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Vaccine Development Against HIV-1

Current Perspectives and Future Directions

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Abstract

The development of an efficacious vaccine against the human immunodeficiency virus (HIV) is of great urgency, because it is accepted that vaccination is the only means capable of controlling the AIDS pandemic. The foundation of HIV vaccine development is the analysis of immune responses during natural infection and the utilization of this knowledge for the development of protective immunization strategies. Initial vaccine development and experimentation are usually in animal models, including murine, feline, and nonhuman primates. Experimental vaccine candidates are closely studied for both efficacy and safety before proceeding to human clinical trials. There are a number of different therapeutic and prophylactic vaccine strategies currently being studied in human clinical trials. Vaccine strategies that are being tested, or have previously been tested, in humans include subunit, DNA plasmid, and viral vector, and combinations of these various strategies. Some of the results of these trials are promising, and additional research has focused on the development of appropriate chemical and genetic adjuvants as well as methods of vaccine delivery to improve the host immune response. This review summarizes the vaccine strategies that have been tested in both animal models and human clinical trials.

Key Words

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Introduction

The human immunodeficiency virus (HIV), the causal agent of the acquired immune deficiency syndrome (AIDS), continues to infect people worldwide despite the development of effective drug therapies and increased educational awareness, efforts meant to slow the progression to AIDS. However, therapeutic drugs alone have no likelihood of halting the AIDS pandemic. Only a safe and efficacious vaccine against HIV will accomplish this goal.

Development of an appropriate animal model for HIV vaccine study has been a formidable challenge. Lower mammals are susceptible to infection by species–specific retroviruses, whereas nonhuman primate models such as chimpanzees and monkeys can be infected by HIV and simian immunodeficiency virus (SIV), respectively. Nonhuman primate models are more cost prohibitive than other animal models, and there is a continuing need to develop and evaluate smaller animal models for vaccine development.

Currently, most HIV candidate immunogens in clinical trials include subunit vaccines, with envelope (Env) glycoprotein–based proteins as the major preferred target *(1)*. Other proteins that have been targeted are the Pol (polymerase) and Gag proteins. However, the Env proteins are widely targeted because of their expression on the surface of the virions and particularly their role in viral–host interactions. Specifically, neutralizing antibodies are directed almost exclusively against the Env proteins. The widely studied recombinant Env proteins are the full-length glycoprotein 160 and the external glycoprotein 120 (gp160 and gp120). Normally, expression of recombinant proteins for use as immunogens is achieved in yeast or bacterial cells, then purified and formulated in adjuvant for later immunization. In addition to being tested as single vaccines, subunit vaccines are used as boosting immunogens following primary immunization with another vaccine type. Such boosting may result in a synergistic effect on the overall anti-HIV immune response.

Because the correlates of protection from HIV infection are not fully understood, studies continue on immune responses elicited by experimental vaccinogens to potentially identify some of these important immune correlates. There is debate regarding the importance of the role of humoral responses in the protection against or control of HIV infection. Experimental evidence has suggested the necessity of eliciting potent $CD8^+$ anti-HIV cytotoxic T lymphocyte (CTL) activity by an effective vaccine. Unlike the hepatitis B virus (HBV) subunit vaccine, where anti-HBV antibody titers are closely correlated to protection, a strong humoral response to HIV elicited by a vaccine has not been demonstrated to correspond to protection from infection nor to slower progression to AIDS. Most HIV vaccinologists believe it is prudent to develop a vaccine that elicits both potent humoral and cellular immune responses. The vaccine strategy most likely capable of inducing this strong immune response is a live attenuated vaccine. Because of the obvious safety issues associated with this method, other delivery systems have been utilized such as vaccinia, adenovirus, rhinovirus, and canarypox. Vaccinia virus, particularly because of its large genome, historically has been used to deliver other vaccines.

Most recently, the DNA vaccine, also called "naked DNA" or plasmid DNA vaccine, has been developed as a vaccination method against infectious diseases such as AIDS/HIV and cancer antigens.

Table 1. Animal Models for HIV Vaccines

Animal Model	Infectious agent used with model	Subunit, peptide, recombinant protein	Live-attenuated, whole-killed, fixed-cell	Viral vector (non-replicating)	DNA vaccine (naked plasmid)
Murine and other rodents	Freund's Leukemia Virus (FLV) or Murine Acquired Immune Deficiency (MAIDS) virus	*2, 66, 68, 70, 102*	*3*	*72–74*	*4, 81, 86, 87, 89–94, 99. 101–103, 110–115*
Domestic cat	Feline Immunodeficiency Virus (FIV)	*11–15*	*6–9, 18*	*18*	*10, 16, 17*
Monkey (cynamologous, pig-tailed, or rhesus macaques) and baboon	Simian Immunodeficiency Virus (SIV)	*23, 24, 71, 116*	*25–33*	*36, 37*	*34, 35, 81, 88, 96*
Chimpanzee	Human Immunodeficiency Virus (HIV)	*44, 50*	*45*		*46–49, 80, 91, 98, 104, 105*

Italic numbers in table refer to appropriate references.

Animal Models for Vaccine Development against HIV-1

Several T lymphotrophic lentiviruses causing immunodeficiency syndromes analogous to HIV have been characterized and appear to target similar cells and induce pathogenic manifestations similar to HIV. These viruses and the corresponding infectible animals have been utilized, to varying degrees, as models for HIV research and development. The animal models most commonly studied are murine, feline, and nonhuman primates. In most of these models, live attenuated and whole-killed virus vaccine preparations have been studied. Although these strategies will likely never be used in humans owing to risk of virulence or reversion to a pathogenic strain, the study of these vaccines is important for the determination of potential immunologic factors that may mediate protection. Chimeric viruses have been developed for more directed studies in the monkey model. Finally, the chimpanzee model is used because of their susceptibility to HIV infection. All animal studies described in this article are summarized in Table 1.

