envelope depleted virus vaccine in HIV-infected individuals suggests the possibility of an antiviral effect from the vaccine *(94)*.

Likewise, there are no examples of a vaccine that can prevent disease transmission from infected vaccinees to susceptible contacts But passive transfer of antibodies to infected pregnant women has been discussed as a potential means for reducing maternal-fetal transmission of several Infectious agents, including HIV.

There has been discussion of development of a therapeutic vaccine for HIV-infected women of childbearing age to prevent infection of their offspring, since there is a 15 to 50 percent probability of transmission of HIV infection from untreated infected mothers to their newborns, Recently, however, a clinical trial showed that the antiviral drug AZT (zidovudine), when given to infected mothers during pregnancy, was able to reduce the rate of maternal-fetal HIV transmission from *24* to 8 percent (Pediatric ACTG Protocol 076), Thus, the efficacy of AZT in reducing maternal-fetal HIV transmission is the standard against which the efficacy of any vaccine to reduce maternal-fetal transmission will be compared.

(continued)

BOX 2-1: The Spectrum of Possible Strategies for Use of HIV Vaccines (Cont'd.)

5. *Vaccines to reduce infectivity* Another strategy involves the vaccination of uninfected members of high risk groups to reduce their infectivity in the event of subsequent infection; in this case, the vaccine is not expected to actually prevent chronic infection in subsequently exposed individuals, but to decrease their infectivity by reducing the rate of viral replication. Presumably, the reduction in the rate of viral replication would probably be accompanied by a decreased rate of disease progression, and so this vaccination strategy represents a variant of the classic prophylactic vaccination strategy. There are no examples of human vaccines that follow this strategy, but an analogous situation occurs naturally in some diseases (e.g., tuberculosis, Hepatitis B infection), where persistently infected individuals that mount a strong immune responses have been shown to have decreased infectivity This decreased infectivity has also been shown to occur in vaccinated monkeys that are infected with SIV (85, 86). HIV vaccines that do not clear all virus (achieve "sterilizing immunity") may also reduce infectivity, although this has not been demonstrated.

This vaccination strategy has not yet received much attention from experts in the field of HIV vaccine research. Investigators would have difficulty demonstrating the efficacy of a vaccine to reduce infectivity because it would require a clinical trial that followed not only a large number of high-risk vaccine and placebo recipients, but the recipients' contacts as well.[1] In addition, conclusions about the effect of the vaccine on the transmissibility of infection could only be drawn from observation of incidence of HIV infection among those persons whose only risk for HIV infection is from contact with a vaccine trial participant (e.g., the vaccinee's offspring or monogamous sexual partners). Nevertheless, this may be the vaccination strategy that has the greatest chance of success in controlling the AIDS epidemic in the foreseeable future. Therefore, designing the necessary studies to test the efficacy of vaccines to reduce infectivity is important.

The efficacy of a vaccine to reduce infectivity could be tested, for example, in a clinical trial involving intercity truckers in India. These truckers are at high risk for HIV infection due to their frequent contact with female sex workers. The wives of these truckers, however, tend to be monogamous. Such a trial would require investigators to monitor incidence of HIV infection not only in the truckers participating in the trial, but in their wives as well. Another way to test this strategy would be to vaccinate uninfected women of child-bearing age who are at high risk of acquiring HIV, and then monitor HIV infection incidence in these women and their offspring.

The efficacy of this vaccine in reducing the infectivity of subsequently infected individuals may also be approximated by testing the vaccine in individuals that are already infected with HIV. Such a trial would require enrollment of far fewer participants. To demonstrate the efficacy of such a vaccine in reducing infectivity, however, one would still need to follow up the vaccinees' monogamous sexual contacts. Furthermore, a vaccine may not be nearly as effective in reducing infectivity when given after infection as it is when given before infection.

SOURCE: David Schwartz, "Analysis of 'Worst Case Scenarios' for Theoretical Risks Associated with Experimental HIV Vaccines," unpublished contractor report prepared for the Office of Technology Assessment, US. Congress, Washington, DC, July 7, 1994.

[1] For example, assuming a 5-percent annual incidence of HIV infection in the high-risk target population, and 5-percent annual transmission from this population to their monogamous sexual partners, there would be a 0.25 percent annual incidence of HIV infection among the sexual partners. More than 40,000 participants from the high-risk target population would be required for a Phase Ill efficacy trial of a vaccine using this strategy.

TABLE 2-1: Immune Response Elemen

Type of Immune response	Elements	Function of elements
Humoral immunity	Antibody produced by B lymphocytes	Inactivates free virus
Cellular immunity	T lymphocytes	Helper cells
	CD4+	Cytotoxic lymphocytes
	CD8+	
	Macrophages	Immune intermediary cells
Mucosal immunity	Antibody plus immune cells	Blocks mucosal invasion

SOURCE. Office of Technology Assessment, 1995.

bacteria) or its components, in a safe form, designed to protect against future disease. Administration of a vaccine stimulates the body's immune system to generate protective defenses specifically directed against the microorganism. This vaccine-induced protective immune response is rapidly restimulated when a vaccinated individual is subsequently exposed to the microorganism. Thus, the vaccine "primes" the immune system to respond to a microorganism, so that upon exposure to that microorganism, spread of the microorganism through the body is dampened before it can cause disease (51). This is the mechanism by which traditional vaccines protect against establishment of infection.

Immune Response Elements

Selection of starting material for a vaccine begins with identification of the important sites on the microorganism that stimulate the immune system. These sites are known as *antigens,* which are usually composed of proteins, which are long chains of amino acids.[6] The term *epitope* describes the specific amino acid sequence and configuration of the antigenic protein. Epitopes are the functional sites that are recognized by the body's immune defense system, and that induce the body to produce an immune response. These epitopes are incorporated in various forms into the vaccine.

Knowledge of the nature of the elements of the immune system and how each element functions is important in understanding how a new vaccine is designed. The immune system can be thought of as having three major response elements: 1) *humoral immunity,* the immune response to foreign substances from antibody[7] circulating in the blood; 2) *cellular immunity,*[8] immune response from a network of immune white cells in the blood and tissues, and 3) *mucosal immunity,* a specialized system of antibody and immune cells located at the smooth, moist mucous membranes (mucosa) that cover-inner body surfaces, including the routes of sexual transmission of HIV: the vagina, anus, and penile urethra (table l-l).

Each of the three immune response elements plays a unique role and each may be stimulated differentially by altering the design of the vaccine or its method of administration (1, 62, 63). One type of immune white cell, the B lymphocyte, produces antibody. Each antibody is antigen-specif-

[6] Proteins are composed of long chains of amino acids. A protein's shape, properties, and biological functions are determined in part by the specific sequence of its constituent amino acids. *Peptides* are short amino acids.

[7] Antibodies are blood proteins produced in B lymphcytes, a type of white blood cell, in response to the introduction Of a specific antigen (e.g., an invading virus, incompatible red blood cells, inhaled pollen grains, or foreign tissue grafts). Once produced, the antibody has the ability to combine with the specific antigen that stimulated antibody production, and thereby render the antigen harmless, a process called neutralization.

[8] Cellular immunity is also called *cell-mediated* immunity.

TABLE 2-2: Design of Contemporary Viral Vaccines

Preparation	Vaccine
Live attenuated virus	Adenovirus
	Measles
	Mumps
	Polio
	Rubella
	Smallpox (vaccinia)[b]
	Varicella[a]
	Yellow Fever
Inactivated whole virus	Hepatitis A[a]
	Japanese Encephalitis
	Polio
	Rabies
Protein subunit (recombinant)	Hepatitis B
Protein subunit (purified)	Influenza

[a] Under review for licensure.
[b] No longer recommended; smallpox globally eradicated

SOURCE: Office of Technology Assessment, 1995.

ic and can bind and inactivate ("neutralize") virus particles that are free in the circulation but cannot inactivate virus located inside of infected cells. Another type of white cell, the T lymphocyte, participates in cellular immunity. Subtypes of T lymphocytes include CD4+ (helper T) lymphocytes and CD8+ (cytotoxic T) lymphocytes. Cytotoxic T lymphocytes can kill cells undergoing active viral infection. CD4+ (T helper) lymphocytes are necessary for the development of mature functional lymphocytes. A third type of immune white cell, the microphage, is an important intermediary in the development of the immune response.

Historically Successful Vaccines

Review of the design of contemporary viral vaccines provides background for understanding the strategies available for the design of an HIV vaccine. Contemporary viral vaccines, in fact, follow only a few basic designs (table 2-2). Eight are live attenuated (weakened) vaccines, four are inactivated (killed) whole virus vaccines, and two are protein subunit vaccines. Hepatitis B is the sole vaccine prepared using recombinant biotechnology (gene splicing) techniques. Both attenuated and inactivated poliovirus vaccines are available. Most successful viral vaccines are live attenuated and, less frequently, inactivated whole-virus products.

A common feature of vaccines currently in use is their ability to induce durable circulating antibody, usually persisting for many years. A low level of antibody directed against the virus maybe sufficient for protection against establishment of viral infection. For some viruses, such as measles virus, the rapid immune recall due to vaccine priming may be sufficient to protect against infection; for protection against other viruses, such as influenza virus, a preexisting threshold level of virus-specific antibody is necessary. For other vaccines, a vaccine-induced cytotoxic T lymphocyte response may also participate in protection (e.g., varicella).

Currently used vaccines are capable of preventing the initial viral infection from becoming established and progressing to *disease;* they are not capable of preventing the initial viral *infection.* This distinction is important to understanding the requirements for an effective HIV vaccine. Live attenuated vaccines, composed of live virus that has been altered to make it incapable of producing disease, most closely reproduce the immune state seen after natural infection. Attenuated vaccines may induce, in addition to circulating antibody, a

TABLE 2-3: HIV Structural Elements and Their Function

Structure	Viral Function	Immune Significance
Envelope proteins	Cell attachment and penetration	Induce antibody
gp160	Precursor of gp120, gp41	
gp120	External protein	
gp41	Membrane anchor	
Internal proteins		Important CTL sites
gag	Structural, viral assembly	
pol	Facilitates replication	
Auxiliary proteins (6) e.g., *nef*	Regulate level of virus activity	Selective deletion produces attenuated virus vaccine
RNA genome	Genetic code for all viral proteins (virus has a latent DNA stage in host chromosome)	Use of infectious DNA as vaccine

KEY: CTL = Cytotxic T lymphocytes

SOURCE: Office of Technology Assessment, 1995

cytotoxic T lymphocyte response and mucosal immunity. Further, unlike nonreplicating vaccines, live attenuated vaccines generally do not require multiple primary and booster doses. In practice, before the era of modern biotechnology, inactivated whole virus and live attenuated viruses were usually tried empirically, and live attenuated vaccines were preferred as a more reliable source of long-term protection.

▮ Historically Unsuccessful Vaccines

The number of infectious agents for which we have failed to develop a satisfactory vaccine, even those targeted as high priority (49), is far greater than the number for which we have been successful. Examples of viruses for which we have failed to develop a vaccine include the viruses herpes simplex, infectious mononucleosis, cytomegalovirus, respiratory syncytial virus, and rotavirus; vaccines against many sexually transmitted disease agents, such as syphilis and gonorrhea; vaccines against parasitic diseases, such as malaria and schistosomiases; and vaccines against numerous bacterial infections, including tuberculosis. Individually, these infections are characterized by such features as chronic persistence of the organism, restriction of the organism to mucosal sites, genetic variability of the organisms, and lack of spontaneous recovery from the disease that they cause. Vaccines that have been successful are more likely to be directed against acute, self-limiting systemic[9] infections, where immune responses can readily clear residual organisms.

