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Review Article

HIV vaccines: current approaches and new developments

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Abstract

The challenge of developing an acquired immune deficiency syndrome (AIDS) vaccine continues to be paramount as the global AIDS epidemic progresses. Traditional vaccine strategies that induce broadly neutralizing antibodies have so far been ineffective, and focus is now turning toward more novel approaches that seek to elicit T-lymphocyte responses. Recent results from the STEP vaccine trial, a test-of-concept trial of an adenoviral serotype 5-based vaccine, failed to show a protective effect despite clear induction of human immunodeficiency virus (HIV) immunogenicity and early promising results in nonhuman primate studies. The results from the STEP trial demonstrate that innovative approaches and a better understanding of the immunopathogenesis of HIV disease are needed for the goal of a safe, globally effective HIV vaccine to be realized.

Introduction

The global acquired immune deficiency syndrome (AIDS) epidemic is now entering its third decade, and in that time it has accounted for over 20 million deaths (1). Latest estimates indicate that the number of people living with human immunodeficiency virus (HIV) has risen to 33.2 million, with some 2.5 million people newly infected just within the last year. Although potent antiretroviral therapy has dramatically improved outcomes, practical limitations have made it difficult to deliver therapy to all areas in need. Worldwide, only 1 in every 4 HIV-positive individuals living in resource-limited settings is on antiretroviral medications, and for each person who begins therapy there are an estimated 6 people who become newly infected (2). Because of the economic and logistical constraints of widespread antiretroviral therapy, the need for an AIDS vaccine remains paramount. It has been estimated that even a vaccine with only 50% efficacy, delivered to just 30% of the population, could reduce annual infections by up to one-third, averting 17 million infections over the next 15 years (3). The scientific barriers to the development of a safe and globally effective AIDS vaccine, however, remain daunting. To date, attempts at inducing HIV envelope-directed neutralizing antibody responses have proven ineffective. Meanwhile, it remains unclear if vaccines that generate cell-mediated immune responses can prevent infection or possibly delay disease progression.

Natural history of HIV infection

HIV is most commonly transmitted via mucosal exposure during sexual contact. Studies in nonhuman primate models suggest that the first cells to become infected at mucosal entry sites are resting memory $CD4^+$ T cells that express the HIV coreceptor CCR5 (4, 5). The virus replicates locally within mucosal sites and is also transported to draining lymph nodes, where it can be detected about 1 week following mucosal exposure. Dendritic cells have been implicated in the transport of HIV to lymph nodes, and they may directly facilitate transfer of virus to T cells, thus increasing the efficiency of T-cell infection (6-8). Starting at about 7 days after exposure, the virus is detected hematogenously and a robust burst of viremia peaks at around 3 weeks (Fig. 1). This viremia seeds peripheral sites, particularly gut-associated lymphoid tissue, which houses an abundance of $CD4^+$ memory T cells, over half of which are destroyed during the first 4-10 days of acute infection (4, 9, 10).

The initial adaptive immune response to acute infection consists primarily of HIV-specific $CD8^+$ T lymphocytes, which expand to approximately 10% of all circulating $CD8^+$ T cells during the first weeks after exposure and likely mediate the initial control of viremia (11-14).