Rodent Models of Immune Deficiency Syndromes

The use of mice for the in vivo study of immunologic responses to antigens from pathogenic organisms has widely been utilized and studied. In mice, the murine acquired immune deficiency syndrome virus (MAIDS) and Friend's leukemia virus (FLV) have been used as model systems. These viruses not only infect mice in vivo, but also result in pathology sim-

ilar to HIV in terms of the cytopathogenicity induced in the cells of the immune system. Other rodents such as rats and guinea pigs have also been utilized.

A variety of different vaccine strategies have been tested in the murine model, including subunit *(2)*, whole, killed and live, attenuated virus *(3)*, and DNA vaccines encoding HIV proteins *(4)*. Strategies to improve immunogenicity of vaccine candidates include improved delivery, as with liposomes *(2)*, and choice of delivery site *(4)*. Chemical adjuvants have also been studied, including incomplete Freund's adjuvant (IFA) and Detox PC *(3)*.

The ease of care, high accessibility, and extensive knowledge of the murine immune system make mice a good initial candidate for vaccine immunogenicity studies. However, since immunocompetent rodents cannot be infected with HIV, the use of the murine model is considerably limited. Cytokine and immunoglobulin subtyping reagents are increasingly available in this model, making the detailed study of immunogenic responses to HIV vaccines much easier.

Feline Immunodeficiency Virus and the Cat Model

Infection of cats with feline immunodeficiency virus (FIV) is another model for the study of HIV. Viral challenge in vaccinated cats is frequently performed with culture-isolated FIV intravenously or intravaginally; however, FIV isolated from infected cats proves to be a more difficult agent to protect against *(5)*.

Different vaccine strategies have been tested in the cat model, including whole inactivated virus, fixed with paraformaldehyde *(6)*. This vaccine strategy was found to be protective in the absence of neutralizing antibodies *(7)*; however, this protection is short-lived and difficult to subsequently boost *(8)*. Other studies with whole inactivated nonfixed cell virus have failed to result in protection *(9)*, possibly due to more effective presentation of antigens by infected cells; whereas FIV depleted of proteins necessary for replication have been found to be protective *(10)*. Subunit vaccines against FIV have been linked to enhanced infection *(11,12)*. However, other studies demonstrate delayed infection but not protection *(13)*, even when formulated in immune-stimulating complexes (ISCOMS) *(14)*. FIV subunit vaccines containing T_{H1} enhancing adjuvant such as Freund's complete adjuvant were found to be protective *(15)*. DNA plasmid vaccines have been shown to elicit T_{H1} and T_{H2} immune responses. However those vaccines encoding *env* demonstrated enhanced infection in vaccinated cats *(16)*; co-administration of vaccines encoding nucleocapsid proteins appear to inhibit this enhancement *(17)*. Viral vector vaccines utilizing canarypox alone were not protective, whereas when boosted with inactivated FIV-infected cell vaccine, the cats were protected *(18)*.

Infection of cats with FIV has been a more studied model due to their low cost and the increasing availability of feline immunologic reagents. It would appear, however, that unlike other animal models, the T_{H2} immune response alone has a deleterious effect on the viral infection, possibly limiting the usefulness of this model.

Simian Immunodeficiency Virus and the Nonhuman Primate Model

Monkeys and SIV provide one of the leading models for HIV infection. Similar in genomic structure to HIV-2, SIV asymptomatically infects many African monkey species *(19)*, while symptomatically infecting Asian monkey species such as cynomologous, pigtailed, and rhesus macaques *(20)*. SIV infection has been found to result in pathogenesis and infection mechanisms similar to

HIV infection in humans *(21)*, leading to $CD4^+$ lymphocyte depletion and opportunistic infection within 1–2 yr of infection *(22)*.

Some subunit vaccines have been shown to protect against disease but not infection *(23)*, while others have protected from viral challenge *(24)*. Live, attenuated virus has been the most consistently successful vaccine strategy in this model *(25–27)*, although some studies suggest this protection is only elicited after a lengthy interval *(26,28)* with variable protection from variant SIV field strains *(29)*. Live, attenuated SIV with *nef*-depletion has successfully protected *(30,31)*; however, in some cases in vivo repair of *nef* resulting in pathogenicity has occurred *(32)*. However, multiple gene deletions were unable to revert to pathogenic strains *(33)*. Studies demonstrate DNA plasmid vaccines elicit T_{H1} and T_{H2} immune responses in monkeys, resulting in rapidly declining low levels of immune responses unable to protect against infection *(34)*. In contrast, another study demonstrated that a DNA vaccine encoding gp160 elicited in vivo protection *(35)*. Nonreplicating viral vector vaccines utilizing Semliki Forest virus (SFV) were not protective *(36)*; while some vaccinia virus vaccines were protective, at least in part *(37)*.

Although monkeys and SIV provide an excellent model for HIV study, their cost and maintenance are often prohibitive for wide-scale research. Monkey reagents and immunological study are becoming more prevalent, and their use will likely provide invaluable keys in the search for an effective HIV vaccine.

Simian–Human Immunodeficiency Virus

A relatively new chimeric virus using HIV envelope proteins with SIV core proteins and genome has been developed, called the simian–human immunodeficiency virus (SHIV), which allows for the study of HIV immunogens in the monkey model. Although early SHIV strains were limited by slow replication and cytopathogenicity *(38)*, more recently developed virulent SHIV strains result in immunodeficiency-related disease progression in macaques *(39,40)*. Other chimeric viruses have been developed to infect baboons *(41)*, some specifically against clade E, a common Asian strain *(42)*.

Chimpanzees as a Model of HIV Infection

Humans and chimpanzees share over 98% of genetic homology, and as such respond similarly to antigens in an immunologic sense. HIV has been determined to infect chimpanzees resulting in pathological immune deficiency and mortality *(43)*. However, despite some associated mortality most HIV vaccinologists consider HIV to be nonpathogenic in chimpanzees.

Various vaccine strategies have been studied in chimpanzees, including subunit, whole-killed virus, and DNA plasmid. Recombinant HIV-1 gp120 was found to protect against homologous viral challenge, whereas rgp160 (recombinant gp160) did not *(44)*. Whole-killed virus vaccines have been demonstrated to elicit immune responses and to delay infection *(45)*. DNA plasmid vaccines encoding *env*, *rev*, and *gag/pol* of HIV-1 are safe and nontoxic in the animals tested *(46,47)*, and protected chimpanzees from a high dose heterologous HIV-1 challenge *(48)*. This DNA plasmid preparation had immunotherapeutic qualities when tested in HIV-1 seropositive chimpanzees, including increased humoral responses and decreased viral load *(49)*.

