Current Advances in HIV Vaccines

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Development of a safe and preventive HIV-1 vaccine is a
high priority. Recent advances in HIV vaccine development
include an improved understanding of HIV envelope struc-
ture, development of techniques that enable a detailed anal-
ysis of vaccine-induced immune responses in humans,
expansion of the pipeline of promising candidate vaccines,
and completion of the first vaccine efficacy trials. A com-
mon feature of several preventive vaccine strategies in early
clinical trials is their ability to attenuate clinical disease
rather than completely prevent HIV infection in nonhuman
primates. One or more candidate vaccines will likely
advance into efficacy trials within the next few years, while
efforts to identify new designs that induce broadly neutral-
izing antibodies continue with incremental success.

Introduction

Human immunodeficiency virus is the world's deadliest
infectious disease with approximately 40 million individu-
als living with HIV, approximately 14,000 new infections
daily, and approximately 3 million deaths last year alone.
Populations that have had access to highly active antiretro-
viral therapies (ART) have experienced a dramatic drop in
AIDS deaths. Many resource-poor nations provide ART to
prevent maternal-to-child transmission of HIV and are
beginning to make ART more widely available to HIV-
infected persons through international and national donor
initiatives. However, HIV continues to spread virtually
unabated in many parts of the world. Safe and effective
preventive vaccines are a proven public health approach
for stopping the spread of viral disease. The potential value
of a preventive HIV vaccine to protect individual recipients
from AIDS and stop HIV transmission is indisputable.

Preventive HIV vaccine trials have been conducted in
the United States since 1987. Worldwide, more than 65
different vaccine products and more than a dozen experi-
mental adjuvants have been evaluated in clinical trials
enrolling more than 12,000 HIV-negative individuals. This
review summarizes different vaccine strategies, describes
recent technologic developments to measure immune
responses, and previews vaccine products that are in or
close to clinical trial.

Humoral Immune Responses

A critical challenge in HIV vaccine development is the
design of an immunogen that induces broadly neutralizing
antibodies. Neutralization epitopes reside on the surface
glycoprotein (gp)-120 and transmembrane gp-41 envelope
(Env), which are derived from a gp-160 precursor mole-
cule. These glycoproteins form a trimeric complex and
bind to host receptor and coreceptor to trigger a series of
membrane-fusion events that culminate in virus entry [1].
HIV-1 is neutralized when antibodies bind critical regions
on either glycoprotein, thus disrupting the entry process.
However, during the course of HIV infection, the virus
mutates to escape antibody recognition and persist in the
host. Longitudinal studies of autologous neutralizing anti-
body indicate that the antiviral capacity of antibody
responses can be of sufficient magnitude to completely
replace circulating neutralization-sensitive virus with suc-
cessive populations of neutralization-resistant virus. Fur-
thermore, specific genetic changes in Env permit the
acquisition and rearrangement of glycan moieties, result-
ing in an evolving glycan shield, which can evade antibody
recognition [2•,3•].

Despite the enormous genetic variability and continu-
ous evolution of HIV-1 variants that escape humoral
immune surveillance, a small number of human mono-
clonal antibodies (mAbs) derived from HIV-1–infected
individuals (*eg*, b12, 447-52D, 2G12, 2F5, and 4E10) have
broad and potent cross-clade neutralizing activity [4].
These mAbs recognize epitopes in gp-120 or gp-41 that are
highly conserved across the genetic subtypes, and they also
display highly unusual features. For example, the b12 and
2F5 mAbs have an unexpectedly long complementarity
determining region loop. Passive administration of combi-
nations of these neutralizing antibodies prevented infec-
tion in animal models [5–7]. Neutralizing activity
appeared to be associated with the ability to bind to func-
tional trimeric Env but not isolated gp-120 or gp-41 sub-
units [8]. A corollary of this observation is that an
immunogen should be able to elicit neutralizing antibod-
ies that bind with reasonable affinities to conserved parts
of the Env trimer.