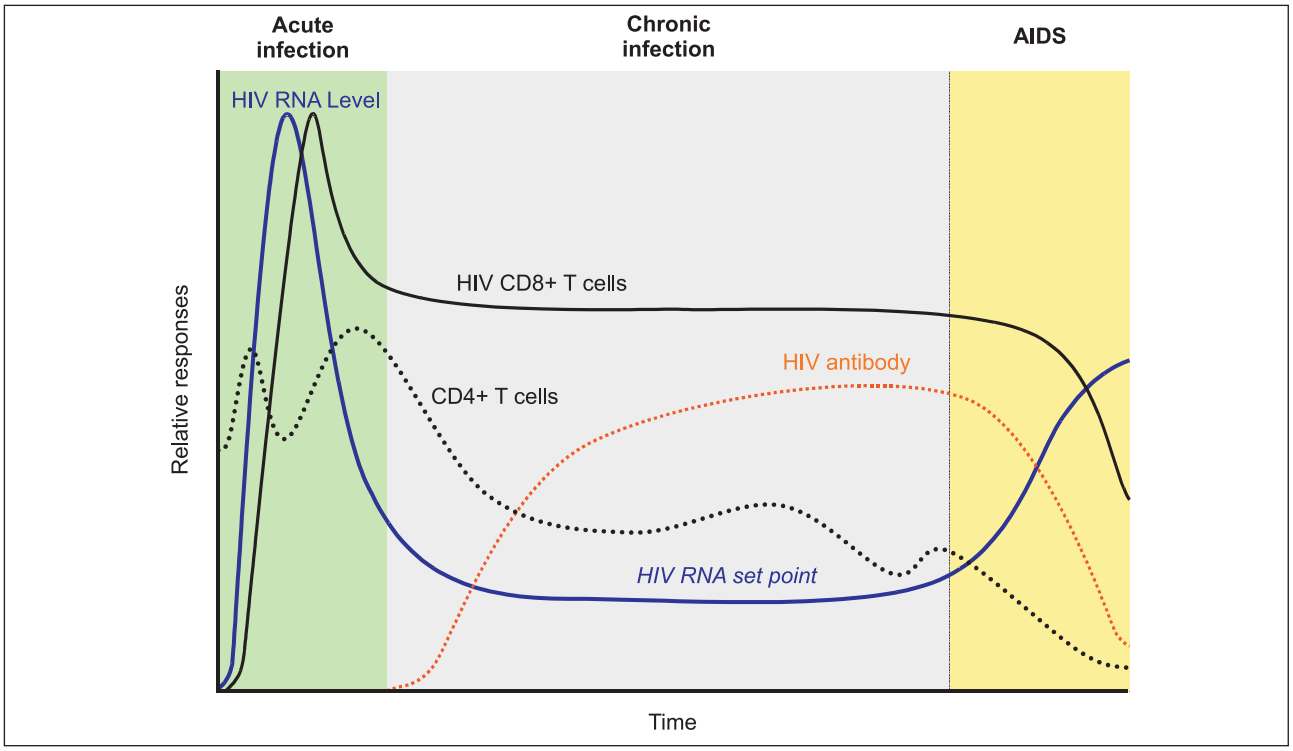

Fig. 1. Immune response to human immunodeficiency virus (HIV) infection. Relative changes in HIV RNA level, HIV-specific CD8+ T-cell response, HIV-specific antibody response and CD4+ T-cell levels during HIV infection.

Consistent with this hypothesis is the observation that depletion of CD8+ T cells in macaques acutely infected with simian immunodeficiency virus (SIV) results in high levels of sustained viremia and accelerated clinical decline (15, 16). CD8+ T-cell responses are eventually able to suppress plasma viremia within 2-6 months to levels 10- to 100-fold less than those achieved at the peak of acute infection. In almost all patients, however, virus remains detectable and a viral set point is established. This viral set point is inversely correlated with the rate of disease progression (17).

During the chronic phase of infection, CD8+ T-lymphocyte responses continue to be necessary for suppression of virus. HIV develops mutations in the dominant epitopes targeted by CD8+ T cells, however, thereby causing shifting patterns of immunodominance that reflect viral adaptation to selective pressure and reciprocal alterations in the specificity of the cellular immune response (18-20). At 1-2 months after the initial exposure to virus, HIV antibodies appear. This antibody response likely has little role in controlling acute infection, although it is accompanied by adaptive changes in the viral envelope. These changes appear to have little functional cost to viral fitness, unlike escape mutations that occur in response to CD8+ T lymphocyte-driven selective pressure (21-25). A plateau phase of infection is eventually achieved in which patients may remain clinically stable for years. The latent reservoir of infected resting CD4+ T cells, however, persists, even with long-term antiretroviral therapy that suppresses plasma viremia to undetectable levels (26). Without antiretroviral therapy, the disease is almost invariably progressive and fatal.