Chimpanzees offer the only animal capable of being infected with HIV. However, several disadvantages in this model include a limited infection compared to humans in addition to being somewhat prohibitively expensive and limited in terms of availability

(owing to the endangered species status). HIV vaccine studies have demonstrated that chimpanzees react somewhat similarly immunologically to humans, however Berman, et. al. *(50)* found human anti-HIV antibodies to have a higher half-life, whereas chimpanzee anti-HIV antibodies demonstrated more avidity.

Subunit Vaccines

The administration of proteins or peptides called subunit vaccines has been found to successfully protect against a variety of viral infections; however, they have been unable to consistently and effectively protect against HIV infections. Subunit vaccines are likely processed as endogenous proteins by cells in vivo resulting in a primarily humoral response.

A number of human clinical trials have been conducted on the therapeutic administration of HIV Env proteins to HIV seropositive individuals. Recombinant gp160 (rgp160) administered to HIV seropositive patients induced vaccine-associated lymphoproliferative responses *(51)*, as well as statistically significant boosts in anti-HIV cytotoxic T lymphocyte (CTL) activity *(52)*. HIV seropositive adults were administered rgp120 or rgp160, resulting in HIV-specific lymphoproliferation *(53)*. Pinto et al. *(54)* demonstrated that the administration of peptide segments of gp160 in seropositive volunteers resulted in HIV-specific $CD4^+$ responses and increased anti-HIV antibody titers. In another study, administration of three HIV glycoproteins, rgp120 SF-2, rgp120 MN and rgp160, to HIV seropositive children were studied for safety and immunogenicity, and demonstrated good toleration and stimulated lymphoproliferative responses *(55)*. In a study conducted in asymptomatic HIV-1 pregnant women, a vaccination with rgp120 was well tolerated and caused no postpartum adverse effects on the infants, but had no effect on the rate of vertical transmission of HIV to the infants *(56)*. Despite the vaccine-associated induction of immune responses in these studies, the rate of progression to AIDS as well as the $CD4^+$ counts appeared to be unaffected by the administration of the vaccine compared to placebo.

Administration of an inactivated gp120 depleted immunogen was found to increase anti-HIV p24 proliferation of $CD4^+$, $CD8^+$, and natural killer cells *(57)*. Also encouraging was a study in which multiple immunizations with an HIV-1 immunogen jointly with antiretroviral agents was well tolerated and resulted in a reduction of plasma HIV-1 RNA. Based on results from this adjunctive therapy, there appears to be an inverse correlation between HIV-1 specific antibody titers and levels of plasma HIV-1 RNA *(58)*. In a similar study, a therapeutic p24-VLP (Gag virus-like particles) subunit vaccine was co-administered with zidovudine (ZDV) and found to increase HIV-specific CTL activity, while either p24-VLP or ZDV alone did not significantly broaden CTL activity *(59)*. Patterson et al. *(60)* compared the levels of HIV-1 RNA and DNA in both $CD4^+$ T cells and monocytes after administration of IFA plus highly active antiretroviral therapy (HAART), or HAART plus an HIV-1 immunogen (gp120 Env and Gag). The results indicated that there was a reduction in HIV-1 DNA and mRNA for Gag and Pol proteins in volunteers vaccinated with HIV-1 immunogen plus HAART compared to those administered IFA and HAART. These results and others support the usefulness of co-administration of therapeutic vaccines with antiretroviral drugs.

Another subunit vaccine approach involves using rgp160 as a priming vaccine, followed by boosts with rgp120 in HIV-1 seropositive volunteers. Previous data demonstrated that rgp160 induced production of $CD4^+$ memory cells and rgp120 induced production of anti-HIV neutralizing antibodies. The vaccine

recipients with the highest titer of neutralizing antibodies were those primed with rgp160, then boosted with rgp120 *(61)*. Another synergistic strategy aimed at debilitating HIV was conducted by Kang et al. *(62)* in which chimeric *gag* genes, including the hypervariable V3 region of HIV-1 were constructed. These *gag–env* chimeras were slightly larger than the Gag protein itself and were recognized by seropositive HIV-1 human sera, indicating their potential usefulness as a vaccine candidate for HIV-1. In a more peripheral experiment conducted by Cox et al., *(63)* antibody-dependent cellular cytotoxicity (ADCC) was measured in HIV-1 seropositive subjects after receiving rgp160 envelope protein in alum (an adjuvant that promotes a T_{H2} bias). Ultimately, the experiment demonstrated no difference in ADCC activity in either those volunteers receiving rgp160 plus alum or placebo. This study also demonstrated that ADCC activity itself did not correlate with a lack of progression to AIDS.

Prophylactic administration of HIV envelope proteins in HIV seronegative volunteers has also been conducted. Immunization with monomeric rgp120 in HIV seropositive and seronegative volunteers demonstrated similar anti-HIV binding titers against monomeric rgp120 as well as those antibodies thought to inhibit gp120 binding to CD4 *(64)*. The employment of two subunit proteins administered in priming and boosting doses was studied and found to promote a strong humoral response. In this study two types of rgp120 (MN and IIIB) were administered to HIV-seronegative volunteers to measure the induction of anti-HIV antibodies. Booster immunizations with rgp120 MN induced the highest levels of antibodies against a panel of the 6 B subtype strains of HIV-1 in vitro. Additionally, lower levels of vaccine elicited antibodies against other envelope subtypes of HIV-1 were also generated, including subtypes A, D, and EA (a glycoprotein sequence representing both subtypes E and A) strains *(65)*.

Subunit vaccines composed of the V3 region of HIV gp120 have been tested in the HLA DQ6 murine model, in which the MHC class II expressed was only DQ6. In vitro studies demonstrated neutralizing antibodies were generated following vaccination, possibly suggesting that DQ6 positive individual may be protected from infection using this vaccine candidate *(66)*.