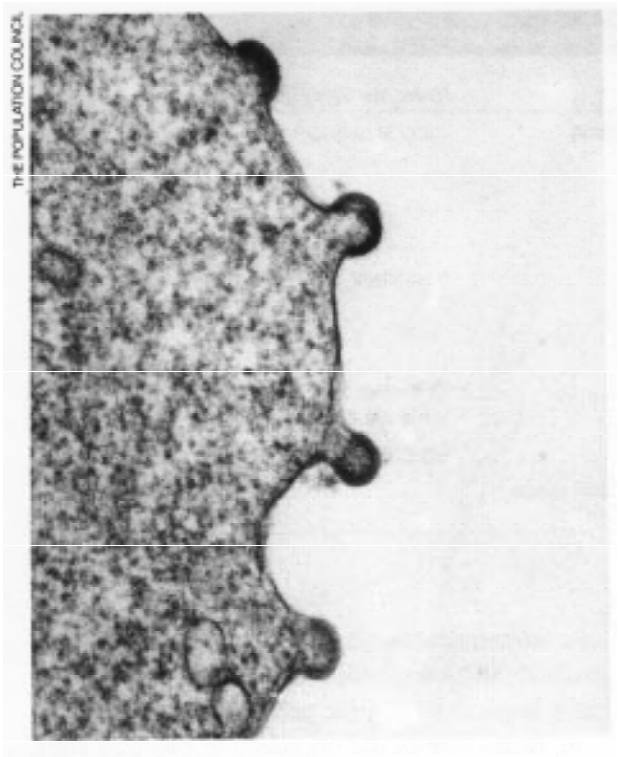
HIV ISA UNIQUE VIRUS

▮ HIV Structure: Starting Point for Vaccine Design

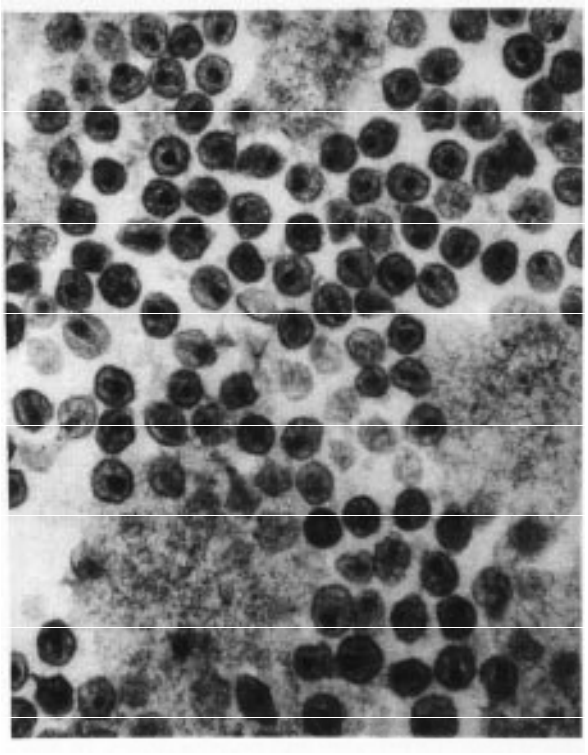
A brief description of HIV structural elements and their function will facilitate later discussion. The virus is bounded by a membrane with the gp160 protein projecting through the membrane surface or envelope (see table 2-3 and Figure 2-1). The envelope gp160 protein is composed of, and is precursor to, the gp120 and gp41 envelope proteins. [10] The envelope protein gp120 bears the V3

[9] Systemic infections involve the whole body, in contrast to localized infections, which may involve one specific organ or body part.

[10] The "gp" refers to its composition of glycoproteins (proteins bound with sugars), and the numbers 160, 120, and 41 refer to a measure of each glycoprotein's weight.



Electron micrograph of HIV virions budding from an infected cell.



Electron micrograph of free HIV virions.

loop, which is the site of attachment of the human immunodeficiency virus to its receptor on the surface of the CD4+ lymphocyte. The V3 loop of the gp120 protein is also the site for induction of neutralizing antibody (antibody that specifically binds to, or "neutralizes," the antigen); antibody to gp120 can block HIV from entering and propagating in cells.

The viral membrane encloses two major internal components, the *gag* and proteins, and several small auxiliary proteins that control the rate of virus replication[11] (see figure 2-l). The genetic information, or genome, of HIV is composed of ribonucleic acid (RNA); by contrast, the human genome (and that of most other species) is composed of deoxyribonucleic acid (DNA). The RNA genome of HIV is associated with the internal proteins. Epitopes on the gp120 and *gag* proteins, as well as those on other internal proteins, can induce cytotoxic T lymphocyte responses necessary for cellular immunity **(4).**

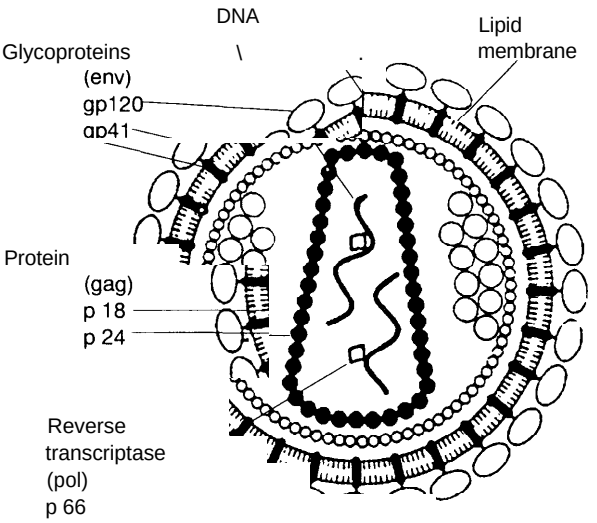
■ Properties of HIV That Handicap Vaccine Development

Because of several unique features of HIV, the model for an effective HIV vaccine is much more complicated than the model for contemporary vaccines. HIV is endowed with an unusual set of capacities that enable it to evade or manipulate normal immune defenses (table 2-4). These capacities are listed below:

1. HIV incapable of evading immune surveillance by integrating its genome into the genome of infected cells. During replication, the human immunodeficiency virus undergoes a stage " where its RNA genome is transcribed into DNA by a process called "reverse transcription." As a necessary part of its life cycle inside

11 These small auxiliary proteins are called *regulatory* or *accessory* proteins.

FIGURE 2-1: Morphology of HIV-1 and Diagram of the Structural Relation of Major Viral Proteins



SOURCE: G C. Schild, and P D Minor, "Human Immunodeficiency Virus and AIDS, Challenges and Progress, " *Lancet* 335 1081-1084, 1990

TABLE 2-4: Mechanisms by Which HIV Evades Immune Control

- Latency in chromosomal DNA
- Extensive genetic diversity
- Virus infects and destroys critical immune cells
- Spread by microphage and direct cell fusion
- Silent transmission during prolonged latent infection
- Transmitted by three routes, as free- or cell-associated virus
- Recovery from infection not known, providing no clues to protective mechanisms
- Primate models offer no clear guidance to protective mechanisms

SOURCE Off Ice of Technology Assessment, 1995

the cell, HIV DNA must integrate into the DNA of the human chromosome in the cell nucleus. While the HIV genome is integrated into the human genome, it is hidden from immune surveillance and cannot be recognized and eliminated. While it is integrated, the HIV genome is latent and not replicating. HIV may persist in this sanctuary, later to reactivate, replicate, and shed new virus from the cell.

2. The virus can undergo genetic change through a process of rapid genetic mutation and selection of viral mutants resistant to preexisting antibody. Viral mutations can occur at epitopes, the key sites normally recognized and attacked by antibody and immune cells. These mutations may render the epitopes unrecognizable, allowing the virus to avoid immune elimination. During the lengthy course of infection in a single individual, new genetic variants of HIV emerge.

Globally, at least six major subtypes (clades) of HIV have been identified based upon genetic analysis (70). Subtype B has been isolated in the Americas, Western Europe, and in parts of Southeast Asia. Substantial genetic variation is found even within each subtype of HIV (73). A significant consequence of the genetic diversity of HIV is that the immune response directed to one HIV strain may not necessarily protect an individual from other subtypes of HIV or from different strains within the same subtype of HIV. Therefore, there is consensus that HIV strains used to prepare vaccines must match HIV specimens that are freshly isolated from infected individuals in the region where the vaccine is to be used (so called "fresh primary field isolates") (103).

3. Virus spreads through the body soon after initial contact with the surface mucus membranes (the mucosa) of the vagina, anus, and penile urethra, the sites of sexual transmission. The virus selectively invades and can injure the very cells that play central roles in immune defense, the CD4+ (T helper) lymphocytes and the macrophages.
4. Virus that infects and is sheltered by macrophages may spread to other sites, such as the central nervous system, a body compartment

where access of immune cells and antibody is poor.[12] Virus can also spread by direct cell-to-cell contact through a process of direct fusion, again avoiding immune inactivation.

5. HIV infection is chronic, with a variable number of years of apparent clinical wellness preceding the onset of HIV-related illnesses. Despite the presence of vigorous, sustained antibody and cytotoxic T lymphocyte responses to HIV, the virus continues to multiply to high concentrations (titers) in immune cells in lymphoid tissues of the body. The virus remains silently transmissible. When a sufficient number of CD4+ lymphocytes are injured and lost, the acquired immunodeficiency syndrome, AIDS, becomes clinically apparent, with eventual death. The progression of HIV-related immune dysfunction is classically monitored by measuring the fall in concentration of circulating CD4+ lymphocytes.
6. HIV can be transmitted by three different routes, which, in itself, can complicate the task of developing a vaccine that can induce an effective immune blockade. HIV is acquired by sexual contact with mucosa of the vagina, rectum, or penis; by direct inoculation into the blood stream; or by transfer from mother to fetus or infant through the uterus, at birth, or through breast milk. Protecting the mucosa against infection presents special challenges because of the difficulty in inducing mucosal immunity through vaccination. Virus may be transmitted as free virus or as virus carried inside cells (see photos 2-1 and 2-2). It is more difficult to block the transmission of virus in infected cells; different immune mechanisms are required.
7. Unlike other viral infections that are self-limited, there are few, if any, instances of recovery from HIV infection to offer clues for understanding the key immune response elements that are necessary for protection from the virus.
8. Animal models of human HIV infection, using monkeys and other primates, have not yet yielded definitive guidance to the immune elements necessary for protection.

ANIMAL MODELS

What Has Been Learned from Animal Models?

Animal models of infection historically have contributed to the development of vaccines in two general ways: 1) use of animal models has helped to define interactions between the virus and the infected organism or *host*, particularly in understanding the immune responses necessary for control of infection; and 2) animal models have provided a system to predict the behavior of a candidate vaccine in man. The primate model can be used to provide an initial assessment of vaccine concepts, test a vaccine's immune potential, provide evidence of protection against challenge virus, and screen the vaccine for safety. Scientific opinion varies concerning the significance and validity of primate studies as a guide to HIV vaccine development and as a criterion for judging the eligibility of a vaccine for participation in efficacy trials (83). However, as our understanding expands, patterns of primate infection are emerging that should permit more focused studies.

