AIDS Rev 2003;5:131-139

AIDS Vaccine Development: the Long and Winding Road

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Abstract

Development of a vaccine that provides sterilizing immunity against HIV infection remains an elusive goal, due primarily to the difficulty in generating neutralizing antibodies to primary HIV isolates. In lieu of a present solution to this problem, recent approaches to develop vaccines against HIV/AIDS have focused not on preventing infection outright, but on eliciting potent antiviral CD8+ T-cell responses to limit HIV replication in individuals who become infected after vaccination. Successful control of HIV replication *in vivo*, enabled by vaccine-elicited immune responses should, in turn, attenuate an individual's rate of progression to AIDS while reducing their likelihood of subsequently transmitting HIV.
Recent pre-clinical evaluation of CTL-based vaccines in non-human primate models of AIDS has shown several different vaccine modalities (e.g. heterologous 'prime/boost' strategies such as DNA + recombinant viral vectors) to be capable of eliciting high-level cellular immune responses that are associated with limitation of virus replication and protection against disease following challenge with select pathogenic virus isolates. However, it is not currently known to what extent these protective effects, observed under optimal experimental conditions in select animal models, can be translated into relevant protection of humans against AIDS. In this article we discuss the promise, potential limitations, and scientific challenges that currently provide the context for efforts to develop and successfully employ a safe and effective AIDS vaccine.

Key words

HIV. SIV. SHIV. AIDS. Vaccine. CD4+ T-cell. CD8+ T-cell. CTL. Macacca mulatta.

Worldwide, it is estimated that over 42 million people are now infected with HIV and over 14,000 more become infected each day[1]. A majority of these new infections occur in developing nations that lack the economic resources and infrastructure to acquire and successfully deliver effective antiretroviral therapy to those most in need. As a result, it is generally accepted that development of a safe and effective vaccine against HIV represents the single best hope for curtailing the human devastation wrought by AIDS and may be the only sustainable means to contain the AIDS pandemic. While nearly twenty years have passed since HIV was discovered and shown to be the cause of AIDS, medical science still grapples with challenges surrounding the de-

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velopment of an effective vaccine – why is this so? In part, it is due to our incomplete understanding of the correlates of immune protection against HIV infection[2]. In an overwhelming majority of cases, natural infection with HIV does not result in natural immunity – rather, it results in fatal infection. As such, the natural history of HIV infection does not provide scientists with a clear road map to follow toward vaccine-emulation of successful natural immunity. While vaccines have been successfully developed against certain other viruses that occasionally (e.g. HBV) or reproducibly (e.g. VZV) establish lifelong infection of humans, these infections differ substantially from HIV and certain critical aspects of the biology of HIV infection present novel, heretofore insurmountable challenges to the development of an effective vaccine (Table 1).

HIV is a difficult target for antibody neutralization

Development of a vaccine that provides sterilizing immunity against HIV infection remains an elusive goal, due primarily to the difficulty in generating neutralizing antibodies to primary HIV isolates. Immunological shielding of critical epitopes on the virion envelope glycoprotein (gp120) through extensive carbohydrate masking, mutational variation of variable loop sequences, occlusion due to gp120 oligomerization, and conformational (entropic) masking of receptor binding sites[3], contribute to a refractory protein structure that resists antibody neutralization (reviewed in[4]). Furthermore, neutralizing antibody responses that do arise during the natural course of HIV infection are quickly rendered ineffective due to rapid virus evolution and the emergence of neutralization-resistant mutants[5,6]. While studies in non-human primate models of AIDS indicate that neutralizing antibodies, experimentally administered to achieve high concentration *in vivo*, can provide passive protection to macaques against infection with a challenge virus (SHIV)[7-9], the limitations currently facing HIV vaccine development are the paucity of relevant immunogens that can elicit neutralizing antibody responses *in vivo* that are broadly reactive against primary HIV-1 isolates and the ability of HIV to escape from neutralizing antibody responses.

Vaccine augmentation of antiviral CD8+ T-cell responses may control HIV infection

Alternatively, recent approaches to the development of HIV/AIDS vaccines have focused on eliciting cellular immune responses, particularly antiviral CD8+ T-lymphocyte responses, towards controlling the level of HIV replication in infected individuals, rather than preventing HIV infection *per se*. Because "setpoint" levels of HIV replication provide a strong predictor of the ensuing rate of progression to AIDS[10], CD8+ T-cell-based anti-HIV vaccines that successfully and substantially lower the setpoint level of HIV in infected individuals are expected to slow the rate of progression to AIDS while simultaneously reducing the likelihood of subsequent HIV transmission[11,12]. If widely implemented, such vaccines may have a significant impact on improving the quality and length of life for HIV-infected individuals, while at the same time reducing the rate at which HIV continues to spread throughout the human population. However, to be effective such vaccines will need to elicit CD8+ T-cell responses that are broadly reactive against genetically diverse HIV strains and that are qualitatively better (if not also quantitatively larger) than the CD8+ T-cell responses that normally arise during the natural course of HIV infection.