Proven vaccine strategies and early attempts at an HIV vaccine

A major challenge to the design of an effective HIV vaccine is the seemingly inexhaustible ability of the virus to mutate and generate escape variants. This has made the use of classical vaccine strategies frustratingly problematic. Vaccines currently licensed for use in humans utilize live attenuated virus, inactivated virus or recombinant viral proteins (27). Each of these strategies has shortcomings when it comes to designing an HIV vaccine.

Live attenuated viruses replicate in vivo but are weakened so that they do not cause disease. This replication induces CD8+ T-cell, CD4+ T-cell and antibody responses to viral antigens. Yellow fever, oral polio, measles, mumps, rubella and varicella vaccines are all examples of live attenuated vaccines currently in clinical use. In non-human primate models, live attenuated vaccines for SIV have been shown to effectively induce CD8+ T-cell responses and to provide significant protection from viral challenge (28-30). Such live attenuated strains, however, can revert to pathogenic variants and cause disease (31-33). For this reason, the strategy is considered too risky for use in humans.

Vaccination with nonreplicating immunogens avoids the potential pathogenicity of live attenuated viruses. Nonreplicating immunogens predominantly elicit CD4+

T-cell and antibody responses, without inducing $CD8^+$ T-cell responses. If high titers of neutralizing antibody responses could be elicited against HIV, they might protect against infection. Studies in nonhuman primate models of HIV show that passive immunization with HIV-specific monoclonal antibodies, albeit at very high antibody concentrations, is protective in macaques which are challenged with an SIV–HIV chimeric virus (SHIV) either intravenously or intravaginally (34, 35). Similarly, intravaginal administration of HIV antibodies followed by vaginal challenge of virus also demonstrates protection from infection (36). Antibody administration after infection, however, is not protective (37).

There are two main strategies to elicit antibody responses against viruses using nonreplicating immunogens: immunization with whole inactivated viruses or recombinant viral proteins. Examples of successful whole inactivated virus vaccines are those against hepatitis A, influenza and polio (specifically, the inactivated polio vaccine). Recombinant viral proteins have been used to develop vaccines against hepatitis B and human papillomavirus. Both of these strategies have been pursued in developing an HIV vaccine, without success to date.

In nonhuman primate models of HIV, the use of inactivated virions initially appeared promising, until it was discovered that the protective immunity that was seen was due to antibody responses directed against human proteins that had been incorporated in the outer membrane of the virus during its production in human cell lines (38, 39). When virus was produced in simian cell lines, whole inactivated virions no longer elicited neutralizing antibody responses. Although an inactivated HIV vaccine has been studied in HIV-infected patients to determine if virus-specific immune responses can be elicited or viral replication controlled, this strategy has not been tested for use as a preventive vaccine (40-42).

In contrast, using recombinant viral proteins was one of the earliest approaches to developing a preventive HIV vaccine. Recombinant soluble HIV envelope (Env) protein was tested in the first and only phase III clinical trial of an HIV vaccine completed to date. The reagent failed to protect men who have sex with men or injectable drug users from seroconversion despite eliciting HIV antibodies in 90% of those who received the vaccine (43, 44). The recombinant gp120 is currently being tested as part of a prime–boost strategy in conjunction with an engineered canarypox vector encoding genes for HIV gag, pol and gp120.