Primate Systems

The chimpanzee is the only animal in which HIV will replicate. However, chimpanzees have severe limitations as animal models. Chimpanzees are expensive and their supply is limited; a typical study may involve two chimpanzees given experimental vaccine and one chimpanzee receiving placebo vaccine for comparison. In the chimpanzee, the virus causes a minimal persistent infection, waning over time, with no disease manifesta-

[12] The central nervous system includes the brain and spinal cord, and is separated from the other body compartments by the "blood-brain" barrier. Certain immune response components, including certain white cells and antibody, are limited in their ability to traverse this brain barrier.

tions. Some fresh human HIV isolates may actually fail to infect chimpanzees.

Macaque monkey infection with simian immunodeficiency virus (SIV) provides an important parallel to HIV infection in humans. SIV, a retrovirus that is in the same virus family as HIV, is highly virulent in macaques, with induction of high concentrations (titers) of antibodies and persistent infection leading to an AIDS-like syndrome within 6 to 24 months of the infection. The rapidity of disease progression varies with the level of virulence[13] of the SIV strain used. Unlike chimpanzees, the macaque is readily available.

Human immunodeficiency virus, type 2 (HIV-2) causes human AIDS restricted to West Africa. HIV-2 is more closely related to SIV than HIV-1, grows poorly in monkeys, and does not grow at all in chimpanzees.

Protection Under Optimal Conditions

There are examples of vaccine protection or partial protection in primates, largely under optimal circumstances, for example where vaccinated primates were exposed to virus immediately following the final dose of vaccine (which corresponds to the height of the immune response elicited by the vaccine), where vaccinated primates were "challenged" with virus that was homologous to (i.e., of the same strain as) the virus used in the preparation of vaccine, and where small doses of cell-free virus were inoculated directly into the blood stream by the intravenous route (8, 30, 47, 83, 101). Also, large doses of antibody administered to the chimpanzee have been shown to provide passive protection from infection with HIV for several hours, but no longer (24).

Studies using the SIV/macaque model have shown that it may not be necessary for a vaccine to attain sterilizing immunity (to clear all virus) to protect against disease (44). If this is also true of HIV in humans, it may lower the requirements for an effective HIV vaccine. Importantly, vaccine protection against SIV infection of the vaginal mucous membranes of macaques has been accomplished recently using microspheres, which permit slow release of antigen (58).

Live attenuated vaccines show a high level of protection against SIV infection in macaques. The promise of live attenuated vaccines and their safety concerns are discussed later in this chapter (19, 20, 21).

Inconsistent Results in Primate Studies

Primate studies conducted over the past decade have been subject to inconsistent results that are sometimes difficult to duplicate. It is now appreciated that the outcome of a vaccine challenge experiment can vary depending on the relative virulence of viral infection in different primates, the choice of virus strain, the dose of virus, the route of viral inoculation, the history of the virus, and other specific conditions of viral challenge (10, 83). Understanding these variables allows investigators to select primate systems that pose higher or lower hurdles for vaccine protection. For example, protection against HIV infection in the chimpanzee (the HIV/chimpanzee model) appears to be more readily accomplished than protection against the more lethal SIV infection in the macaque (the SIV/macaque model). Success in the less virulent HIV/chimpanzee model frequently cannot be duplicated in the more virulent SIV/macaque model. Both models are helpful in understanding HIV in humans. The HIV/chimpanzee system models silent persistent HIV infection of humans; the SIV/macaque model parallels HIV disease progression in humans.

IMMUNE CORRELATES OF PROTECTION

Knowledge of the specific elements of an immune response required to protect against HIV infection (the immune correlates of protection) would help guide the design of an effective HIV vaccine. Two approaches to understanding such correlates are available: 1) experiments using experimental vaccines in primates, and 2) observations that may

[13] The *virulence* of a microorganism refers to its capacity to produce disease.

suggest the development of a protective immune response in human HIV infection. While primate studies have shown examples of protection under limited circumstances, as yet the immune responses required for a successful HIV vaccine remain undefined. Levels of antibody induced in primates by vaccines are, in themselves, not well correlated with protection against HIV infection.

What is the evidence for natural immunity to HIV infection in man? Studies of the natural history of long-term survivors of HIV infection have helped us know what are the clinical indicators of sustained favorable prognosis in HIV infection. But these studies have been less useful in helping us understand the requirements for a protective immune blockade to HIV infection (57). Studies of individuals who have remained seronegative[14] despite intense exposure to HIV, such as infants of seropositive mothers (78) and multiply-exposed men (17) have shown that some of these individuals have developed protective patterns of immune response, suggesting that "natural immunization" to HIV infection may occur.

DEVELOPMENT AND CLINICAL EVALUATION OF HIV VACCINES

U.S. Program of HIV Vaccine Development

The U.S. Public Health Service established a program of discovery, development, and clinical trials directed toward making available a safe and effective preventive HIV vaccine. The effort is centered at the National Institutes of Health (NIH) with the National Institute of Allergy and Infectious Diseases (NIAID) as the lead institute. Fundamental and applied studies of HIV molecular biology, pathogenesis, and immunopathology and of HIV vaccine development have been fostered by a variety of funding strategies, enabling interactive research among scientists in the U.S. and abroad.

The NIAID Division of AIDS (DAIDS) created a network of primate centers to study HIV infection in the chimpanzee and SIV infection in lower primates. The DAIDS AIDS Vaccine Clinical Trial Network (AVCTN) has several components. The AIDS Vaccine Evaluation Group (AVEG) includes six AIDS Vaccine Evaluation Unit (AVEU) trial sites at university research centers. Each unit has an associated Community Advisory Board. Other AVCTN elements include a Central Immunology Laboratory, which develops standards and performs most of the immunological assays, a Mucosal Immunology Laboratory, and a Data Coordinating and Analysis Center. A Data and Safety Monitoring Board exercises independent oversight of HIV vaccine trials.

The process of testing a candidate vaccine in clinical trials is initiated by a sponsor, which presents preclinical data to the Food and Drug Administration's (FDA's) Center for Biologicals Evaluation and Research (CBER) for review. FDA assesses data from laboratory studies of the vaccine, data from animal studies, and other "preclinical data" for evidence of the vaccine's safety, potency, and potential for efficacy. The FDA is also responsible for approval and oversight of experimental protocols as vaccines progress through clinical trials.

Vaccine sponsors may present data from preclinical studies of their vaccines to the AIDS Vaccine Selection Group; the group will consider this material in determining which vaccines will be entered into federally funded AVEG trials. A unified approach to trial design, clinical assessment, laboratory assays, and data analysis permits direct comparisons among multiple vaccine strategies and products.

Other major participants in HIV vaccine development include the National Cancer Institute, the

[14] An individual that is *seronegative* for HIV infection has a negative result on a test for HIV infection, and a *seropositive* individual has a positive test. The enzyme-linked immunosorbant assay (ELISA) is the most commonly used screening test for HIV infection. The ELISA indirectly determines whether one is HIV infected by testing for the presence of antibodies to HIV. Because antibodies to HIV may not appear for two or more weeks after initial HIV infection, some "seronegative" individuals may actually be infected with HIV.

Centers for Disease Control and Prevention (CDC), vaccine manufacturers, universities, the World Health Organization (WHO), and the Department of Defense. These participants contribute capacities for research, product development, and conduct of clinical trials in the United States and other developed countries, as well as in the developing world.

Design of Clinical Trials (Phases I and II)

Promising candidate vaccines are selected for initial assessment of immune responses and safety in carefully monitored, prospectively randomized, double-blind, placebo-controlled clinical trials.[15] Phase I and II are described below, and Phase III (large controlled clinical trials of a vaccine's efficacy) are described in a later section. The FDA approval process involves three phases.

Phase I focuses on an assessment of vaccine safety and the immune responses to the vaccine. Phase I study protocols involve 25 to 100 individuals who are randomly assigned to either a placebo control group or one or more experimental groups. Recruitment for Phase I studies involves selection of healthy noninfected individuals who are prescreened and undergo a full physical and laboratory examination. Volunteers are selected to be at low risk for HIV infection to minimize their potential for acquiring confounding HIV infection during the trial. Trial participants receive detailed individual counseling and education on the experimental nature of the vaccine, the design of the trial, and possible adverse consequences of the vaccine. Informed consent for trial participation is obtained from each volunteer. The effects of varying the vaccine's dose of antigen, schedule of administration, and ratios of adjuvant to antigen are determined in Phase I studies.

If the immune responses to the vaccine and the safety profile of the vaccine warrant further studies, it may undergo Phase II trials, which involve up to a few hundred individuals. These studies refine and enlarge the database, may directly compare products or sequences, or may include individuals at higher risk of acquiring infection.

Role of Industry

The role of U.S. industry, traditionally a world leader in vaccine development and marketing, deserves special comment. There is a long list of candidate vaccines in trials or in development (tables 2-5, 2-6, 2-7). Not all vaccines in development will be eligible for Phase I trials. HIV vaccine sponsors, to a large extent, are small biotechnology companies, private research institutions, and universities (98). Some of the large pharmaceutical manufacturers in the Unites States are not sponsoring an HIV vaccine. There may be different market forces affecting large companies and small companies that affect their decisions to become involved in HIV vaccine development. Some have argued that the compelling global progression of the AIDS epidemic warrants exploration of special incentives to attract increased participation of both small and large companies.

Corporate decisions to invest in the development of an HIV vaccine are based on several considerations, including the opportunity costs of vaccine development relative to drug development, the potential market for an HIV vaccine,

[15] A prospective, randomized, double-blind, placebo-controlled study design minimizes threats to the validity of the study. Prospective studies are ones where the investigator observes the participants from the beginning of the study on; in retrospective studies, observations are made after the study is completed. A randomized trial refers to one in which participants are randomly assigned to experimental and control groups; random assignment helps ensure that each of the groups are equivalent. A double-blind trial is one in which both the clinician and the subject are unaware of the group to which the subject has been assigned; this minimizes the risk of bias that may be introduced when either the clinician or subject is aware of the subject's assignment. A *controlled trial* is one in which one group of participants (the control group) is assigned to a receive a either a placebo or a standard comparison treatment. A *placebo* is an inert substance which, in the context of a controlled trial, is made to appear identical to the active experimental treatment. Comparison of one or more *experimental* groups with the control group allows the investigators to determine the impact of the experimental treatment.