CD8+ T-cells recognize infected target cells through specific binding of their TCR to foreign antigen that has been proteolytically processed into 8-11 amino acid epitopes. These epitopes are presented on the target-cell surface in a trimolecular complex with a MHC class I molecule and β2-microglobulin (β2m). Upon recognition of cognate epitope/MHC-I/β2m on a target cell, effector CD8+ T-cells may exert their antiviral effects in several ways: through killing of target cells via apoptosis[13]; through secretion of cytokines (IFN-γ, TNF-α) to induce an antiviral state in infected cells[14,15]; or through secretion of chemokines (MIP-1α, MIP-1β, RANTES) to block co-receptor molecules (CCR5, CXCR4) that are required for HIV entry[16-19], thereby inhibiting infection of additional susceptible target cells. Because CD8+ T-cells recognize CD4+ target cells only after they are infected, it is unlikely that antiviral CD8+ T-cells alone will be

Table 1. *Challenges to developing an AIDS vaccine*

- Natural infection with HIV does not result in protective immunity
- HIV is difficult to neutralize with antibodies
  - Carbohydrate shielding of critical gp120 epitopes
  - Occlusion of envelope epitopes via oligomerization
  - Conformational (entropic) masking of receptor binding sites
  - Mutational variation of envelope variable loops
- HIV infection results in progressive destruction and impaired regeneration of CD4+ T-helper cells, key mediators of antiviral immune responses
- HIV rapidly evolves *in vivo* to escape from cellular and humoral immune responses
- High levels of HIV genetic diversity will likely require ≥ 1 vaccine preparation for use globally

able to prevent the establishment of persistent HIV infection[20].

Several lines of evidence from studies of HIV-infected humans or SIV-infected macaques indicate that CD8+ T-cell-mediated responses may, at least transiently, constitute potent antiviral responses against infection with CD4+ T-cell-tropic lentiviruses. These include a temporal correlation between the emergence of virus-specific CD8+ T-cell responses and decline of primary viremia[21-24], and the emergence of CTL-escape mutants during acute infection[25-28]. Inverse relationships between the level of setpoint HIV viremia and levels of HIV-specific CD8+ T-cell responses during chronic infection has been observed in some[29], but not all cohorts[30,31], suggesting that, in certain contexts, HIV-specific CD8+ T-cells may also control HIV replication during chronic infection. While the potential protective mechanism is unclear, it has also been suggested that HIV-specific CD8+ T-cell responses detected in cohorts of HIV-exposed/uninfected health care workers[32,33] and serially exposed/uninfected female prostitutes[34-37] may contribute to preventing HIV-1 infection. Because others have not observed analogous HIV-specific CD8+ T-cell responses in HIV-exposed/seronegative cohorts[38,39], this hypothesis may not be generalizable. Finally, experimental, antibody-mediated depletion of CD8+ cells during acute[40,41] or chronic[42] SIV infection of macaques results in marked increases in levels of SIV replication, indicating that CD8+ T-cells play a role in control of virus replication.

HIV genetic diversity presents challenges to vaccine design

Two fundamental aspects of the biology of HIV infection, rapid evolution *in vivo* and progressive impairment/destruction of CD4+ T-helper cells, contribute synergistically to the elusiveness of this pathogen from host antiviral responses and remain as formidable challenges to the development of an effective vaccine against HIV/AIDS. The capacity of HIV to evolve rapidly *in vivo* to escape recognition by host cellular and humoral immune responses is due to the high error rate of reverse transcriptase acting in concert with a high level of virus replication/turnover that is typically seen during untreated HIV infection[43,44]. It has been estimated that, during chronic infection, HIV variants harboring single-nucleotide mutations at every position of the viral genome have the potential of arising thousands of times per day[45] (reviewed in[4]). In a relatively short time, such enormous capacity for generating diversity results in a vast pool of mutants from which host immune responses may select for resistant variants. Thus, to curb the generation of (and establishment of latent infection by) potential immune escape variants, vaccine-elicited CD8+ T-cell responses will likely need to control HIV replication at the very earliest stages of infection.

In order to achieve early immune recognition and containment of HIV, vaccine-elicited CD8+ T-cell responses should target a maximal number of epitopes that are identical (or nearly so) to those in the strain of HIV most likely to be subsequently encountered. However, choosing which antigens to include in candidate vaccines is further complicated by the enormous worldwide diversity of HIV variants. Phylogenetically diverse HIV subtypes are composed of HIV variants whose gene products may diverge by 15% (*Gag*) to 30% (*Env*) at the amino acid level[46]. In contrast, amino acid changes of less than 2% between a vaccine strain and circulating forms of influenza virus necessitate a change in vaccine strains[46]. High levels of genetic recombination further increase the diversity of HIV by generating viruses with inter-subtype mosaic genomes (circulating recombinant forms, CRFs). Because phylogenetically diverse HIV subtypes are prevalent in different geographical locations[1], the use of HIV subtype-consensus sequences as vaccine immunogens has been proposed as a scientifically justifiable and feasible approach towards trying to diminish the problem that diversity poses against selecting relevant antigens for inclusion in candidate HIV vaccines[46-48].