The V3 loop of HIV gp120 has been evaluated in a phase II clinical trial with HIV seropositive adults. In vitro studies demonstrated that following exposure to the vaccine, syncytia formation was impaired in human $CD4^+$ cells expressing Env through a mechanism independent of Env biosynthesis. Although the vaccine did not cause conformational changes in the receptor binding site or in gp120 during Env biosynthesis, it is proposed that the interference of SPC3 in HIV infection is due to a process occurring after the binding of HIV to $CD4^+$ cells, perhaps by interfering with gp120 binding to its secondary chemokine receptors (CCR5 and CXCR4) *(67)*.

Insertion of a linear peptide sequence of a gp41 epitope into V1, V2, and V4 regions of gp120 molecule appears to increase immunogenicity as well as demonstrate a higher affinity to anti-gp41 monoclonal antibodies than native Env *(68)*. Thus, it has been demonstrated that the manipulation of peptide sequences may promote a more powerful immune response.

The Gag protein, p17, was prophylactically administered to seronegative volunteers and resulted in the elicitation of anti-Gag humoral and CTL responses in 45% and 55% of volunteers, respectively. In the same study, memory lymphocytes from immunized volunteers were administered to SCID mice resulting in 78% of the mice being protected from heterologous challenge *(69)*. This study

demonstrates the potential importance of Gag as an effective immunogen.

Significant results have been demonstrated by the study of vaccines targeting HIV replication proteins including Tat, a regulatory protein demonstrated to cause cellular damage in HIV infected individuals by acting as an extracellular toxin *(70)*. Therapeutic vaccination with a biologically active Tat protein in SIV seropositive cynomologous monkeys has been found to be a safe preparation that elicits broad vaccine-associated humoral and cellular responses. Additionally, this preparation reduced SHIV infection by preventing $CD4^+$ depletion in vaccinated animals *(71)*. Thus far, this preparation has not been tested in humans.

A Tat toxoid vaccine has been developed and therapeutically administered to HIV seropositive, immunocompromised volunteers. This preparation was found to be well tolerated and elicited HIV-specific T_{H1} and T_{H2} immune responses. The Tat toxoid vaccine may be an important therapeutic tool in the future, by possibly limiting the deleterious toxic effects of extracellular Tat in HIV seropositive individuals *(70)*.

Viral Vector Systems for HIV Protein Products

Another method of vaccination utilizes nonreplicating viral vectors encoding HIV proteins, such as adenovirus, rhinovirus and canarypox. These vectors mimic natural infection and are therefore potentially powerful tools as HIV vaccines.

A vaccine utilizing the adenovirus vector has been demonstrated to induce anti-HIV humoral and cellular responses. Env expression was increased in this vaccine by encoding the regulatory gene, *rev*, bicistronically (one mRNA can produce multiple proteins) and insertion of the stimulatory *tat/rev* 5′ splice donor site. Using the bicistronic system, a dosage dependent T_{H2} response was observed, whereas a monocistronic system stimulated a low CTL response *(72)*.

Using an attenuated and molecularly cloned strain of Venezuelan equine encephalitis (VEE), the matrix/capsid (MA/CA) coding region of HIV-1 was inserted with VEE 26 S viral subgenomic RNA promoter into two sites in the VEE genome to determine where optimal expression of the foreign HIV protein occurred. Higher levels of HIV-1 MA/CA were expressed from a site downstream of the VEE E1 gene at the 3′ end of the VEE genome as opposed to the alternative insertion site just upstream of the naturally occurring 26 S VEE promoter. In BALB/c mice, both sites of foreign HIV gene insertion were stable and expressed after VEE passage through baby hamster kidney cells *(73)*. However, the downstream insertion induced a stronger humoral response despite both sites expressing similar CTL responses. Ultimately, this experiment demonstrated that downstream VEE, acting as an HIV expression vector, can maintain high serum antibody titers against HIV-1 MA/CA. Anti-HIV CTLs were displayed in all mice, further supporting previous data demonstrating that fewer viral proteins need to be expressed in order to elicit a TH1 immune response against HIV.

Another viral vector system, human rhinovirus (HRV) 14, has been developed by inserting HIV-1 MN V3 loop of gp120 in combination with a natural epitopic region of the rhinovirus. Immunologically relevant combinations were selected from the generated library of chimeric HIV V3 loop/HRV-14 based on the virus's ability to be neutralized with four anti-V3 loop Mabs. Out of the eight chimeric viruses studied, seven were able to induce anti-HIV-1 MN V3 loop neutralizing antibody responses *(74)*.

A canarypox viral vector encoding HIV gp120, gp41, *gag*, and *protease* was administered to HIV seronegative volunteers alone and with an HIV subunit vaccine. The viral vector preparation induced anti-HIV CTL activity, whereas the subunit vaccine alone was found to be poorly immunogenic *(75)*. Another canarypox virus vaccine (ALVAC) encoding gp120, gp41, *gag*, *protease*, two *nef* genes, and three *pol* regions was administered as a priming vaccine, followed by a rgp120 booster, in 140 HIV-seronegative volunteers. The viral vector preparation induced anti-HIV CTL activity that lasted as long as 3–6 mo after the priming administration and for greater than a year in 50% of vaccinated volunteers. The addition of subunit boosting doses elicited earlier, more potent anti-HIV humoral responses and did not appear to interfere with the elicitation of anti-HIV CTL activity *(76)*.

HIV DNA Vaccines

The DNA vaccine strategy refers to the administration of "naked" plasmids that encode specific protein(s) under the control of a mammalian promoter. The aim of this vaccine method is the in vivo expression of immunogenic proteins and elicitation of immune responses against the expressed protein. It is hypothesized that DNA vaccines mimic a live viral infection without the pathogenic potential from the viral genome.

Several methods for the administration of DNA vaccines have been utilized. These include intramuscular, intranasal and intravaginal routes of administration. In addition, epidermal inoculation of DNA-coated gold particles using a gene gun in different animal models has been used *(77)*. Thus far, these strategies have been well tolerated and able to elicit different types of immune responses.