Novel Approaches to Antibody Induction

Human immunodeficiency virus-1 vaccines based on components of Env glycoproteins have not generated potent neutralizing antibodies with desirable breadth and magnitude against primary HIV-1 isolates. Vaccine-induced antibodies have demonstrated a limited specificity for T-cell–line adapted strains of virus that closely resemble the vaccine strain [9]. Env candidates may have induced mostly subtype-specific antibodies because they were directed against monomers or because the conserved Env core is shielded by large surface-exposed variable loops and sugar moieties that mask conserved neutralization epitopes.

Knowledge of the atomic structure of gp-160 is helping guide the design of Env candidates that mimic the native trimeric Env structure on the virion surface. The approaches include mimicking the native Env spike and modifying monomeric, gp-160, or gp-140 by hyperglycosylating Env, deleting variable loops from Env, and partially deglycosylating Env [10,11]. Other approaches include CD4 Env complexes, CD4 mimetic, and CD4-independent Env that may expose the conserved coreceptor binding site [12].

Approaches that trigger and freeze conformational intermediates formed during binding and fusion of gp-120 and gp-41 to the cell membrane are also under investigation. Antibodies raised against several gp-41 regions involved in fusion lack neutralizing activity in conventional assays. Major constraints to their efficacy are an inability to gain access to epitopes within the limited space between the attached virus and the cell surface and the inability to bind in time to trap the transient fusion intermediate.

Other approaches include using polyvalent Env, polyvalent peptides, consensus Env, and ancestral Env as immunogens in an effort to minimize the genetic and antigenic differences between vaccine and field isolates. Early studies have shown that artificial consensus strains fold properly, retain some functional activity, and retain key epitopes [13]. Another approach has been to immunize animals with a mixture of 23 recombinant vaccinia viruses, each expressing distinct Env glycoproteins to maximize the breadth of neutralizing antibody response [14]. However, the optimal number of different Env immunogens to elicit this response or the forms of Env protein for inclusion in the cocktail remains uncertain because little information exists regarding HIV neutralization serotypes.

Novel Neutralizing Antibody Assays

Several new neutralizing antibody assays have been reported. The new assays use genetically engineered cell lines containing viral protein-activated reporter genes, such as Tat-activated luciferase reporter gene. The engineered cell lines are more reproducible compared to fresh peripheral blood mononuclear cells (PBMCs) and permissive to infection by a wide range of HIV-1 variants. A recent comparative study of these novel assays in five laboratories suggested that the assays were equivalent and reliable and yielded qualitatively similar outcomes. However, quantitatively the assays appeared different and this requires further investigation [15]. Although these assays do not substantially enhance sensitivity compared to PBMC-based assays, they offer a simple cost-efficient, quantitative, and high throughput format. Standard clade-specific panels of HIV-1 isolates and polyclonal reference reagents needed to compare antibody-inducing vaccine candidates are under development.

Clinical Evaluation of Envelope Protein

The first two phase III double-blinded placebo-controlled clinical trials of two candidate HIV-1 gp-120 subunit protein vaccines were completed. Both trials evaluated alum-adjuvanted bivalent protein subunit vaccines containing recombinant gp-120 from two strains of HIV-1: T-tropic HIV-1 (clade B) and M-tropic HIV-1 (clade B or E). The first trial (VAX004; B/B product) was conducted in North America, Canada, and the Netherlands, predominantly in a population of men who had sex with men. The second trial (VAX003; B/E product) was tested in Thailand in injection drug users. Vaccine-induced protection from HIV infection was not observed in either trial. The trial sponsors initially reported in the media that gp-120 was efficacious in Hispanic, African-American, Asian, and self-described "other" subjects in VAX004. Further analysis demonstrated that efficacy in these groups was not significant and that no differences in host factors, risk behaviors, or viral or antibody response factors supported the plausible efficacy in these groups [16]. In other studies of different Env candidates, neutralizing antibody responses did not differ between whites and African-Americans, supporting the conclusion that there is no biologic basis to racial or gender differences in these trials [17].