TABLE 2-5: Current U.S. and Foreign Phase I/II Clinical Trials[a] of HIV Vaccine Candidates in Noninfected Adults

Vaccine	Developer	Trial sites or sponsor
Envelope proteins		
rgp160-LAI [b] (insect) [c]	MicroGeneSys	AVEG[d]/LIR
rgp160-LAI (mammalian)	Immuno AG	AVEG
rgp160-MN (mammalian)	Immuno AG	AVEG
rgp120-LAI (mammalian)	Genentech	AVEG
rgp120-MN (mammalian)	Genentech (Phase II)	AVEG
rgp120-SF2 (yeast)	Biocine	AVEG
rgp120-SF2 (mammalian)	Biocine (Phase II)	AVEG, SFGH
Virus-like particles		
Ty.p24.VLP	British Biotechnology, Ltd.	London, UK
Peptides		
V3-MAPS	United Biomedical, Inc.	AVEG, SFGH, China, Australia
V3-MAPS (15 component)	United Biomedical, Inc.	AVEG
V3 peptide PPD conjugated	SSVI	SSVI
V3 peptides PPD conjugated	SSVI	Israel
V3 peptides conjugated to *Pseudomonas aeruginosa* toxin A	SSVI	Switzerland
HGP-30 (p17 peptide)	Viral Technologies, Inc.	SFGH/United Kingdom
Vectors		
Vaccinia-gp160-LAI	Bristol-Myers Squibb	AVEG/University of Washington
Canarypox-gp160	Pasteur-Merieux-Connaught	AVEG
Combinations of Vaccines		
Vaccinia-gp160 plus rgp160	Bristol-Myers Squibb, MicroGeneSys	AVEG, University of Washington
Vaccinia-gp160 plus rgp120 (yeast) or rgp120 (mammalian cell produced)	Bristol-Myers Squibb, Biocine	AVEG
Vaccinia-gp160 plus rgp160 plus 3 envelope peptides	G. Beaud, Institut Jacques Monod; A. Burney, University Libre de Bruxelles	Paris, France
Vaccinia-gp160 plus rgp160 or rgp12 (MN, LA1 or SF2)	Bristol-Myers Squibb; Immuno AG; Genentech; Biocine	AVEG
Canarypox-gp160 plus rgp160	Pasteur-Merieux-Connaught (Virogenetics, Transgene)	PMSV/ANRS
Vaccinia - *env, gag, pol*	Therion	AVEG
rgp160 plus V3 peptide	Pasteur-Merieux-Connaught (Transgene)	PMSV/ANRS
rgp120 (LA1)) plus rgp120 (MN) (sequentially or simultaneously)	Genentech	AVEG

[a] All vaccines listed are in Phase I trials, unless otherwise indicated .
[b] HIV-strains represent a group of clade B isolates from the United States and Europe, which includes LAI, IIIB, MN, and SF2.
[c] Cell substrate for recombinant subunit protein.
[d] The AIDS Vaccine Evaluation Group is a component of the AIDS Vaccine Clinical Trials Network, The network includes Johns Hopkins University, Baltimore, MD; St. Louis University, St. Louis, MO; University of Rochester, Rochester, NY; University of Washington, Seattle, WA; Vanderbilt University, Nashville, TN. Former members were Baylor University, Houston, TX and University of Maryland, Baltimore.

KEY: AVEG = AIDS Vaccine Evaluation Group of AIDS Vaccine Clinical Trials Network; LIR = Laboratory of Immunoregulation; SFGH = San Francisco General Hospital, CA.

SOURCE: Adapted from M.C. Walker and P.E. Fast, *Clinical Trials of Candidate AIDS Vaccines* (in press).

TABLE 2-6: Current Clinical Trials of HIV Vaccine Candidates in Noninfected Adults that Compare Adjuvant Formulations.

Vaccine	Developer	Adjuvants Compared	Adjuvant Source	Trial Site
rgp120-MN	Biocine	Alum	Superfos/AS	AVEG
		MPL	Ribi lmmunoChem Res.	
		Liposome-encapsulated MPL with alum	C. Alving/WRAIR	
		MF59	Biocine	
		MF59 + MTP-PE	Biocine	
		SAF/2	Syntex/Biocine	
		SAF/2 + MDP	Syntex/Biocine	
rgp120-MN	Genentech	Alum	Reheis	AVEG
		QS21	Cambridge Biotech	
		Alum + QS21	Reheis/Cambridge Biotech	

KEY: alum = Aluminum hydroxide; AVEG = AIDS Vaccine Evaluation Group of AIDS Vaccine Clinical Trials Network; MDP = Muramyl dipeptide; MF59 = Microfluidized oil-in-water emulsion; MPL = Monophosphoryl lipid A; MTP-PE = Muramyl tripeptide-phosphatidylethanolamine, QS21 = Purified saponin adjuvant; SAF = Syntex adjuvant formulation; WRAIR = Walter Reed Army Institute of Research

SOURCE: Adapted from M.C. Walker, and P E Fast, *Clinical Trials of Candidate AIDS Vaccines,* in press

whether the development of an effective HIV vaccine is scientifically feasible, and potential liability for unforeseen adverse reactions to HIV vaccines. Of the disincentives to HIV vaccine development, scientific feasibility is a primary concern. The development of an HIV vaccine is hampered by a lack of clear scientific objectives, a consequence of the undefined protective immune requirements for an HIV vaccine.

Concerns surrounding the safety of an effective vaccine may also play a role in corporate decisions. Notably, manufacturers have pursued the development of HIV vaccines composed of envelope subunit proteins, which have inherently more limited immune capability than HIV vaccines composed of whole inactivated virus or live attenuated virus. Manufacturers have not, however, pursued the development of inactivated virus vaccines or live attenuated virus vaccines because of the greater inherent potential for safety problems from these vaccines. This is despite the fact that HIV vaccines based on these more classical vaccine designs are far more promising. Recognizing this, research on attenuated virus vaccines for HIV has been supported by the DAIDS program. (Recently, some manufacturers have expressed interest in developing an inactivated virus vaccine.)

There appears to be no unanimity on the relative importance of concerns about potential liability in corporate decisions to invest in the development of an HIV vaccine. Some cite potential for liability as a part of the "cost of doing business," to be considered along with scientific feasibility, marketing potential, and other business considerations. Industry may need further encouragement through special incentives to undertake unusual risks.

ADVERSE REACTIONS

Safety Lessons Learned from Experience with Traditional Vaccines

Safety Standards for Prophylactic Vaccines

The standard of safety applied to prophylactic vaccines is higher than that applied to other tools in the medical armamentarium. Historically, vaccines, especially those designed for universal use in children, have been held to extremely high safety standards. A vaccine is given to uninfected, healthy individuals to prevent potential disease for which the vaccinee mayor may not be at risk at a future time. In this setting, any significant injury, even occurring in one in a thousand or million recipients, may be considered unacceptable.

TABLE 2-7: Vaccine Strategies and HIV Vaccine Candidates in Preclinical Development

Candidate	Expression system/ production method	Adjuvant or delivery system	Developer
Strategy: Targeting of immune response to specific HIV neutralization (B cell) epitopes and/or cytotoxic T lyrnphocyte (CTL) epitopes.			
rgp160	Mammalian	Oil/water, 3-deacyl monophosphoryl Lipid A	SmithKline Beecham
rgp120	Insect	Oil/water, 3-deacyl monophosphoryl Lipid A	SmithKline Beecham
V3-MAPS[a]	Synthetic	Alum (slow release for mulation)	United Biomedical, Inc.
Ty.V3.VLP	Yeast	Alum/none	British Bio-tech., Ltd.
T1 -SPIO(A)	Synthetic	IFA	B. Haynes, Duke University
V3-T helper epitope peptides (PCLUS 3-18, PCLUS 6-18)	Synthetic	IFA, QS21	National Cancer Institute,
CLTB-34, CLTB-36, p24E-V3MN	Synthetic chimeric V3-p24 gag peptides	Alum, QS21	Connaught
V3 and gag peptides[a] coupled to lysine copolymers	Synthetic	Alum	Yokohama City University, Japan
V3-BCG	Recombinant mycobacteria	—	Nagasaki and Osaka Universities, Japan
V3-BCG[a]	Recombinant mycobacteria	—	NIH, Japan
V3 peptide coupled to Mycobacterium protein	Synthetic	10K mycobacterium protein	SSVI
env peptides coupled to beta-gal	E. coli	IFA	WRAIR-Univax
CD4 binding domain peptomer	Synthetic, conformationally constrained	Alum	F.A. Robey, NIDR
HBcAg-v3 particles	E. coli	—	Max V. Pettenkofer-Institut, FRG
Recombinant rhinovirus - HIV V3 peptides	Recombinant human rhinovirus (HRV14)	—	Rutgers University
Recombinant mengovirus - HIV, V3, V4 peptides	Recombinant murine mengovirus (attenuated)	—	Institut Pasteur

(continued)

TABLE 2-7: Vaccine Strategies and HIV Vaccine Candidates in Preclinical Development (Cont'd.)

Candidate	Expression system/ production method	Adjuvant or delivery system	Developer
Strategy: Mimicry of attenuated or inactivated **HIV**			
Whole inactivated HIV	Inactivated with betapropiolactone, BEI, formaldehyde	Digitonin	Retroscreen, Ltd./ISI
HIV env, *gag, pol* pseudovirions[b]	Mammalian/vaccinia	Alum	Therion Biologics
HIV *env, gag, pol* pseudovirions[b]	Mammalian (Vero)	—	Connaught
Gag-V3 virus-like particles	Insect cells/baculovirus	—	Universitat Regensburg, FRG
p55gag/V3 chimeric vaccinia	Recombinant vaccinia	—	Universitat Regensburg, FRG
TBC-3B, (vaccinia-HIV *env, gag, pol)*[b]	Recombinant vaccinia	—	Therion Biologics
Vaccinia-HIV *env, gag, pol* [b]	Attenuated recombinant vaccinia (NYVAC)	—	Pasteur-Merieux-Connaught (Virogenetics)
Canary pox-HIV *env, gag, pol* [b]	Recombinant canarypox (ALVAC)	—	Pasteur-Merieux-Connaught (Virogenetics)
HIV expression vector coated with 1.0 micron gold particles	DNA	particle acceleration device	Agracetus
pM160, (HIV envelope gp160 DNA construct)	DNA	—	University of Pennsylvania School of Medicine
Strategy: Induction of mucosal immune responses in gastrointestinal and genitourinary tracts.			
Adenovirus-Hiv env or gag	Recombinant adenovirus (Ad4, Ad5, Ad7 vaccine strains)	—	Wyeth-Ayerst
Poliovirus-HIV	Recombinant poliovirus	.	—
Poliovirus-HIV envelope peptides	Recombinant dicistronic poliovirus	—	SUNY, Stony Brook
Poliovirus-HIV nef, gag, env	Recombinant poliovirus (Mahoney type 1, Sabin types 1 and 2)	—	Gladstone Institute, UCSF
Encapsidated recombinant poliovirus HIV env, gag, or pol minireplicons	Encapsidate recombinant poliovirus	—	UAB

(continued)

TABLE 2-7: Vaccine Strategies and HIV Vaccine Candidates in Preclinical Development (Cont'd.)

Candidate	Expression system/ production method	Adjuvant or delivery system	Developer
Strategy: Induction of mucosat immune responses in gastrointestinal and genitourinary tracts. (Cent'd.)			
Mengovirus-HIV nef	Recombinant mengovirus (attenuated Ml 6 Murine strain)	—	**Gladstone Institute UCSF**
Shigella-V3 peptide	Recombinant Shigella flexneri (attenuated strain SC602)	—	**Institute Pasteur, France**
Salmonella-HIV gp120, p24, nef	Recombinant Salmonella typhi (CVD 908 vaccine strain)	—	**University of Maryland**
BCG-HIV env peptides	Recombinant BCG	—	**Medimmune,** Inc.
BCG-HIV	Recombinant BCG	—	—
Recombinant Lactococcus-V3 peptide	Fusion of V3 peptide to TT fragment C in Lactococcus lactis	—	University of Cambridge, UK
Env-PND-gag-HGP-30 conjugate	Synthetic peptide	Cholera toxin B	Viral Technologies, Inc.; Alpha-1 Biomedical:
rgp 120	Recombinant protein	Liposome/Cholera toxin	UAB/Connaught
V3-MAPS[a]	Synthetic	Microparticles	United Biomedical, Inc.
Tetravalent MAP-gp120 sequence coupled to a ipophilic moiety	Recombinant protein	Synthetic lopophilic moiety	Vanderbilt University
Strategy: Development of HIV-2 vaccines			
Whole inactivated HIV-2	Triton or formalin inactivation	IFA, alum, RIBI adjuvant, ISCOMS	National Bacteriological Laboratory, Sweden
gp125	Purified native glycoprotein	ISCOMS, RIBI adjuvant	National Bacteriological Laboratory, Sweden
gp130	Purified native glycoprotein	IFA, alum	National Bacteriological Laboratory, Sweden
rgp160	Baculovirus	—	German Primate Center, FRG
Vaccinia-HIV-2 env, gag, pol	Attenuated recombinant vaccinia (NYVAC)	—	Virogenetics
Canarypox-HIV-2 env, gag, pol	Recombinant canarypox (ALVAC)		Virogenetics
Vaccinia HIV-2 env	Recombinant vaccinia	—	German Primate Center, FRG
Salmonella-HIV-2 env, gag	Recombinant Salmonella typhimurium	—	National Cancer Institute

[a]Contains non-clade B strains.
[b]Multiple genetic deletions introduced for Safety.