Significant advantages of DNA vaccines over subunit vaccines include the cost of production and stability of the product. In addition, there is substantial flexibility in the incorporation of changes to the genes encoding the immunogenic proteins.

This section discusses possibly mediating immune responses elicited by experimental HIV-1 DNA vaccines. Additionally, we will discuss different strategies used for improving the immunogenicity of the DNA vaccines and the current state of DNA vaccine trials in nonhuman primate models as well as in humans.

Immune Response Elicited by HIV-1 DNA Vaccines

In order to design an optimum vaccine, DNA-based or otherwise, it is ultimately important to determine which immune responses mediate protection against infection. Although data demonstrate the likely relevance of both humoral and cellular immunity, these two types of responses appear to target different events of HIV infection *(77–79)*. It is suggested that humoral immunity protects from infection by neutralization of HIV infectious particles, keeping them from attaching to host cells as evidenced by the reduction in infectious particles due to anti-gp120 antibodies in HIV-infected chimpanzees *(79)*. Cellular immunity through CTL response is directed against already infected target cells to prevent further viral spreading and replication.

Some data suggest the importance of humoral immunity in mediating HIV infection, such as the correlation of high maternal antibody titers against the C-terminus region of gp41 and the lack of vertical transmission of HIV from infected mother to child *(47)*. In contrast, another study found neutralizing antibodies did not confer protection, as demonstrated by the inability of these antibodies to protect chimpanzees from infectious challenge

with primary isolates. Beneficial clinical effects of neutralizing antibodies may be correlated with the conformational states of gp120, as well as specificity to conserved regions of gp120 and gp41, since antibody affinity is the primary determinant of neutralization *(79)*.

Vaccine-associated increases in certain cytokines may mediate protection against HIV infection. For example, an HIV-1 DNA vaccine was able to induce increases in HIV-1 suppressive beta chemokines *(80)*, providing another positive correlate for an effective vaccine.

The elicitation of a cytotoxic response against conserved regions of HIV proteins is a potentially important vaccine strategy, by allowing the immune response to be focused on, and not diverted by, variable sequences. DNA vaccines encoding all of the HIV epitopes for $CD8^+$ T cells to which the human population can develop an immune response have been developed *(81)*. One advantage of eliciting $CD8^+$ T cell responses is the ability to target intracellular viral proteins, such as accessory and regulatory proteins (Nef, Rev, and Tat) *(82)*, which may reduce HIV spreading by killing infected cells before the production of new viral particles.

CTL responses appear to control early steps of the infection process as evidenced by exposed but uninfected healthcare workers and Gambian sexual workers with high levels of HIV-1-specific $CD8^+$ T cells. In addition, long-term nonprogressor HIV seropositive patients display high levels of HIV-specific CTL responses *(83,84)*.

Amplification of Immune Response to HIV-1 DNA Vaccines

Chemical adjuvants are compounds with physicochemical properties that increase the immune response in different ways, such as recruitment of antigen-presenting cells (APCs), the induction of cytokines, or the facilitation of DNA entry into the host cells. Several chemical adjuvants are used experimentally, including monophosphoryl lipid A (MPL), QS-21 saponin, ubenimex, cationic lipids, mannan-coated liposomes, microparticles, and bupivacaine *(85)*. When used with HIV DNA vaccines, most of these agents enhanced T_{H1} and T_{H2} immune responses.

DNA vaccines co-administered with genetic adjuvants can amplify and direct the immune response to either the T_{H1} or T_{H2} type. Genetic adjuvants are divided into two classes: cytokines (including chemokines), and cell adhesion or costimulatory molecules. Various molecular cytokine constructs, including T_{H1} (IL-2, IL-12, IL-15) and T_{H2} (IL-4, IL-10) interleukins, and GM-CSF (granulocyte-macrophage colony stimulating factor), have been administered with plasmids encoding different HIV-1 proteins. In the murine model, HIV DNA vaccines co-administered with IL-2 or 4 induced the best anti-HIV humoral response, whereas IL-12 appeared to shift the IgG2a/IgG1 ratio to favor the production of IgG2a (a T_{H1} humoral marker) *(86)*. The stimulatory effect of the co-administration of IL-12 plasmids with HIV DNA plasmids (*env*, *vif*, and *nef*) on CTL responses resulted in significant increases in HIV-specific lysis *(87)*.

In the monkey model, IL-2 plasmids co-administered with HIV DNA vaccines demonstrated in vivo peripheral T cell activation, increased anti-HIV antibody titers, and increased $CD8^+$ levels *(88)*. IL-15, which shares biological properties with IL-2, co-administered with HIV DNA vaccine in mice increased HIV-specific CTL activity, favored IgG2a antibody production, and increased levels of IFN-γ (a T_{H1} cytokine) *(89)*.

T_{H1} cytokines constructs of IL-2, GM-CSF, and IFN-gamma were co-administered with a DNA HIV vaccine encoding vpr and vpu, as well as partial deletion fragments of *pol* and

tat, in rats *(90)*. GM-CSF increased the anti-HIV immune response, while IFN-γ had little effect.

Plasmids encoding HIV-suppressive beta chemokines MIP-1 α and β and RANTES were co-administered with HIV DNA vaccines encoding *env/gag/pol* in mice and chimpanzees. Both chemokines induced HIV-specific CD8+ proliferation, but RANTES elicited higher levels of cytolytic activity and IFN-γ in both animal models *(91)*.

Cell adhesion, or costimulatory molecules, include CD154 (CD40L), CD80 and CD86 *(92–94)*. CD154 gives a signal required by B cells for activation and immunoglobulin class switching. Mice vaccinated with a DNA construct encoding human CD154 and HIV DNA plasmids encoding *env* and *rev* elicited higher anti-HIV humoral and CTL response *(92)*. In contrast, DNA constructs encoding CD80 or CD86 co-administered with HIV DNA plasmids encoding *env/gag/pol* in mice did not appear to elicit HIV-specific humoral responses; however, CD86 elicited strong anti-HIV CTL responses *(93,94,95)*.