Novel Technologies to Measure Vaccine-induced T-Cell Responses

Evidence from HIV-infected humans and simian immunodeficiency virus (SIV)- or simian-human immunodeficiency virus (SHIV)-infected rhesus macaques suggests that T-cell responses are important in controlling HIV replication. Consequently, much effort has been devoted to the design of HIV-1 immunogens capable of eliciting T-cell responses. To determine whether a cellular immune function correlates with vaccine-induced protection in humans will require a standardized and validated assay that is quantitative, highly sensitive, specific, quality-controlled, and amenable to evaluation of large numbers of cryopreserved cell samples generated in a vaccine efficacy trial. The most readily accessible source of T cells is the peripheral blood. Therefore, collection, processing, cryopreservation, storage, and manipulation of biologically viable PBMCs are key components for laboratory-based assessments of vaccine candidates.

Traditionally, measurement of T-cell responses used chromium-release lytic assays or lymphoproliferative assays. A new generation of single-cell techniques, such as

interferon-γ Elispot assay, major histocompatibility complex peptide tetramer binding, and flow cytometric analysis of intracellular cytokine production, are replacing older technologies. These newer assays display an improved sensitivity to enumerate antigen-specific T cells in immunized individuals, are easier to perform, amenable to an automated format, require fewer cells, and enable quantitation of lineage-specific cell surface phenotypes.

Cellular Immune Responses

Several key observations in humans have demonstrated a link between cellular immunity and protection against disease progression. High levels of CD8^{+} cytotoxic T lymphocytes (CTL) measured after primary infection were closely associated with the drop in plasma viremia. Published work demonstrated an inverse association between HIV-specific CD8^{+} T cells and the steady-state plasma viral load or viral set point. In addition, viral set point inversely correlated with survival time after infection [18]. Therefore, lowering this set point may be an important target of a successful vaccine. The breadth and clonal diversity of the primary CD8^{+} T-cell response is predictive of disease progression: broad polyclonal responses are associated with more favorable outcome [19]. HIV-specific CTL was detected in commercial sex workers and other individuals who were repeatedly exposed to HIV-1 but remained uninfected. However, some exposed seronegative patients have been described who have extremely low levels of HIV, exhibit HIV-1–specific cytotoxicity, and remain seronegative [20•]. In addition, CD8^{+} T cells from long-term nonprogressors had a greater capacity to proliferate and this effector function was coupled to perforin expression [21]. HLA specificity can significantly influence the rate of HIV disease progression and the responses to candidate vaccines. HLA types HLA-B27 and HLA-B57 are associated with clinical long-term nonprogression and positive responses to vaccines, whereas HLA-B35 is associated with faster disease progression [22–24]. Recent simultaneous evaluation of viral genotypes and HLA alleles in 473 patients with chronic HIV infection indicate that polymorphisms in HIV-1 are most prevalent at sites of the least functional or structural constraint and are associated with particular HLA class I alleles [25].

Despite convincing observations that potent CD8^{+} CTLs help control HIV, uncertainties remain as to whether vaccine-induced responses will be sufficient to achieve long-lasting effective control of HIV replication. Vaccines that depend strictly on a CTL response may or may not fail eventually as a result of the emergence of epitope-escape variants that allow the virus to escape detection by T cells. This response was documented in a vaccinated macaque, in addition to humans in which mother-to-child transmission of HLA-B27–restricted Gag escape mutants remained stable and the infants showed impaired viral control [26,27]. However, recent work has identified two escape mutations within an immunodominant HLA-B57 restricted Gag epitope, one of which undergoes reversion and the other is stable in the absence of the B57 allele that drives its selection [28]. This work highlights the potential for diverse outcomes and confirms the need to clarify the fate of escape mutations to determine which immune responses remain relevant and which are irrevocably lost through escape mutation.

T cells and Vaccine Design

In HIV-1 infection, CD4 T cells are preferentially infected and the lack of adequate T-cell help may be a key reason for the functional impairment of protective memory cells and the eventual failure of immune control of viremia in HIV-infected patients [29–31]. Early antiviral therapy of acutely infected patients appears to preserve virus-specific CD4^{+} T-cell responses and allows selected patients to maintain low viral loads [32]. This result suggests that early control of viral replication by an effective vaccine-induced immune response may help to preserve critical CD4^{+} T-cell function, which could then coordinate effector CD8^{+} T-cell and B-cell memory responses.