KEY: BCG = Bacille-Caimette Guerin; bovine tuberculosis; IFA = incomplete Freund's adjuvant; LAl = group of closely related HIV isolates that includes LAV, IIIB, BRU, etc ; MAP = multiple antigent peptide; MAPS = multiple antigen peptide; MAPS = multiple antigen Presentation system; NIDR = National Institute of Dental Research, National Institutes of Health, SSVI = Swiss Serum and Vaccine Institute, Berne, Switzerland; SUNY= State University of New York; 11 = tetanus toxin; UAB = University of Alabama at Birmingham; UCD = University of California, Davis; UCSF = University of California, San Francisco; WRAIR = Walter Reed Army Institute of Research.

SOURCE: Adapted from Walker, MC., Fast, PE.,*Clinical Trials of*Candidate AIDS Vaccines, in press

By contrast, there is greater tolerance for adverse reactions accompanying the administration of a therapeutic drug given as treatment for an existing disease. Further, this tolerance is proportionate to the severity and unfavorable prognosis of the illness treated. For example, severe side effects may be considered acceptable in cancer chemotherapy.

The concept of an "acceptable" risk has not been applied to vaccines. Good public health practice at the population level may at times be in conflict with the goal of near-zero risk to the individual. Attenuated polio vaccine has eradicated poliomyelitis from the Americas, yet each of the few vaccine-associated paralytic cases annually has given rise to a compensation claim.

Types of Adverse Events Seen with Traditional Vaccines

Vaccines are prepared from biologically active starting materials with inherent potential for harmful effects. Early adverse reactions, occurring within hours or days after vaccination, may be local (e.g., sore arm) or systemic (e.g., fever, malaise), and typically are minor, transient, and without residual effects. Severe reactions have occurred very rarely to vaccines currently in use; these include anaphylaxis (a severe allergic hypersensitivity reaction) (e.g., tetanus toxoid) and neurologic disease (e.g., pertussis vaccine).

Causal relationships with illnesses occurring long after vaccination may be particularly difficult to document and to distinguish from the occurrence of unrelated diseases. Relationships may be perceived between illnesses and vaccination that are not, in fact, causally related. The difficulty in assigning cause is exhaustively reviewed in two reports by the Institute of Medicine (IOM) of the National Academy of Sciences (48, 49).[16] The IOM reports are based on accumulated experience with millions of doses of licensed vaccines used worldwide, many in use for decades. The findings provide the basis for compensable awards by the Vaccine Injury Compensation Program (Statutory Basis for the National Vaccine Plan: Title XXI of the Public Health Service Act, Public Law 99-660). The IOM reports point to need for: 1) research on mechanisms of induction of adverse events; and 2) prospective, long-term, post-marketing surveillance. Both undertakings are expensive and technically difficult.

Despite the inherent potential for injury from vaccines, licensed vaccines in the United States have a record of remarkable safety and have provided a highly cost-effective method of disease control.

Safety Experience in Phase I and II Trials

Trial Design Using Envelope-Based Vaccines

Initial approaches to HIV vaccine have concentrated on envelope proteins gp160 or gp120. Purified proteins have been produced in three different cell types by recombinant techniques. These envelope proteins may be combined with carrier mole-

[16] In a retrospective analysis of worldwide published studies, the weight of evidence for or against causality of possible adverse events was examined for each of the childhood vaccines. There often was difficulty in assigning cause, but difficulty also in proving lack of cause. Four types of primary evidence were considered: a) biological plausibility; b) case reports, case series and uncontrolled observational studies; c) controlled observational studies; and d) controlled clinical trials. Based on these categories of evidence, the presumed adverse events were classified into five levels of certainty: a) no evidence bearing on a causal relation; b) evidence inadequate to accept or reject a causal relation; c) evidence favors rejection of a causal relation; d) evidence favors acceptance of a causal relation; and e) evidence establishes a causal relationship.

These analyses are then reviewed in the context of the compensable injuries covered by the Vaccine Injury Compensation Program established by Congress in 1986. The childhood vaccines have been in widespread use for many years, and millions of doses have been administered. Despite this historical experience, the data was difficult to interpret. The vast majority of adverse events came from uncontrolled studies and individual case reports. The pathologic conditions under consideration often were uncommon or rare in the population. Because comparative age-specific incidence rates and relative risk estimates of the condition in the general population are rarely available, it was not possible to calculate a statistical rate of excess vaccine-related cases, if any. Controlled epidemiological studies are lacking (48, 49).

cules and injected into the individual to produce an immune response. A second method of immunization with envelope protein uses live vaccinia virus as a "delivery vector" (vaccinia/gp 160 vector); the vaccinia virus genome has been genetically altered to incorporate the HIV envelope gp160 gene. Replication of vaccinia virus in the dermal layer of the skin results in expression of gp160 protein, which in turn induces the immune response. From the initiation of the AVEG program in 1988, more than 1,400 volunteers have participated in trials of envelope-based HIV vaccines (tables 2-5 and 2-6). Twelve envelope-based vaccine products or combinations, formulations, and adjuvants[17] were used, prepared by five manufacturers using three subtype B virus strains. Additional independent trials of envelope-based vaccines have been conducted by U.S. and foreign sponsors.

Immune Responses

The immune responses provide an initial measure of the potential value of envelope vaccines and must be considered in context of adverse reactions accompanying the use of these vaccines (5, 4, 38, 53, 60, 61). Envelope-based vaccines have induced antibodies directed against the strains of virus used to prepare the envelope proteins (homologous strains). The titers (concentrations) of antibody induced by envelope-based vaccines were 5- to 10-fold lower than the titers of antibody found in HIV-infected individuals. Antibody titers are not sustained, falling rapidly after each dose of vaccine. Other subgroup B strains (heterologous strains) were neutralized less well, and freshly isolated strains were entirely resistant.

The evasion of neutralization by freshly isolated strains is of concern and remains under intensive study to determine its significance.

Envelope vaccines, with or without adjuvants, produced no consistent cytotoxic T lymphocyte responses. Priming with vaccinia/gp160 vector vaccine followed by a booster dose of envelope-based vaccine resulted in modest cytotoxic T lymphocyte responses in a few recipients. Envelope-based vaccines that were combined with new adjuvants to enhance vaccine immunogenicity produced modest increases in titers of neutralizing antibody; this enhanced immunogenecity occurred at the expense of an increased rate of local or systemic reactions in some of these vaccines.

Thus, envelope-based vaccines preferentially generated antibody responses and were disappointing in that they failed to generate substantial cytotoxic T lymphocyte responses. The antibody responses elicited by envelope-based vaccines have been judged by many scientists to be marginal with respect to their magnitude, duration, and cross-reactivity with other strains.

Safety Overview

Adverse reactions following vaccination with envelope-based products have been minimally greater than adverse reactions following placebo vaccination. (Eighteen percent of participants in trials of envelope-based vaccines received a placebo vaccination.) In general, the experience with envelope-based HIV vaccines suggests that they have a benign adverse reaction profile, similar to currently licensed vaccines. Sequential measures of biochemical, hematological, and immunological status and kidney and liver function tests showed no significant vaccine-related abnormal findings. Importantly, there has been no evidence of adverse effects on immune function, including CD4+ and CD8+ lymphocyte counts.

Early Self-Limited Adverse Reactions

Envelope-based vaccines with alum adjuvant were associated with local reactions at the injection site, consisting of mild pain, tenderness, redness, and swelling for one to two days. The incidence and type of systemic complaints, such as fever and malaise, were similar to those of placebo

[17] In immunology, an adjuvant is a substance, such as alum, that is added to a vaccine to non-specifically enhance the vaccine's immunogenicity (the vaccine's ability to produce an immune response).

recipients. Addition of some of the new adjuvants, Genentech QS21 and Chiron/Biocine SAF/2, induced transient moderate to severe local reactions and febrile flu-like illnesses for one to three days in a number of recipients (53). None of the vaccinees dropped out of the trials, missed school or work, or had residual consequences. No further studies were undertaken with these adjuvants.

Ten vaccinees developed a rash to several products, and one also developed painful joints (arthralgias). A positive antinuclear antibody (ANA) test (which may at times be associated with autoimmune disease, such as rheumatoid arthritis) was found in a few individuals. However, further testing ruled out any vaccine-related disease. Despite careful screening and counseling, 14 pregnancies occurred. There was no evidence of vaccine-related adverse effects.

Level of Attenuation of the Vaccinia Vector

The trials permitted comparison of the side effects of vaccinia/gp160 vector with the commercial vaccinia strain used to prevent smallpox, from which it had been derived. Smallpox vaccine virus, injected into the dermal layer of the skin, can spread and cause severe or fatal disease in rare instances, especially in individuals with compromised immune systems. The vaccinia vector has been attenuated (rendered incapable of producing disease) as measured in laboratory tests. Reactions to the vaccine resembled those seen following classical smallpox vaccination in individuals who had not been vaccinated previously (36). There were no differences in rates of pustule development at the inoculation site, regional lymph node swelling, or systemic symptoms. The vaccinia virus did not appear to be attenuated and, thus, could carry the risk of vaccinia complications known to occur with classical vaccination (75). Under the controlled conditions of the trial, occlusive dressings were used over the inoculation site, and no secondary spread to other individuals was observed. With broad use of an HIV vaccine, substitution of a more attenuated poxvirus vector, such as canarypox virus, is preferable.

Neoplasms

As of May 1994, 10 neoplasms (tumors) were observed in 9 different protocols (52). One of the neoplasms was benign. At the time of review, more than 1,300 volunteers were in AVEG trials, 18 percent of whom were assigned to a placebo control group. Those neoplasms that were malignant tended to occur in older groups. Analysis by the Data and Safety Monitoring Board and an ad hoc expert committee found no evidence that these neoplasms were linked to any vaccine. The wide variety of tumor types seen in these vaccinees was judged to be biologically incompatible with the hypothesis that there was a causal relationship between these neoplasms and vaccine. The occurrence of such coincidental events exemplifies the need for placebo-controlled trials of HIV vaccines, with careful long-term followup and independent review.