Thus far, the results from studies with new approaches for improving the immunogenicity of HIV DNA vaccines in different animal models have been very encouraging. Co-administration of IL-2 and IL-4 constructs with HIV DNA vaccines encoding *env/gag/pol* in monkeys demonstrated significant increases in HIV-specific humoral immune responses *(96,97)*. IL-12 expressing plasmids co-administered with the same DNA vaccine in chimpanzees resulted in T_{H1} responses, including HIV-specific CTL activity and proliferation *(98)*.

In another study IL-2 coadministration with codon optimized *gag* and *env* induced stronger cellular immune responses in a rhesus study. Increasing the expression of HIV-1 antigens by DNA vaccines have been examined also. HIV utilizes a highly biased codon usage, which is not easily recognized by the mammalian cell machinery, resulting in a reduced level of expression of these genes. To increase expression, all HIV-1 MN gp160 and gp120 codons were exchanged with mammalian high expression codons, and the modified versions were utilized in DNA vaccines. Higher levels of expression of the immunogens resulted and favored development of a T_{H2} response, with the soluble gp120 inducing more humoral response than the gp160 *(99)*. Recently studies by Pavlakis have supported that the block to expression is actually mRNA dependency on *rev*. Codon changes modify RNA secondary structure and thus allow high levels of expression *(100)*.

Self-replicating expression vectors for an HIV DNA vaccine encoding the regulatory protein, *nef*, were found to express this protein at high levels for up to 3 wk. In addition, better T_{H1} and T_{H2} immune responses were elicited in mice vaccinated with self-replicating constructs than with a non-self-replicating DNA vaccine *(101)*.

Since HIV DNA vaccine boosting injections generally elicit weak immune responses and resistance to increased T_{H1} and T_{H2} immune response, strategies using DNA vaccines to prime and other strategies to boost have been tested. Upon testing a variety of combination regimens including HIV DNA plasmids, recombinant vaccinia virus, and subunit vaccines in mice, an optimal protocol was determined. The best immune responses were elicited by priming with a DNA vaccine encoding *env* followed by boosting with recombinant vaccinia virus encoding *env* with a subsequent boost with Env protein. A shorter period between immunization was found optimal for T_{H1} and T_{H2} immune responses; however, despite increased B cell and CTL activity, there was a poor response to a heterologous Env antigen possibly due to poorly conserved sequences in Env. The utilization of *env* cocktails rather than the administration of single-

env vaccines may have elicited more favorable results *(102)*.

Use of conserved sequences of HIV proteins Nef, Rev, and Tat may help to overcome poor immune responses to heterologous HIV clades. Since Tat has been determined to elicit lymphoproliferative and neurological AIDS associated disorders, a *tat* gene was developed to mutate the transactivation domain to discontinue its transcriptional activity. This mutated version elicited both T_{H1} and T_{H2} immune responses against wild-type Tat in mice *(103)*, which may block pathogenesis caused by the protein and may provide an avenue of therapeutic usage.

A DNA segment encoding a multi-CTL epitope of HIV, including three human CTL epitopes (20 epitopes restricted by 12 different HLA alleles), three macaque epitopes, and one mouse epitope, was incorporated into a plasmid vaccine, and the viral vector MVA (modified virus Ankara). A regimen of DNA plasmid followed by the MVA preparation resulted in increased T_{H1} and T_{H2} activity in mice and macaques. Human CTL clones recognized and lysed target cells expressing the epitopes. Thus, this is a potential approach for designing an effective DNA vaccine against HIV, based on the feasibility of identifying CTL epitopes that include the entire repertoire of human HLA alleles. Moreover, by using CTL epitopes for 10 HLA alleles, it is possible, in theory, to induce immune responses in most of the human population *(81)*.

HIV DNA Vaccines in Nonhuman Primate Models and Human Clinical Trials

DNA vaccines encoding *env/rev* and *gag/pol* genes from HIV-1 IIIB were administered to chimpanzees and elicited anti-gp120, anti-V3 loop of 120, and anti-gp41 antibody responses, whereas *gag* was not a significant inducer of antibody response *(104)*. Anti-HIV in vitro neutralization correlated to anti-HIV antibody response; however, anti-HIV CTL activity in each animal was variable. The two chimpanzees that elicited the strongest T_{H1} and T_{H2} immune response were infectiously challenged with HIV-1 IIIB and successfully protected. This same vaccine was administered to pregnant chimpanzees and found to induce T_{H1} and T_{H2} immune responses as well as placental transfer *(105)*. Importantly, the same vaccine elicited reduction in the viral load of infected chimpanzees *(47,49)*. The results obtained in the study of DNA vaccines in primate models suggest that relative protection and control of viral replication in HIV infection is possible with this approach *(97,106)*.

Only a few studies to date have been conducted using HIV DNA vaccines in human clinical trials. The first human trial used the plasmid APL 400-003, encoding *env/rev* genes from the HIV-1_{MN} isolate. The vaccine was administered to HIV-seropositive volunteers without AIDS, and who had not received HAART within the last 3 mo, and were homogenous based on $CD4^+$ and $CD8^+$ lymphocyte profiles. Three groups of five volunteers were administered three intramuscular injections of vaccine at the same doses (30, 100, or 300 μg). Each group was compared based on the doses they received. No pattern was determined in vaccine-associated effects on plasma viremia or $CD4^+$ and $CD8^+$ T cell populations. Subjects receiving 100 μg or 300 μg doses demonstrated increases in anti-HIV antibody responses and CTL activity, including an increase in anti-V3 loop antibodies after the second dose of 100 μg of this vaccine. Vaccine safety, the major goal of a phase I study, was demonstrated, and no significant adverse effects were reported *(107,108)*.