Memory CD8^{+} T cells will need to persist for long periods through homeostatic proliferation. The speed at which an effective anamnestic immune response is generated and whether it will be sufficient to prevent generation of resting T cells harboring integrated provirus remains unknown. Continued immune control may be necessary to prevent disease progression. However, if sufficiently high levels of memory T cells exist before HIV exposure and if those memory T cells yield effector T cells that prevent the formation of latently infected resting T cells, then establishment of chronic infection may be prevented.

Novel Approaches to Induce Cytotoxic T Lymphocytes

Several vaccine strategies have proven effective in inducing high-frequency CD8^{+} T-cell responses in monkeys that effectively control, but do not resist or clear, viral infection. If these nonhuman primate studies prove predictive of what will be observed in humans, patients who have vaccine-elicited CTL responses and are subsequently infected with HIV-1 will contain the virus more effectively compared to nonimmunized individuals. A vaccine that results in containment of viremia may also lower or prevent transmission because control of virus replication in HIV-infected patients is associated with decreased rates of disease progression and sexual transmission.

Published reviews have adequately described several first-generation viral vectors designed to elicit CTLs [33]. Therefore, this review will focus on new vaccine strategies in or close to human trial.

DNA Plasmids

Several DNA plasmid candidates, some of which have demonstrated promising results in animal models, are in

clinical trials. Some DNA vaccines express a single HIV antigen, whereas other vaccines express multiple antigens. Most candidates are based on the HIV subtypes circulating in the proposed trial population and most are codon-optimized to maximize expression of HIV genes in human cells. In nonhuman primates, plasmid DNA has generally been safe when injected intramuscularly by needle or when adhered to gold beads and delivered epidermally using a gene gun. Animals immunized with HIV *gag* DNA vaccine adjuvanted with interleukin (IL)-2/immunoglobulin G fusion protein or IL-2/immunoglobulin G encoding plasmid maintained their CD4$^+$ T-cell numbers, exhibited low to undetectable viral loads, and were protected from disease [34]. One of these vaccinated animals later progressed to disease, and this progression was associated with detection of a CTL escape mutant, further supporting the role of CTL in controlling infection [26]. A human version of this plasmid has entered phase I trials. Other DNA vaccines are being developed with additional cytokine gene adjuvants, such as IL-15 or IL-12. These vaccines are complex and include a mixture of various HIV genes or peptides representing CTL epitopes and the cytokine-expressing plasmid. Immunization of rhesus macaques with a *gag*-expressing DNA plasmid and a IL-15–expressing plasmid resulted in an increase in magnitude of Elispot responses. Similar results were observed with an IL-12–expressing plasmid adjuvant [35].

A DNA vaccine produced by Merck Research Laboratories (MRL; Whitehouse Station, NJ) and expressing clade-B *gag* genes is in a phase I trial in the United States. In addition, two different *tat* DNA vaccines are undergoing separate evaluations in phase I trials in Italy and Finland. One vaccine, based on clade-B HIV, expresses *env*, *gag*, *pro*, reverse transcriptase, *tat*, *rev*, and *vpu*, is in a phase I trial in the United States. Another clade-B–based multigene DNA vaccine expressing *env*, *gag*, reverse transcriptase, *rev*, *tat*, and *vpu* is in a phase I trial in Australia, with plans for clinical trials of a clade-E–based candidate in Thailand. One clade-A DNA vaccine encodes HIV-1 p17, p24, and a polypeptide of 25 CTL epitopes from *env*, *gag*, *pol*, and *nef*, and it is undergoing evaluation in phase I/II trials in the United Kingdom and phase I trials in Kenya, Uganda, and the United Kingdom. The vaccine has been well tolerated and immunogenic. Preliminary results suggest that cellular immune responses were directed predominantly to the Gag insert rather than the polyepitope [36]. Another polyepitope DNA vaccine encodes 21 CTL supertype epitopes of HIV recognized by multiple HLA alleles and is in a phase I trial in the United States and Botswana [37].