HIV Infections Among Trial Volunteers

A Phase II trial of envelope-based vaccine was conducted in 300 noninfected individuals from groups at high risk for HIV infection. These included men who have sex with men, injection drug users, sexual partners of infected individuals, and teenagers engaged in high-risk sexual behavior. A control group of individuals at low risk for HIV infection was also included for comparison. The trial has provided experience with recruitment, counseling, cohort retention, and compliance. It has also provided information about the acceptability of the vaccine and the effect of vaccine trial participation on risk behaviors. The trial was not designed to determine the efficacy of the vaccine because inadequate numbers of individuals were included. Despite counseling, HIV infections have occurred among vaccinees. “Breakthrough cases” of HIV infection in all protocols have been entered into a special study.

To date, 12 of the 1,400 individuals in AVEG trials since 1988 have become infected with HIV (37). Of the 12 breakthrough cases, three received placebo vaccine, eight an envelope-based vaccine, and one received a vaccinia/gp160 vector

vaccine boosted with rgp160 vaccine. Five breakthrough cases received one to two doses of vaccine, and only four breakthrough cases received an adequate series of three to four vaccine doses. Notably, five of nine breakthrough cases occurred among volunteers enrolled in vaccine trials involving low-risk groups. Three additional infections occurred among individuals enrolled in an intramural NIAID trial, and two others occurred among individuals enrolled in non-NIAID vaccine trials, so that a total of 17 volunteers have become infected in envelope-based vaccine trials. Envelope-based vaccines of all participating manufacturers were involved (Genentech, Chiron/Biocene, Bristol-Myers Squibb/Oncogen and MicroGeneSys) (95).

Breakthrough infections among vaccine trial participants were to be expected because: 1) some volunteers received placebo; 2) the protective efficacy of the vaccine, if any, is not known; 3) maximum protection is afforded only after a full vaccine dosage schedule (involving 3 or more doses); and 4) antibody-dependent enhancement of infectivity must be considered as a possible reason for breakthrough infections.

Despite intensive counseling, on retrospective review, all HIV infections among vacinees accompanied high-risk behavior (5). Intensive study of recipient and donor viruses and of immune titers may provide clues to mechanisms of protection or failure.

Antibody-Dependent Enhancement

Some experts have questioned whether priming with an HIV vaccine can potentiate subsequently acquired natural HIV infection (12). The historical prototype giving rise to this concern is dengue virus, a tropical viral disease. The presence of serum antibodies induced by a first attack of mild dengue can facilitate the development of severe disease on subsequent infection with a related dengue virus (40). This "antibody-dependent enhancement" (ADE) of infection can be demonstrated in the laboratory by an increase in growth of virus in cell culture in the presence of antibodies from the serum of exposed individuals.

Recipients of envelope vaccines have been shown to develop small amounts of enhancing antibodies (66). The clinical significance of HIV vaccine-induced ADE is unclear. No direct evidence exists at this time that ADE has any clinical significance. Many scientists consider it to be an unrelated laboratory phenomenon only. Enhancement of disease has not been duplicated with HIV-1 or SIV in primate experiments, although it has been recommended that studies in primate models should continue (59, 67).

Other Mechanisms of Enhanced Disease

Historically, two other vaccines have been associated with an accompanying subsequent natural infection that is atypically severe: an experimental respiratory synitial virus (RSV) vaccine and a licensed measles virus vaccine (27, 54). Both were vaccines composed of whole virus inactivated by formalin. While the mechanisms of disease enhancement remain unclear, they both appear to occur by mechanisms unrelated to ADE of the dengue fever type. The enhanced disease experiences with these vaccines were wholly unexpected and have had a significant effect on further vaccine development. For measles, a live attenuated vaccine has supplanted the inactivated vaccine, and currently there is no licensed RSV vaccine. It has been suggested recently that inactivated RSV vaccine may induce inappropriate cytokines, or cell-to-cell communication substances, that are responsible for enhancement (35).

These experiences with vaccine-related enhancement of disease severity have only theoretical implications for HIV vaccines, such as inactivated whole-virus vaccines.

Induction of Autoimmunity

HIV vaccines may have potential for causing an immune reaction against the body's own tissues. Such "anti-self" antibodies could, in theory, be the basis for autoimmune injury (56, 84). Concern arises because HIV shares several envelope protein sequences that are identical (homologous) to sequences on human tissues, a phenomenon known as molecular mimicry. One example is the

similarity of an HIV envelope protein region to a normal human blood type protein (32). Immunization with such viral structures can induce immune responses to the cells of vaccinated individuals. Adverse effects of the autoimmune type have not been observed among HIV vaccine recipients to date, although, in theory, autoimmune phenomena could appear months to years after vaccination.

NEW GENERATION VACCINES: IMPLICATIONS FOR SAFETY

Immune Goals Drive Vaccine Design and Enlarge Potential for Risk

As has been discussed, the immune determinants of protection against HIV infection remain undefined. The unique ability of HIV to evade immune controls in natural disease and in experimental systems suggests that all avenues of immune containment should remain on the research agenda. Based on classical theory, three elements may be required to prevent infection: 1) neutralization of free virus would be more effective with a more vigorous, broadly strain-reactive, sustained antibody response; 2) destruction of infected cells requires induction of cytotoxic T lymphocytes that recognize multiple HIV epitopes; and 3) protection against sexual transmission of HIV requires an antibody and cellular response at genital and rectal mucosal surfaces.

New vaccine strategies may be needed to fulfill these immune requirements (14). Some of the new-generation concepts are novel, never before applied to vaccines used in humans. Each vaccine formulation or variation on a formulation is regarded as a new product by the FDA, and separate evaluations of each are required. New approaches may carry special risks, some unique to that system. The potential for minimizing known, suspected, or theoretical risks is limited. Tests of vaccine *in vitro* laboratory studies and in animal models can be poor predictors, particularly of infrequent or late events. The major types of experimental vaccines in development are addressed below, along with implications for their safety (table 2-8).

Synthetic Peptides

Defined epitopes on viral proteins are simply and cheaply duplicated by artificial synthesis of short amino acid chains (41, 99). Specific B and T lymphocyte epitopes selected to stimulate antibody and cytotoxic T lymphocytes may be combined. Vaccines directed at multiple epitopes (multivalent vaccines) have been prepared containing subtypes of HIV that are endemic in diverse regions of the globe. Immune responses have been improved by arranging peptides into complex structural forms, as well as by adding new adjuvants or carrier molecules. Peptide-based vaccines have induced cytotoxic T lymphocyte responses in the SIV/macaque model. Clinical reactions to peptide products have been benign in initial clinical trials.

Live Vectors Carrying Genes Coding for Immunizing Antigens

A *vector* is a living virus or bacterium used as a carrier to express one or more “foreign” genes encoding desired antigens. Vectors under study include canarypox virus (a relative of vaccinia virus), adenovirus (a cause of respiratory disease), BCG (an attenuated bovine tuberculosis organism), Salmonella or Shigella (typhoid-like bacteria), and attenuated poliovirus. Canarypox can be altered to express HIV antigens, but canarypox does not itself multiply in the human. Canarypox therefore serves as a safe substitute for vaccinia as a vector (3, 15, 16, 69, 74, 91).

Live vectors have important advantages in inducing protective responses. First, protein antigen synthesized in a vector can induce cytotoxic T lymphocyte responses not expected with antigen administered as inert protein. Second, vectors carrying multiple *env*, *gag*, and *pol* genes but not RNA or other sequences essential for viral replication can assemble into a viral configuration, or *pseudovirion* (55). The nonreplicating structure of the pseudovirion is designed to duplicate advantages of a whole inactivated vaccine but eliminate

its risks. Vaccines using *virus-like particles* (VLP) have also been produced without use of live vectors (102). Third, vectors that grow on body surfaces, such as adenovirus or Salmonella, can induce HIV local mucosal immune responses.

Live vectors also carry inherent safety concerns. The vector must be: 1) stably attenuated and unable to produce the natural human disease caused by the vector, 2) safe from unwanted spread to contacts and community at large, and 3) safe for individuals with impaired immunity. The safety problems that have occurred in licensed smallpox (vaccinia virus) vaccines allow us to predict potential safety problems with vaccines using live vaccinia virus vectors. These may include severe skin and mucous membrane infections, invasive and neurological diseases, and even death in susceptible immunosuppressed individuals (75).

Infectious DNA

The development of vaccines composed of pure viral genetic material, infectious or "naked" DNA, is a novel departure from traditional vaccines. Viral DNA coding for a single or multiple genes, injected directly into the muscle or skin, provides the genetic code for synthesizing new protein, which in turn behaves as a potent antigen. Persistent antibody and cytotoxic T lymphocyte responses have been induced in laboratory animals (42, 100). Mechanisms leading to the potent immune responses are not understood. Safety questions, which are highly theoretical at this time, involve possible tumor formation, production of autoimmune disease, or even the possibility of DNA transmission to the fetus.

Inactivated Whole Virus Vaccine

Development of inactivated as well as live attenuated HIV vaccines, using classical approaches, were seriously considered in early deliberations. Historically, the empiric use of either of these two pathways was generally successful with other viruses. These strategies have not been applied to HIV by vaccine manufacturers because each may carry significant risk.

TABLE 2-8: Vaccine Concepts and Their Stages of Development

Stage of development	Vaccine design
Phase I/II Trials	Envelope proteins (gp160, gp120)
	Vaccinia vector/gp160
Currently entering trials	Synthetic peptides
	Live vectors/multiple proteins
	Virus-like particles
	Pseudovirions
	Immune modulators/delivery systems
Preclinical research	Infectious DNA
	Inactivated whole virus
	Live attenuated virus

SOURCE Off Ice of Technology Assessment, 1995

Preparation of a safe inactivated whole-virus vaccine, exemplified by the Salk-type of inactivated polio vaccine, requires inactivation of a high-titered preparation of live virus using gentle physical-chemical means to preserve full immunogenicity, yet ensuring inactivation of all live viruses. The process must guarantee absence of even a single infectious dose in large volumes (hundreds of thousands of patient units) of vaccine. There is a narrow margin between surviving virus and the destruction of viral immunogenicity; this was highlighted early in the use of licensed polio vaccine when a number of vaccinated individuals developed paralytic poliomyelitis from vaccine lots containing residual live virus (71). The safety problem was resolved by simple refinements in the inactivation process. By contrast, assuring inactivation of all HIV particles could prove difficult. In particular, concern exists as to whether cell cultures or animal models are sufficiently sensitive to detect the minimal residual live virus capable of infecting humans. There has also been theoretical concern regarding residual reactive viral DNA in the product.

In addition, the safety of the "lymphoblastoid" cell lines used to prepare the virus is unknown. "Adventitious agents," that is, unwanted agents growing silently in the cell cultures used to prepare vaccine stock, have posed safety problems in

the past. As an example, SV_{40}, a monkey tumor virus, contaminated early lots of inactivated polio vaccine prepared in monkey cells (68).

The safety of an inactivated whole-virus vaccine for HIV was reviewed at a workshop in 1990. It was the consensus that a safe product is technically feasible but that product development should proceed with caution (82).

Live Attenuated Vaccine

Vaccines using live attenuated virus, exemplified by polio or measles vaccines, are capable of producing immune responses that closely mimic the solid, long-term protective immune response afforded by natural viral infection. In addition to a more vigorous and broader antibody response, attenuated virus vaccines may more effectively induce cytotoxic T lymphocytes and mucosal immunity compared with vaccines composed of inert antigens, such as envelope protein vaccines.