DNA vaccines encoding HIV regulatory proteins *nef*, *rev*, or *tat* were therapeutically administered to HIV-seropositive, asymptomatic patients with no prevaccination antibody

Table 2. Human HIV Vaccine Clinical Trials

Type of Vaccine	Vaccine Candidate	Cohort	References
Subunit	rgp160	HIV seropositive adults	*51,52*
	rgp160 segments (PCLUS 3-18MN and PCLUS6.1-18MN)	HIV seropositive adults	*54*
	rgp160 (plus alum)	HIV seropositive adults	*63*
	rgp160 and rgp120	HIV seropositive children	*55*
		HIV seropositive adults	*53,61*
	rgp120	HIV seronegative adults	*64,65*
		HIV seropositive pregnant women	*56*
	rgp120 (SPC3, V3 loop)	HIV seropositive adults	*67*
	p24 (HGP30)	HIV seropositive adults	*69*
	Gag/Env chimera	HIV seropositive adults	*62*
	HIV-1 immunogen)	HIV seropositive children	*58*
	HIV-1 immunogen (gp120 depleted) and rp24	HIV seropositive adults	*57*
	p24 (plus Zidovudine)	HIV seropositive adults	*59*
	rgp41 and Gag (plus HAART)	HIV seropositive adults	*60*
Toxoid	Tat toxoid	HIV seropositive (200–500 $CD4^+$ count)	*70*
Viral vector & subunit	ALVAC-HIV vCP205 (canarypox with gp120, *gag*, protease) + rp24	HIV seronegative adults	*75*
	ALVAC-HIV vCP300 (canarypox with gp120, gp41, *gag*, protease, *nef*, *pol*) and rgp120 subunit	HIV seronegative adults	*76*
DNA plasmid	APL 400-003 (HIV *env/rev*)	HIV seropositive adults	*107,108*
	HIV *nef*, *rev*, or *tat*	HIV seropositive adults	*82*
		HIV seropositive adults on HAART	*109*

responses to these proteins. As with the previous HIV DNA vaccine trial, these nine volunteers displayed no clear pattern of change in viral load. The *tat* vaccine elicited a slight increase in $CD4^+$ cells. Importantly, all three vaccines elicited increases in HIV-specific CTL precursors in eight of the nine volunteers studied, and the *nef* vaccine elicited the best anti-HIV CTL activity *(82)*.

HIV DNA vaccines administered in combination with HAART have also been studied. The volunteers were HIV-seropositive, had previously been immunized with rgp160, and had no significant antibody response to HIV-regulatory proteins. This regimen resulted in a low production of anti-HIV antibodies, and increases in CTL activity and IFN-γ production. HAART alone was found to reduce viral load, but did not increase anti-HIV CTL activity *(109)*.

Human clinical vaccine trials against HIV are summarized in Table 2.

Current Vaccine Trials

In general, phase I clinical HIV vaccine trials are designed to evaluate the safety and immunogenicity of candidate HIV vaccines. If the vaccine is well tolerated and immunogenic, then testing is expanded phase II, with larger cohorts and expanded regimens. A phase III study is used to evaluate safety and efficacy in a larger volunteer group. Except for several clinical trials being conducted by

pharmaceutical companies such as Wyeth-Lederle, Glaxo and Merck, most others are being sponsored by IAVI (International AIDS Vaccine Initiative) or the federal government. Those being conducted by the National Institute of Allergy and Infectious Diseases (NIAID) include those from AVEG (AIDS Vaccine Evaluation Group) and HIVNet. Recently, the William and Melinda Gates Foundation has been instrumental in providing support to IAVI for several ongoing and planned clinical vaccine trials. Some examples of ongoing trials are listed in Table 3. The table is not comprehensive since some trials, such as those sponsored by Merck and Glaxo, have just begun so no data on the trials are yet available.

Prophylactic vaccines designed to overcome HIV persistence and escape are being tested. In many currently conducted vaccine trials different candidate vaccines are combined to activate both arms of the host immune system. It is hoped that this combination approach to immunization will result in synergism capable of protective results.

C4-V3 peptides of HIV gp120 in IFA are being tested in HIV-seronegative volunteers (AVEG20), to examine the vaccine-associated anti-HIV T_{H1} and T_{H2} immune responses. Additionally, the study will determine the induction of HLA-B7 and HLA-A2 restricted $CD8^+$ CTLs, as well as HIV-specific delayed-type hypersensitivity (DTH) responses. The objectives of VaxGen's subunit vaccine (AVEG 36) are to evaluate and compare the safety and immunogenicity of monovalent vs bivalent HIV-1 candidate vaccines when formulated with QS21 or QS21 plus alum, and to determine with a new preparation of QS21 in polysorbate 80 is more reactive than in previous QS21 preparations.

Delivery of vaccines via alternative mucosal routes is being studied in two different trials using recombinant vectors and subunit vaccines. In these trials, either a live recombinant canarypox (AVEG 27) or attenuated *S. typhi* bacterial vector is used to express HIV-1 gp120 or truncated gp120, followed by rgp120 MN in alum boosting doses (AVEG28). Anti-gp120 mucosal secretory IgA and lymphoproliferative immune responses will be measured, as well as measuring titers of neutralizing anti-HIV antibodies and CTL activity.

Conclusion

The study of potential vaccines against HIV continues to be of international importance and as such has been both aggressive and extensive. As new vaccine strategies are characterized, it is increasingly important to perform a wide scale study of efficacy and safety of these candidates to most efficiently find an appropriate and effective vaccine. In general, large studies of experimental vaccine design must be tested in animal models before testing in humans. Although the murine model does allow for facile and inexpensive study of the potential immunogenicity of HIV vaccines, their genetic variance from humans is significant and limits the usefulness of determining vaccine efficacy. The cat model provides a better model; however, although FIV is analogous to HIV in many respects, there are significant differences that hinder the usefulness of anything but preliminary vaccine studies.