Challenges to inducing broadly neutralizing antibody responses

The failure of HIV inactivated virion or recombinant subunit vaccines to induce protective immunity is likely due to their inability to stimulate broadly neutralizing antibody responses. If this can be achieved, the development of a vaccine that stimulates broadly neutralizing antibody responses to HIV would be the most optimal approach. Native gp120 exists as a homotrimeric complex on the surface of virions in which conserved regions of gp120 reside within thermostable crypts that are masked by variable loops and regions of heavy glycosylation. The 24-35 *N*-linked glycosylation sites within gp120 create a protective barrier around the surface envelope that reduces its immunogenicity. This is in sharp contrast to the influenza hemagglutinin protein, which contains less than seven glycosylation sites (45, 46). The critical importance of HIV envelope glycosylation in immune evasion is supported by experiments in which macaques were infected with an SIV carrying an envelope that had particular glycosylation sites mutated (47). Animals infected with this mutated virus initially demonstrated control of viremia with induction of potent neutralizing antibody responses. Within a few weeks, however, mutant virus arose in which glycosylation sites had reverted.

An additional barrier to the development of broadly neutralizing antibody responses is HIV envelope variability. There are currently nine HIV subtypes, or “clades”, which differ by 25-35% in their Env sequences and show differing patterns of global variation. Unlike influenza virus, for which serum from infected individuals is able to neutralize circulating strains, serum from HIV-infected individuals is generally only effective against the viral isolates that generate the infection (22). Additionally, antibody responses tend to target variable regions on the envelope, with the subsequent rapid selection of escape mutants that appear to have little cost in terms of viral fitness (22, 48, 49). Conserved regions of the envelope do exist, and to date five broadly neutralizing monoclonal antibodies have been identified, suggesting that it is possible to elicit such responses (50). However, it is clear that neutralizing antibody responses are difficult to achieve, and the failure of early vaccine strategies to elicit robust and effective antibody responses has led to greater interest in nontraditional vaccine strategies.

Vaccines that elicit T-cell responses

Greater attention has been placed more recently on vaccine strategies that elicit T-lymphocyte responses. Nonhuman primate models of HIV infection have been used to test several vectors that induce T-cell responses, including recombinant modified vaccinia virus Ankara (MVA), recombinant adenovirus, recombinant vesicular stomatitis virus and plasmid DNA. Although none of these approaches has protected animals from infection, they have shown the ability to blunt levels of peak viremia and lower viral set points (51-55). A vaccine that could achieve comparable results in humans could potentially have the dual benefit of: 1) reducing HIV transmission by maintaining viremia at lower levels; and 2) slowing clinical disease progression and delaying the time when antiretroviral therapy needs to be initiated.

Plasmid DNA vectors have successfully elicited strong T-cell responses in preclinical animal models, but have been weakly immunogenic in humans, requiring large doses to produce measurable responses (52, 56-58). Some strategies to address this limitation include the

Table I: Selected human immunodeficiency virus (HIV) vaccines currently in clinical trials.

Trial phase	Vaccine candidate	HIV target antigens	Sponsor/manufacturer
III	Prime: canarypox Boost: recombinant gp120	Prime: gag, pol Boost: gp120	NIAID, Sanofi Pasteur, VaxGen, Thailand Ministry of Public Health
II	Prime: DNA Boost: Ad5	Prime: gag, pol, nef, env Boost: gag, pol env	HVTN, NIAID, Vical, GenVec
II	Lipopeptide	gag, pol, nef	ANRS, Sanofi Pasteur
II	Prime: canarypox Boost: lipopeptide or canarypox + lipopeptide	Prime: env, gag, pol, nef Boost: gag, pol, nef	NIAID, ANRS, Sanofi Pasteur
I	Prime: DNA Boost: MVA	Prime: env, gag, RT, rev Boost: env, gag, pol	Karolinska Institute, Swedish Institute for Infectious Disease Control, Vecura, NIH
I	DNA ± IL-15, IL-12 or GM-CSF	gag and multiple T-cell epitopes	HVTN, NIAID, Wyeth
I	Prime: DNA Boost: MVA	Prime: gag, pro, RT, env, tat, rev, vpu Boost: gag, pol, env	NIAID, Geovax
I	Prime: DNA Boost: NYVAC	Prime: env, gag, pol, nef Boost: env, gag, pol, nef	EuroVac
I	MVA ± fowlpox	env, gag, tat, rev, nef, RT	NIAID, Therion