The National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center has conducted phase I trials to evaluate a DNA vaccine expressing a Gag-protease fusion protein and a cocktail of four DNA plasmids expressing a *gag-pol-nef* fusion protein from clade B and three modified Env proteins from clades A, B, and C. A clade-C DNA vaccine composed of two plasmids, one encoding *env* and *gag* and the other encoding *pol*, *nef*, and *tat* genes, recently entered a phase I trial in the United States. These and other planned trials will determine if a single clade exhibits cross-clade responses, if inclusion of multiple clades leads to a broader immune response, and if volunteers respond to all of the components of a multi-component vaccine.

Recombinant Viral Vectors

The most extensively studied viral vectors in human HIV vaccine trials belong to the poxvirus family: vaccinia and canarypox. Because of the potential virulence of vaccinia infection in immune-deficient individuals, attention has been focused on poxvirus vectors with very limited or no ability to replicate in human cells. These vectors include attenuated vaccinia vectors, such as modified vaccinia Ankara (MVA) and NYVAC, in addition to avipoxvirus viral vectors, such as canarypox and fowlpox.

A variety of recombinant canarypox candidate vaccines (ALVAC) have entered clinical trials. ALVAC-based vaccines have an excellent safety profile and more than 2000 participants have received ALVAC-HIV vaccines. ALVAC-HIV vaccines have been able to generate CTL in up to 50% of patients. However, the magnitude and breadth of these responses have been relatively weak. A study of ALVAC-HIV vCP1452 completed in the United States focused on evaluating sixfold higher doses compared to doses administered previously. The higher dose resulted in increased local and systemic reactogenicity without a corresponding increase in immunogenicity. A phase III community-based trial involving ALVAC-HIV vCP1521 and AIDSVAX B/E (VaxGen, Inc., Brisbane, CA) is being conducted in Thailand in collaboration with the Walter Reed Army Institute for Research and NIAID. ALVAC 1521 encodes gp-120 from a clade-E HIV and portions of the *gp-41*, *gag*, and protease genes from subtype B. This collaboration is the first vaccine efficacy trial of a prime boost strategy.

In the first stage of a separate collaboration with Thailand, the University of New South Wales, Australia, began clinical trials in 2003 of a fowlpox vector vaccine expressing clade-B HIV *env*, *gag*, *pol*, *tat*, *rev*, and *vpu*. Another poxvirus, MVA, was used as a vector-encoding SIV *gag* and *pol* to immunize rhesus macaques and elicited antigen-specific CTL responses. During SIV challenge, vaccinated animals responded with a vigorous anamnestic CTL response and displayed reduced viremia that inversely correlated with the magnitude of the vaccine-induced CTL response [38]. These findings prompted continued development of recombinant MVA candidates for phase I trials. A highly attenuated form of vaccinia virus (*ie*, NYVAC) expressing *env*, *gag*, *pol*, and *nef* from a clade-C isolate of HIV is undergoing human evaluation in the United Kingdom and Switzerland. Another clade-C vaccine, based on an adeno-associated viral vector, is being evaluated in a phase I trial in Belgium.

Venezuelan equine encephalitis is an alphavirus that synthesizes high levels of recombinant proteins and targets professional antigen-presenting cells. A phase I trial to test a prototype attenuated Venezuelan equine encephalitis replicon vaccine expressing the *gag* gene from a South African clade-C virus strain began in the United States and South Africa in 2003.

Adenoviruses cause a variety of common infectious diseases in normal hosts, including respiratory infections and conjunctivitis, in addition to more serious infections in immune compromised hosts. Therefore, their use as a vaccine vector poses the challenge of overcoming pre-existing immunity to adenovirus and safety concerns of replicating vectors in immunocompromised individuals. Researchers from MRL compared different candidates encoding the SIVmac239 *gag* gene administered to rhesus macaques and subsequently challenged with SHIV89.6P. Animals immunized with the Ad5 SIV *gag* vaccine before SHIV 89.6 challenge had the highest prechallenge levels of cellular immunity, which was measured by Elispot and tetramer assays, significantly reduced peak viral RNA, no loss of CD4 cells, and were protected from disease [39]. In animal studies, long-term follow-up of parameters, such as peak viremia, viral RNA at set point, slope of CD4 decline, and tissue-based viral reservoirs, will be essential to evaluate the extent and durability of protection during infection.