Nonhuman primates appear to provide us with the most useful animal model for vaccine development against HIV. A variety of monkey models allow us to test immunogenic parameters induced by vaccines, and the development of chimeric viruses (SHIV) provides a promising viral agent to examine vaccine efficacy against HIV. Chimpanzees are clearly the most genetically and immunologically similar to humans, and although HIV does not infect

Table 3. Current HIV Vaccine Human Clinical Trials being Conducted in North America

Type of vaccine	HIV antigen/strain (expression vector/strain)	Vaccine manufacturer	Adjuvant	Enrolling period	Protocol #	Reference
Synthetic peptide (Phase I)	gp120C4V3 MN, EV91, RF, CANO	WLV[1] and Pediatrics	IFA[2]	10/97–3/98	AVEG 020	
Recombinant subunit (Phase I)	rgp120 MN[3], rgp120 bivalent (AIDSVAX™B/E)	VaxGen	QS-21[4] w/w/o Alum	11/98–3/99	AVEG 036	*65*
Viral vector (Phase I)	gp120+TMgp41+*gag*+ protease MN,LAI (Canarypox/vCP205)	PMC[5]		1/00–present	AVEG 038	
	gp120+TMgp41+*gag*+ protease MN,LAI (Canarypox/vCP205) & vCP205+*nef*+*pol* MN,LAI (vCP1433) & vCP1433+2 vaccinia seq. (vCP1452)	PMC[5]		9/99–present	AVEG 034A	*117*
Nucleic Acid (Phase I)	gp160 MN (GeneVax® Plasmid/APL-400-003)	Apollon Inc.[6]	Bupivicaine	11/99–present	96I-50	*91,107,108*
	gp160 MN (GeneVax® Plasmid/APL-400-003)	WLV[1]	Bupivicaine	12/98–present	004	
Combination viral vector + subunit (Phase II)	*env*+*gag*+*pol* MN,LAI (Canarypox/ vCP205) +/–rgp120 SF-2[1]	PMC[5] & Chiron Vaccines	MF59[7]	5/97–1/98	AVEG 202	*61,65,118*
Combination viral vector + subunit (Phase I)	gp120+TMgp41+*gag*+ protease MN,LAI (Canarypox/vCP205) & gp160 MN, LAI-2	PMC[5]	PCPP[8] + Alum	1/00–present	RV124	
	env+*gag*+*pol* MN, LAI (Canarypox/vCP205) with rgp120 SF-2[3] & p24 SF-2 (*Saccharomyces cerevisiae*)	PMC[5] & Chiron Vaccines	MF59[7]	8/99–9/99	AVEG 032	
	env+*gag*+*pol* MN, LAI (Canarypox/vCP205) & rgp120 SF-2[3]	Therion Biologies & VaxGen	Alum	11/97–10/98	AVEG 027	*119*
Combination bacterial vector + subunit (Phase I)	gp120 LAI (*Salmonella typhi*/CVD908) & rgp120 MN[3]	Univ. of MD Center for Vaccine Dev. & VaxGen	Alum	12/97–5/99	AVEG 028	
Combination viral vector + DNA plasmid (Phase I)	*env*+*gag*+*pol* MN, LAI (Canarypox/vCP205) & *gag*+*pol* HXB2 (GeneVax® Plasmid/APL-400-047)	PMC[5] & Apollon, Inc.[6]	GM-CSF[9] and Bupivicaine	1/98–6/98	AVEG 033	*76,120*
	gag+*pol* HXB2 (GeneVax® Plasmid/APL-400-047) & *env*+*gag*+*pol* MN, LAI (Canarypox/vCP205)	PMC[5] & Apollon Inc.	Bupivicaine	7/97–present	AVEG 031	*121*

([1]Wyeth-Lederle Vaccines; [2]mineral oil with mannose mono-oleate; [3]Chinese hamster ovary cell derived; [4]purified component of saponin; [5]Pasteur Merieux Connaught; [6]now called Wyeth Lederle Vaccines; [7]squaline-in-water emulsion; [8]polyphosphazine; [9]granulocyte macrophage colony stimulating factor)

chimpanzees as aggressively as humans, they appear to be the only animal model capable of being infected and pathologically affected by HIV like humans. Chimpanzee use is both limited and prohibitively expensive for wide-scale vaccine safety and efficacy studies; however, accordingly their use should be judiciously weighed.

Subunit vaccine technology can be combined with viral vector delivery systems or utilized as peptide vaccine alone, or as novel approaches that use inserted peptides to create a more immunogenic epitope. However, most of the early focus surrounding subunit vaccine development was directed toward the generation of a humoral response, which has been found to have limited value alone in long-term protection from HIV infection. Currently, subunit vaccines are being designed to elicit both T_{H1} and T_{H2} immune responses. Ultimately, whether or not peptide vaccines alone will prove successful in preventing HIV infection remains to be determined. Promising experimental data suggest that when used with other vaccine strategies, such as viral vectors, peptide preparations may enhance immunogenicity and play an important role in the generation of an efficacious vaccine.

The therapeutic use of HIV DNA vaccines to reduce viral load in HIV-infected individuals is promising. Strategies to increase HIV DNA vaccine immunogenicity and T_{H1} or T_{H2} specific immune responses are being tested, including use of chemical and genetic adjuvants. Use of DNA vaccines together with other vaccine strategies has been tested, for example, priming with DNA vaccines and protein subunit boosting expressed by nonreplicating viral vectors. The immunogenicity and safety of HIV-1 expressing DNA vaccines have been demonstrated in both animals and humans. Importantly, the effect of this approach on modulation of viral load in primates gives optimism to the efforts.

Human clinical trials for testing new HIV vaccine candidates continue to provide valuable data in the search for a safe and effective vaccine. As our understanding of immune correlates of protection from HIV infection increases, the development of an HIV vaccine capable of protecting humans from HIV infection becomes more eminent. Newer adjuvants, delivery methods, and combinational regimens provide encouraging evidence that an effective HIV vaccine will one day be possible.

There has, in particular, been some considerable debate as to whether various vaccine preparations should actively proceed into phase III (i.e., efficacy) human clinical trials. The hesitancy stems from a lack of knowledge of the immunlogical correlates of protection against HIV-1. However, proponents argue that vaccine strategies need to proceed into efficacy trials in order to provide us with important immunogenicity information as well as indications as to which strategies are unlikely to be successful. To this end VaxGen began, in 1998, a phase III human clinical trial with their vaccine (AIDSVAX), which is a gp120 subunit preparation *(122)*. This was the first phase III trial of an HIV vaccine and is likely to pave the way for other preparations to be tested in phase III clinical trials. The results of such trials will have an important influence on where we will be in 5 years in our search for an effective HIV vaccine.

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