Ad5, adenovirus type 5; MVA, modified vaccinia virus Ankara; NYVAC, New York strain of vaccinia; IL, interleukin; GM-CSF, granulocyte–macrophage colony-stimulating factor; RT, reverse transcriptase; ANRS, Agence Nationale de Recherche sur le SIDA; HVTN, HIV Vaccine Trials Network; IAVI, International AIDS Vaccine Initiative; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health.

use of lipofection, gene guns or the coadministration with genes encoding for cytokines (59, 60). Although their poor immunogenicity limits their utility as an unaccompanied vaccine strategy, DNA vectors remain promising candidates as part of heterologous prime–boost regimens. For example, animals primed with a DNA vaccine followed by boost with MVA or fowlpox vectors showed strong induction of $CD8^+$ T-cell responses and lower levels of viremia after parenteral or mucosal viral challenge (51, 61-63).

Although several viral vectors expressing HIV gene products have been explored as a way to induce HIV-specific T-cell responses, the most experience has been obtained with poxviruses such as canarypox and attenuated strains of vaccinia virus, namely MVA and a New York strain of vaccinia (NYVAC). Most poxvirus vectors are effective at inducing $CD8^+$ T-cell responses in nonhuman primates, but are only weakly immunogenic in humans (51, 62). Early studies of MVA vectors in small clinical trials showed only limited immunogenicity, although responses appear to be improved with DNA priming (64, 65). Similarly, only 40-50% of HIV-negative volunteers vaccinated with a canarypox vaccine showed specific HIV responses (66). Nonetheless, a phase III clinical trial is currently under way in Thailand assessing the efficacy of a canarypox prime with a recombinant gp120 boost strategy (67) (Table I). The study is expected to be completed in 2009.

Recombinant adenovirus type 5 (Ad5) appears to be the most immunogenic of the viral vectors being tested to date (53, 68, 69). These replication-incompetent viruses express high levels of HIV proteins under the control of exogenous promoters. A potential problem, however, is the high prevalence of pre-existing immunity to Ad5, particularly in sub-Saharan Africa, where seroprevalence exceeds 90% in some regions (70, 71). The problem of pre-existing immunity may be improved with the use of rare-serotype adenoviral vectors (72). These vectors can evade anti-Ad5 immunity but have been found to be less immunogenic than Ad5 (72, 73). This problem has been addressed by the construction of chimeric Ad5 vectors, in which regions of the hexon proteins are replaced with corresponding sequences from rare-serotype hexon proteins, thus allowing the chimeric vectors to circumvent the pre-existing Ad5 immune response (74). These chimeric vectors have been found to be as immunogenic as Ad5. Evading Ad5 immunity may indeed be critical, especially in light of recent findings from the STEP trial, the first large-scale clinical trial of an Ad5-based vaccine.

Lessons from the STEP HIV vaccine trial

The STEP trial, also known as HVTN 502 and Merck V520-023, was a randomized, double-blind phase IIb test-of-concept trial of Merck's Ad5 vaccine candidate known as MRKAd5. The vaccine consisted of an equal mixture of three replication-defective adenoviral vectors, each containing a near-consensus clade B *gag*, *pol* or *nef* gene. The trial was opened in 2005 and cosponsored by Merck and the National Institute of Allergy and Infectious Diseases (NIAID). The primary endpoints were an assessment of HIV prevention rates and reduction in viral

Table II: HIV infections in STEP trial categorized by pre-existing adenovirus type 5 (Ad5) antibody (ab) titer (75).