Using the SIV/monkey model, attenuated live virus vaccines have been constructed using selective deletions of nonessential auxiliary genes that are required for SIV replication (21). The attenuated virus is stable, not reverting to a virulent form of virus (i.e., a form of virus capable of producing disease) over an observation period of several years. Monkeys vaccinated with an SIV *nef* gene deletion show protection against challenge with large doses of virulent virus. By contrast, the control vaccinated monkeys acquired an AIDS-like disease and died in two years.

Safety Concerns Associated with Attenuated Virus

There are four primary safety concerns about attenuated viral vaccines that have been recognized (11, 22, 104).

1. *Level of attenuation.* Inadequate attenuation (reduction of virulence) of virus may result in a vaccine that induces the disease that it was designed to prevent; over-attenuated virus may fail to induce protective immune responses. However, even an appropriately attenuated virus may show virulent behavior when not constrained by a competent immune system, such as in vaccine recipients with immune systems compromised by cancers, immunosuppressant drugs, and other non-AIDS causes. The highly infectious nature of SIV administered orally to monkeys at birth, before the monkey's immune system has fully developed, has raised new questions about safety of vaccines in immunocompromised individuals (79).
2. *Stability of attenuation.* The vaccine strain could undergo genetic reversion to a more virulent form during the lengthy course of replication in the vaccinee. This risk is of particular concern with vaccines using attenuated strains of HIV, as the human immunodeficiency virus is characterized by rapid and frequent genetic mutations.
3. *Possibility of secondary spread.* Spread of attenuated virus to contacts of vaccinees (secondary spread) may provide the virus with further opportunity to revert to virulence (e.g., vaccine-induced poliomyelitis in contacts of vaccinees). However, if it can be assured that the level of attenuation of the virus remains stable, secondary spread of the virus may be beneficial, because the attenuated virus could induce protective immunity in contacts. Sufficient spread of the attenuated virus would result in the induction of herd immunity (as had occurred with poliovirus vaccine).
4. *Possibility of induction of tumors.* Other members of the retrovirus family regularly produce tumors (e.g., mouse tumors and a form of human leukemia). Theoretically, the prolonged residence of a live attenuated HIV vaccine strain in vaccinees could allow the retrovirus to produce tumors. Recent evidence for a direct role for HIV infection in the etiology of some T-cell lymphomas suggests a need to proceed cautiously while continuing to investigate the long-term potential of these vaccinees to produce tumors (92, 104).

The gene deletion approach to attenuation holds special promise. Deletion of one or more auxiliary genes essential for viral replication should make the risk of reversion to virulence unlikely. Because of safety concerns, viral mutants

with multiple gene deletions are being explored for level of stability and attenuation, duration of protection, and long-term safety. It is hoped that these attenuated viral vaccines will prevent subsequent superinfection with a second, virulent but genetically different HIV strain.

The protective mechanism of attenuated SIV vaccine is unclear. It is not correlated with antibody or cytotoxic T lymphocyte responses, and mucosal immunity is not involved. This observation raises the question of whether another means of blocking virus exists. Attenuated vaccines in the SIV/monkey model offer interesting opportunities to explore immune determinants of protection.

New Approaches to Improve Vaccine Performance

Mucosal Immunity

No vaccine has yet provided an immune barrier at the mucosal membranes of the rectum, vagina, and urethra—the sites of sexual transmission of HIV (62, 63, 64). The mucosal administration of vaccine vectors that grow on mucosal surfaces may provide a critical tool for the prevention of HIV transmission by sexual routes. Antigen uptake from mucosal surfaces is poor compared with injection. New strategies to improve the uptake of antigens from mucosal surfaces involve use of biodegradable microspheres, cholera toxin B, liposomes (phospholipid droplets), and immunostimulating complexes (iscoms) to enhance passage of antigen through cell membranes for more efficient processing (58).

New Adjuvants and Delivery Vehicles

Adjuvants are nonviral materials incorporated into vaccine formulations to augment the magnitude or spectrum of immune responses to vaccines (31). Since the 1940s, however, alum compounds have been the only adjuvants accepted for vaccine products licensed by the FDA. Adjuvants have been discovered largely empirically, and are commonly derivatives of bacteria or plants. They may be combined with chemical surfactants (emulsifiers), forming complexes with specific HIV proteins or individual peptides. The introduction of new adjuvants into clinical practice has been slowed by concerns about the adjuvant's toxicity. Significant transient toxicity was shown in comparative trials of experimental adjuvants (table 2-6).

Exploration of adjuvants is currently undergoing a renaissance in an effort to selectively enhance HIV antibody, cytotoxic T lymphocyte, or mucosal immune responses. The hope is to move from an empiric to a rational approach to attaining specific immune response goals.

The microsphere is a new delivery vehicle that can add flexibility to the antigen's disposition (23, 58, 65). Antigen is coated with an inert plastic polymer, which becomes soluble in body tissues. The microsphere particle size and polymer composition can be altered to target a single dose of antigen to specific tissue sites such as mucous membranes, and to release the antigen in pulses, obviating the need for a multiple dose vaccination schedule.

Cytokines

Cytokines comprise a family of soluble substances (e.g., 1L-2, 1L-4, interferons, etc.) that mediate functions of immune cells. Cytokines can play a significant role in providing protective immune responses following vaccination (18). Specific cytokines may be included in a vaccine, or may be induced in the body by altering the form in which vaccine antigens are presented.

Any of the above approaches to improve vaccine performance may have unexpected side effects. So far, several new adjuvants have caused early transient difficulties and have been withdrawn from use.

SOCIAL HARMS AS ADVERSE EVENTS

Adverse consequences or harms may be expected, not attributable to the biological properties of the vaccine, but rather falling into the realm of "social injury" (2, 90). Vaccines may cause a "false-positive" screening tests for HIV infection. This vaccine-induced seropositivity can result in discrimination against false-positive individuals, such as

in eligibility for military service, employment, health or life insurance, or restriction of travel.

Seropositivity following inoculation with envelope vaccines can usually be distinguished from HIV infection by the Western blot test which is used to confirm the results HIV of enzyme-linked immunosorbant assay (ELISA) tests used in HIV screening. Volunteers in NIAID-sponsored trials have received identification documents certifying their participation in these trails, although AVEG personnel have had to intervene to provide validation of confounding Western blot confirmatory tests (5).

The problem may become more acute in the future as new generation vaccines that include many more types of antigenic proteins than are currently used may render the Western blot test unable to distinguish vaccine-induced seropositivity from true HIV infection. Reliance must then be placed on time-consuming and expensive polymerase chain reaction (PCR) tests which detect the presence of virus directly, and on viral cultures. Simpler methods of distinguishing vaccine-induced immune responses from immune responses induced by natural infection are being actively pursued.

Participation in an HIV trial, in itself, may engender social harms. Others may perceive a volunteer's participation in the trial as implying that the volunteer is in a group at special risk of acquiring HIV infection, and this may result in personal stigmatization of the volunteer. Further, volunteers who are immunized with one candidate vaccine may be precluded from participating in clinical trials of subsequent, possibly more effective, vaccine products. Also, trial participants may assume that they are protected from HIV infection, and as a consequence may increase their risk-taking behaviors. This increased risk-taking behavior may occur despite intensive counseling on the possibility of assignment to placebo vaccine and the unknown efficacy of the trial vaccine.

HIV vaccines will fall short of protecting all recipients. None of the currently licensed vaccines in public health use, even the most effective, vaccines protects all recipients; estimates of protection range from 50 to 70 percent for influenza vaccine, to 95 percent for measles and polio vaccines. Failure of vaccine to protect is expected in clinical trials. These failures may be perceived as vaccine-induced enhancement of infection, manifest as an increased susceptibility or a more aggressive course of infection. Lastly, questions of responsibility and legal liability for vaccine injury, provision of health care, or other services to trial participants remain unresolved (2). The concept of social harms is developed further in the discussion of efficacy trials below. These issues are also discussed in further detail in chapters 3 and 4.

CLINICAL TRIALS IN HIV-INFECTED INDIVIDUALS

Infected Pregnant Women

Prevention of newborn HIV infection by vaccination of the infected mother deserves special note. HIV-infected pregnant women transmit infection to 15 to 40 percent of their progeny. In this complicated situation, vaccination can potentially prevent infection of the fetus or newborn and treat the infection of the mother. The goal of a vaccine in this setting is to favorably alter the immune status of the mother during pregnancy, thereby lowering the risk of transmission of the virus from mother to fetus (vertical transmission) (98).[18]. Possible risks to the mother, fetus, and newborn have not been formally tested in clinical trials of HIV vaccines. Previously, pregnancy has been cause for exclusion in all Phase I and II trials. Despite counseling designed to exclude pregnancy, overall 16 pregnancies have occurred in AVEG trials conducted in uninfected subjects with no adverse events attributable to vaccine.

[18] The use of vaccines to prevent vertical transmission is reviewed by M. Walker and P. Fast, 1995(98).

TABLE 2-9: Current Cinical Trials in HIV-Infected Pregnant Women

Vaccine	Developer	Trial site
In HIV infected pregnant women		
rgp160-LAI	MicroGeneSys	AVEG, ACTG
rgp120-MN	Genentech	AVEG, ACTG
rgp120-SF2	Biocine	ACTG
In infants born to H/V-infected women		
rgp120-MN	Genentech	ACTG
rgp120-SF2	Biocine	ACTG

KEY: ACTG = NIAID AIDS Clinlcal Trial Group, AVEG = AIDS Vaccine Evaluation Group of AIDS Vaccine Clinical Trials Network

SOURCE. Adapted from M C Walker, and P E Fast, *Clinical Trials of Candidate AIDS Vaccines,* in press

While there is no a priori reason to expect adverse events, such as injury to the developing fetus or newborn, from HIV vaccine, the outcomes of these pregnancies will be carefully monitored. Injuries to the newborn that are causally related to the vaccine must be distinguished from the recognized high background rate, approximately 3 percent, of naturally occurring birth defects or developmental problems in newborns. Phase I clinical trials of HIV vaccine in 23 infected pregnant women, using three rgp120 vaccine products, are in progress (table 2-9) (106). The vaccine products were pre-screened for fetal toxicity in rodents. No significant vaccine-related adverse events occurred in mothers or in the 20 infants that have been delivered to date.

In regions of the developing world where there is a high incidence of HIV infection and where effective chemotherapy (Zidovudine) is not widely available, trials of vaccines to prevent vertical HIV transmission remain appropriate. These trials should be a high priority, because HIV-infected infants usually progress rapidly to severe disease.

Trials of Therapeutic Vaccine for Treatment of Established Infection

Use of an HIV vaccine as an agent to treat individuals with established HIV infection (therapeutic vaccination) is based upon concepts that are different from vaccine used as a preventive agent (prophylactic vaccination). In established infection, a vaccine is used for its potential to favorably modulate the immune system. The objective of therapeutic vaccination is to selectively enhance the immune processes that reduce viral replication and increase viral suppression. This, in turn, may control or eliminate persistent virus and delay or prevent disease progression.

However, there has never been a vaccine that has been able to slow progression of an infectious process once the infection has been established. Post-exposure immunization in some viral infections, such as rabies, is only effective if the vaccine is administered early in the incubation period of the virus, before infection is established in the target organ. Approximately 35 Phase I and II trials of therapeutic HIV vaccines are active in the United States and abroad, using envelope and core proteins, novel vectors, inactivated virus, and other products (98).