A MRL-developed replication-defective adenovirus type 5 expressing HIV-1 clade-B *gag* is being evaluated in a dose-ranging study in the United States and internationally. The vaccine appears to be well tolerated and immunogenic. Individuals with pre-existing immunity to adenovirus-5 exhibited lower immune responses to the vaccine. However, a higher dose of the vaccine or a combination with a DNA prime may help overcome prior immunity. Researchers at the NIAID and the National Cancer Institute are also advancing recombinant adenovirus vectors into phase I trials.

Prime Boost Strategies

Recombinant poxvirus vaccines have proven successful in boosting DNA-primed CD8+ T-cell responses to 10 to 100 times higher compared to responses elicited by DNA or recombinant poxvirus immunizations alone in rhesus macaques. Priming with DNA may focus the immune response in the inserted genes, which efficiently expands when poxvirus-infected cells express large amounts of protein and proinflammatory cytokines. In rhesus macaques, a pathogenic SHIV challenge was controlled by the immune responses induced by intradermal saline inoculations of DNA, but not gene-gun–delivered DNA, followed by a recombinant poxvirus booster. Viral control achieved by DNA priming and recombinant poxvirus boosters were superior to viral control achieved by DNA priming and DNA boosting or by DNA priming and boosting with a recombinant protein [40]. In another study, DNA priming followed by recombinant poxvirus boosts controlled

SHIV89.6P administered by a mucosal route 7 months after the last vaccine dose (control persisted for at least 1.5 years after challenge) [41]. In a similar study using an SIV-*gag*–expressing DNA plasmid boosted by a recombinant defective adenovirus vector also expressing SIV *gag*, vaccinated animals were protected against T-cell loss, high viremia, and SHIV-induced disease. However, another DNA/MVA prime boost vaccination regimen in rhesus macaques provided no protection to challenge with SIVmac239 virus [42]. This study differed from the successful studies in two respects: a gene gun rather than saline injections of DNA was used for priming and SIV, instead of SHIV, was the challenge virus. Lower-dose challenge models that may better mimic human exposure are under development. Several groups have designed recombinant viral vectors for use after DNA priming in human trials. Investigators in Australia are conducting trials involving a combination of matching multiantigen DNA and fowlpox vector vaccines. In a similar approach, the International AIDS Vaccine Initiative has supported the conduct of a series of clinical trials in the United Kingdom and Africa to evaluate the combination of the DNA and MVA vaccines.

Priming with one viral vector and boosting with another is an alternative approach. MRL and Aventis (Strasbourg, France) have demonstrated in rhesus macaques that priming with a replication-defective adenoviral vector-expressing *gag* and boosting with a canarypox-expressing *gag* resulted in a more balanced CD4/CD8 response compared to immunization with the adenovector alone (as prime and boost) [43]. This approach entered clinical trials in 2003. Although nonhuman primate vaccine studies suggest that vaccine-induced CD8+ T-cell responses may successfully control viral replication and postpone clinical disease, this control may not prove sufficient to prevent infection or to block the spread of HIV to others. The induction of strong humoral and cellular responses will be necessary to optimize protection. Early clinical trials demonstrated that viral vector priming and protein boost induced cellular and humoral responses. Experiments to elucidate the impact of a combined cellular and humoral immunity in nonhuman primates are ongoing. In one prime boost strategy, researchers are evaluating the combination of a multigene DNA vaccine formulated in microparticles with an oligomeric gp-140 with MF59 as an adjuvant in phase I trials in the United States.

Conclusions

Further understanding of the biology of HIV-1, especially the three-dimensional structure of HIV Env and antibody-binding sites, will continue to inform the design of new candidate vaccines that warrant preclinical and, in some cases, clinical evaluation. Our expanding knowledge of anti–HIV-1 immune responses and the availability of more sophisticated immune assays will enable us to compare vaccine candidates and perhaps minimize controversies

surrounding the initiation of vaccine efficacy trials [44,45]. The development of an effective HIV vaccine that addresses HIV diversity will become a reality only through the conduct of well-designed ethically executed human efficacy trials.

Acknowledgment

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