Ad5 ab titer	< 18 units	19-200 units	201-1000 units	> 1000 units
Vaccine	20/382	8/140	14/229	7/163
	28/522		21/392	
Placebo	20/394	4/142	7/229	2/157
	24/536		9/386	

load in those volunteers who became infected during the study. The original study design called for 1,500 HIV-negative volunteers at high risk of HIV infection who had low pre-existing Ad5 antibody titers. After the trial began, however, data emerged from earlier phase I and II trials that suggested that pre-existing immunity to Ad5 had less of an effect on the induction of HIV-specific immune responses than feared. The trial was therefore expanded to include an additional 1,500 volunteers with high levels of Ad5 antibodies.

In September 2007, a prescheduled interim analysis by the independent data safety and monitoring board for STEP showed that the vaccine failed to prevent HIV infection or lower viral load, and the study was halted (75). In the group that had received the vaccine, there were a total of 49 HIV infections compared to 33 in the placebo group. More concerning, however, was a trend towards increased HIV infection rates in the vaccine group among those who had higher titers of pre-existing Ad5 antibodies (Table II). In subjects who had Ad5 antibody titers of 200 units or less, there were 28 HIV infections in the vaccine group versus 24 in the placebo group. In volunteers with Ad5 antibody levels of greater than 200 units, however, there were 21 HIV infections in the vaccine group compared to 9 in the placebo group. The disparities were not explainable by differences in induction of HIV-specific responses between the various groups, as measured by interferon gamma ELISPOT assays.

It is clear from the data that the Ad5 vaccine did not prevent HIV infection. Whether the vaccine increased susceptibility to infection, however, remains uncertain. It may be that the Ad5 vector increased the activation of $CD4^+$ T cells in those who had pre-existing immunity to Ad5, thus expanding the pool of activated target cells for HIV to infect. There are several confounding factors in the data that remain to be fully analyzed, including age, race, geographic distribution and circumcision rates. Among uncircumcised men, there were more infections in those subjects who were vaccinated compared with those who received placebo, while among circumcised men, HIV infections occurred with equivalent rates (76). The study also raises questions about the utility of current nonhuman primate models of infection and whether better correlates of protection need to be identified. It may be that induction of cytokine secretion by T cells as measured by ELISPOT or intracellular cytokine staining, both of which are frequently cited as surrogate measures of protective responses, is poorly predictive of the ability of a vaccine to deter infection or delay disease progression.

Where do we go from here?

The results of the STEP trial suggest that we need to consider innovative strategies to develop a successful HIV vaccine. New approaches to eliciting T-cell immunity against HIV must be pursued, including tests of heterologous prime–boost strategies. We must also learn more about the correlates of immune protection against HIV, such as innate and mucosal immunity against the virus and genetic factors that protect against HIV infection or control viral replication (27). Studies of patients who control HIV replication in the absence of antiretroviral therapy may also reveal important lessons for the development of an HIV vaccine (77). Finally, novel clinical trial designs must be developed to accelerate testing and evaluation of different candidate vaccine strategies (78).

Conclusions

Although the development of an HIV vaccine that is able to induce broadly neutralizing protective antibody responses remains a tantalizing goal, it is unclear if it will be attainable. It may be necessary to pursue nontraditional vaccine approaches that rely on the induction of cellular immune responses. A vaccine that specifically seeks to generate T-lymphocyte responses has never been developed, and thus it presents unique scientific and regulatory challenges. Such a vaccine may not be protective, but instead may modify disease progression. Nonetheless, this could have significant effects on both HIV transmission rates and the need for antiretroviral therapy. The initial failure of the STEP trial to show sterilizing immunity is disappointing, but it is not clear to what extent the data can be generalized. It certainly is premature to state that the results constitute a condemnation of all T-cell vaccine strategies. The possibility that a candidate vaccine may have actually increased HIV infection rates, however, suggests that current models and correlates of disease progression need to be re-evaluated, and that a deeper understanding of HIV immunopathogenesis will be required to successfully develop a safe, effective and globally deliverable HIV vaccine.

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