Several things can be learned from trials of therapeutic HIV vaccines that bear on the development of a preventive HIV vaccine. First, the more favorable risk/benefit ratio in a treatment setting versus a preventive setting, permits more widespread study of novel products. Second, trials of therapeutic vaccines permit the assessment of the safety and specificity of immune responses to the vaccines (77). Third, there has been no clear evidence that therapeutic vaccines benefit the course of HIV infection, although more definitive randomized, controlled Phase 11 clinical trials are in progress. Finally, there is no evidence that HIV infection has been accelerated or enhanced in recipients of therapeutic HIV vaccines. One study of HIV vaccines in chimpanzees reported a

TABLE 2-10: HIV Vaccine Efficacy Trials

	Total sample size[a]			
	Annual rate HIV infection			
Length of Trial	1%	2%	3%0	4%
2 years	28,896	14,540	9,690	7,290
2,5 years	22,266	11,224	7,496	5,650
3 years	18,294	9,238	6,180	4,668

[a]Two-arm study, 90% power to detect a 30°A reduction m the risk of infection, 10% annual loss to followup.

SOURCE" Wasima N. Rida, Division of AIDS, NIAID, Bethesda, MD, June 1995.

transient rise in HIV-infected cells after vaccination; this transient increase in HIV-infected cells was of unknown significance (28).

PHASE Ill EFFICACY TRIALS

▮ General Concepts of Efficacy Trial Design

The capability of a vaccine to protect against infection is determined in Phase III efficacy trials (96) (97). The quality and quantity of vaccine-induced immune responses measured in Phase I and II trials may predict, but do not demonstrate, efficacy of the vaccine. The second major function of the Phase III efficacy trial is to provide a more definitive assessment of vaccine safety.

Efficacy trials of HIV vaccines will be large, complex, lengthy, and expensive. The design requires a prospectively randomized, double-blind, placebo-controlled study, which will involve several thousand subjects assigned to one or more vaccines or to placebo. The trial site must be prepared with competence in epidemiology capabilities in behavioral, clinical and laboratory roles, and data management skills. The number of subjects, duration of recruitment, and followup are determined by several key variables. These include the number of arms (i.e., vaccine and placebo groups) in the study, seroincidence (annual rate of new infection), length of recruitment period, rate of retention, assumptions about level of efficacy of the experimental vaccines, and the definition of infection or disease endpoint(s) or outcomes that are measured. An example is provided in table 2-10.

Persistent infection accompanied by delay or prevention of clinical disease or reduced transmission of virus requires many years or lifetime followup.

Possible endpoints, including "intermediate endpoints" in vaccine trials, are described in table 2-11. Documentation of the validity of intermediate endpoints as predictors of vaccine protection will require intensive laboratory studies. Multiple efficacy trials will be needed; the initial vaccine formulations may well be less than optimal.

▮ Preparing for Efficacy Trials in the United States

Successive vaccine candidates with potential for improved efficacy and safety will be compared in randomized, double-blind, controlled clinical trials with prior vaccines serving as benchmarks. HIV efficacy trials in the U.S. will be unique in the history of vaccinology. While the underlying epidemiological and statistical principles of trial design are the same as those used in trials of classical vaccines, the groups that are targeted for HIV vaccination and their community settings have special characteristics. This, together with the special biological and social implications of HIV infection, has a great impact on the conduct and outcome of the trial (43, 45, 96, 97).

Populations with high rates of seroconversion (incidence of HIV infection) are required, such as intravenous drug users and men who have sex with men. Such communities may feel disenfranchised and socially stigmatized, have concerns regarding access to health care and other services, and harbor distrust of the government and of

TABLE 2-11: Possible Outcomes of HIV Vaccine Efficacy Trials and Levels of Protection

Possible outcomes of trial	Intertxetation
Sterilizing Immunity	Vaccine has prevented infection.
Minimal infection without antibody	Vaccine has induced immune memory only.
Abortive infection	Early transient viremia and/or antibody response; vaccine has prevented establishment of infection.
Modified infection	Vaccine has decreased viral load, delayed disease, or reduced transmission,
Unmodified infection and disease	Vaccine has failed.
Rapid progression or increased incidence	Immune enhancement of infection as a result of vaccination,

SOURCE: Office of Technology Assessment, 1995

scientific experimentation (90). These underlying ethical, social, legal, and political issues will require sensitive attention.

In anticipation of conducting large-scale efficacy trials, preparatory studies have been initiated (89, 96). Several thousand injection drug users and homosexual and bisexual gay men with a high HIV seroincidence are under study in the HIV Evaluation Network (HIVNET), sponsored by the NIAID, CDC, and the National Institute of Drug Abuse. The goals are multiple: 1) to study sociocultural factors affecting recruitment and retention; 2) to measure the frequency of risk behaviors, to assess the effect of trial participation, counseling, and unbinding on risk behaviors, and to develop strategies to reduce the frequency of risk behaviors (undocumented changes in personal risk behavior can have confounding effects on the apparent efficacy of a vaccine) (87); 3) to determine the basis for attitudes toward vaccine acceptance; 4) to develop educational strategies and consent forms appropriate to the subject groups; and 5) to study the dynamics of trial acceptance and feasibility. Information derived from such studies will enhance the feasibility and readiness to undertake full-scale HIV vaccine efficacy trials in the U.S. Continued research into the measurement of socio-behavioral variables is critical to planning, trial design and data analysis.

Criteria for Selection of a Vaccine for Efficacy Trials

The criteria for selecting an HIV vaccine candidate that merits study in a Phase III efficacy trial has been extensively discussed over the past few years. Because we do not know what specific type of immune response is required to provide protection from HIV infection, the criteria to be used to select vaccine candidates are not sharply defined. Discussions have involved consideration of the following elements: 1) evidence of safety and immunogenicity of the vaccine in Phase I and 11 trials; 2) the vaccines ability to induce high-titered, broadly reactive, and sustained levels of antibody capable of neutralizing primary field HIV isolates; 3) the vaccines ability to induce cytotoxic T lymphocyte responses, and 4) evidence of vaccine protection in a primate model. However, in the face of scientific uncertainty and a rapidly evolving knowledge base, the relative emphasis and stringency given to each of these criteria have varied in successive recommendations. More clearly defined criteria for selection of vaccine candidates for entry into Phase III efficacy trials would be of obvious value.

Envelope Proteins as Candidates for Efficacy Trials

Two candidate vaccines, Biocine SF2 with MF59 and Genentech MN with alum adjuvant have completed Phase II trials. A Phase III clinical trial of envelope vaccine would test the following hypothesis: can neutralizing antibody, with certain limitations in its magnitude, cross-reactivity, durability, and mucosal localization, protect a high-risk population with a measurable level of efficacy?

In June 1994, the NIAID AIDS Subcommittee and AIDS Research Advisory Committee (ARAC)

TABLE 2-12: Factors Considered in Determining Whether to Proceed With Efficacy Trials

Biological factors	Factors favoring efficacy trials	Factors weighing against efficacy trials
Safety	▪ Only minimal transient local and systemic reactions have occurred.	▪ Breakthrough infections; Possibility of immune enhancement.
Immune response	▪ Neutralizing antibody has been induced by envelope vaccines. ▪ CTL may not be essential.	▪ Need increased titer, duration, and cross-reactivity of antibody in response to envelope protein, as well as neutralization of primary isolates. ▪ CTL may be important to protection.
Primate model	• Envelope vaccine protects chimpanzees against mild HIV infection under limited conditions.	▪ Envelope vaccine offered; poor protection in more stringent SIV/monkey disease model.
Social, political, ethical factors	▪ Vaccine need is a public health imperative. ▪ Infrastructure for trials is ready. • Modest protection valuable. ▪ Scientific gains may result, e.g., immune determinants of protection. ▪ Product is ready	▪ An inconclusive trial may result, with loss of public confidence. ▪ A better behavioral database is needed, ▪ Trial may involve large investment of funds and human resources for questionable gains, False security may increase risk-taking. • Trial lacks sensitivity to detect immune determinants of infection, • Setback for industry if trials fail.

KEY: CTL = cytotoxic T lymphocytes

SOURCE Adapted from A. Hause, "Report on the April HIV Vaccine Working Group Meeting," paper presented at the NIAID AIDS Research Advisory Committee (ARAC) meeting, June 17, 1994

recommended that Phase III clinical efficacy trials with the envelope vaccines should not proceed in the United States at that time (25). Factors contributing to the decision included scientific, political, and ethical issues (39) (table 2-1 2). There was a significant level of scientific uncertainty regarding the wisdom of immediate efficacy trials, with advocates on both sides of the question. Two trial designs were discussed (46). A definitive three-armed trial with a sample size of 9,000 high-risk individuals would permit detection of statistically significant protection from a vaccine with only 30 percent efficacy. Alternatively, a smaller trial, involving 4,500 individuals, would allow detection of significant protection from a vaccine with 60 percent efficacy, but have little chance of detecting the protection from a vaccine with 30 percent efficacy. [19]

Phase I and II clinical trials of HIV vaccines continue. New generation products recently entered into Phase I trials or in the preclinical pipeline are designed to expand the quality and quantity of the protective immune response to the vaccine. These products should be available for consideration for Phase III efficacy trails within two to three years.

Monitoring Adverse Events in Efficacy Trials

The long-term followup of large numbers of vaccinees and controls allows for surveillance of events that are infrequent or occur after an interval of years. The prospectively defined populations that participated in vaccine efficacy trials constitute unique epidemiologic cohorts, not easily du-

[19] Larger trials are able to defect smaller degrees of vaccine efficacy.

plicated after controlled efficacy trials are completed. "Vaccinated cohorts" from efficacy trials could be compared to the unvaccinated cohorts that are currently under epidemiologic and virologic surveillance.

Provision for long-term followup should be an integral part of the design of efficacy trials, allowing surveillance of safety issues, such as enhanced infection, autoimmune disease, tumors, or reversion to virulence. Rigorous assessment will be required before acceptance of a causal relationship between a vaccine and adverse events. Despite difficulties and expense, decades of experience with childhood vaccines emphasize the singular need for maintaining followup capability.

Efficacy Trials in the Developing World

While the current document addresses domestic issues, it is clear that HIV-1 efficacy trials at international sites will be an important and integral part of the process of developing and evaluating AIDS vaccine candidates. Such sites provide opportunities to study diverse population groups in highly endemic areas, including heterosexual and maternal-infant transmission of HIV, a variety of cultural and health settings, and vaccines targeting a multiplicity of HIV subtypes. In addition, it affords the possibility of direct benefit to the participating population in a tangible way. Vaccine affordability, ease of administration (given in a few doses or orally), and stability of protection will be critical to widespread use of vaccine. The NIAID and U.S. Department of Defense (DOD) are developing sites in concert with national governments in the Americas, Africa, and Asia. A multivalent peptide vaccine is currently the only approach in advanced stage of development that addresses the diversity of global subtypes. Opportunities for assessing subtype B strains are available in the Americas and Western Europe, as well as in a locus in Thailand.

The June 1994 decision to defer Phase III clinical efficacy trials in the U.S. does not preclude clinical efficacy trials of envelope-based vaccines in the developing world. Applying standards of safety and efficacy to populations with a rapid and uncontrollable rise in HIV infection alters the risk to benefit ratio of the vaccines. While ethical principles of such decisions remain universal, it is recognized that biological circumstances can validly affect the decision process. The attendant risks of adverse reactions or social harms in a developing world setting engender a separate level of issues, involving U.S. industry, institutions, and investigators, as well as the host foreign nationals. Issues surrounding vaccine trials in developing countries are discussed in chapters 3 and 4.

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