HIV infection of CD4+ T-cells presents challenges to vaccine design

Of equal importance to rapid viral evolution is HIV's progressive destruction and impaired regeneration of CD4+ T-lymphocytes (reviewed in[49]), with preferential infection of HIV-specific CD4+ T-cells[50], which presents additional challenges against successful immunological control of infection. CD4+ T-cells recognize epitopes (10-14 amino acids in length) that have been proteolytically processed from exogenous antigens and presented on a target cell's surface in conjunction with MHC class II molecules. CD4+ T-cells, in addition to their role in stimulating humoral immunity, play pivotal roles in maintaining the functionality of antiviral CD8+ T-cell responses during chronic viral infection. During chronic HIV infection, most patients exhibit low, but detectable, levels of HIV-specific CD4+ T-cells[30], and higher levels of Gag-specific-CD4+ T-cells (measured by *in vitro* proliferation assays) have been correlated with lower levels of chronic HIV viremia[51]. During acute HIV infection, combination antiretroviral drug therapy results in augmentation of HIV-specific CD4+ T-helper cell responses[51-53]. Also during acute, but not chronic HIV infection, cyclical on/off treatment of patients with HAART (structured treatment interruption, STI) to stimulate HIV-specific CD4+ and CD8+ T-cell responses through controlled re-exposure to autologous viral antigen, results in subsequent control of virus replication (at least in a subset of subjects) following cessation of therapy[54,55]. In SIV-infected macaques, analogous antiretroviral treatment during acute

infection similarly results in an augmented ability of the host to subsequently control SIV replication upon cessation of therapy by preserving SIV-specific CD4+ and CD8+ cellular immune responses[56,57]. Thus, it appears that early antiretroviral treatment preserves the host's HIV- (or SIV-) specific CD4+ T-cell responses that are otherwise irreparably damaged by unchecked virus replication beginning at the earliest stages of infection. Analogous reduction of early HIV replication by vaccine-induced cellular immunity should also prevent extensive destruction of the host's HIV-specific CD4+ T-cell responses, thereby contributing to effective containment of HIV infection.

Preservation of HIV-specific CD4+ T-cell responses may be a key to effecting control of HIV replication by maintaining HIV-specific CD8+ T-cell proliferative capacity and cytotoxic functionality during both acute and chronic infection[58]. In the murine LCMV model of chronic virus infection, it has been shown that CD4+ T-cell 'help' is required to facilitate CD8+ CTL-mediated clearance of chronic LCMV infection[59] and to prevent the silencing of virus-specific effector CD8+ T-cell responses[60]. Using MHC-class I tetramers to enumerate HIV-specific CD8+ T-cells[61], and intracellular detection of IFNγ to assess cellular responsiveness to antigenic stimulation, Goulder, et al. estimated the proportion of functionally inert CD8+ T-cells during chronic HIV infection to range from 0-60% among HLA-B42-Gag-, A2-Gag-, B8-Nef- and B8-Gag-restricted responses[62]. Additionally, silencing of HIV-specific CD8+ T-cells may be more pronounced for CD8+ T-cell responses directed against other HIV epitopes during chronic infection, or during acute infection as a consequence of early CD4+ T-cell impairment. Loss of functionality of acute CD8+ T-cell responses against HIV due to insufficient CD4+ T-cell help may contribute to the inability of initially robust antiviral CD8+ T-cells to completely clear acute HIV infection[58]. Taken together, these results suggest that optimal vaccine immunogens should induce broad, cross-reactive CD8+ T-cell responses that are accompanied by robust CD4+ T-cell responses to achieve sustained control of HIV replication. To this extent, vaccines that contain complex viral antigens that include CD4+ T-cell epitopes may be expected to elicit more effective and durable antiviral CD8+ T-cell responses than would vaccines designed to elicit only CD8+ T-cell responses against synthetic poly-(MHC class I-restricted) epitopes[63].

Effectiveness of antiviral CD8+ T-cell responses

The ultimate effectiveness in control of virus replication by any single CD8+ T-cell response rests on the ability of the CD8+ T-cell to eliminate/reduce the net production of virus by infected targets[64]. Currently, it is not known whether immunodominant HIV-specific-CD8+ T-cell responses that arise during acute infection[25,65-68], subdominant responses that emerge during chronic infection[65,69-71], or novel vaccine-elicited responses that are not otherwise targeted during the course of infection[72] will prove to be most relevant toward controlling HIV replication. For example, the CD8+ T-cell response against HLA-A*0201-restricted HIV Gag epitope SLYNTVATL, which is not detected during acute HIV infection, commonly emerges as a dominant response during chronic infection[71], and levels of this response have been correlated with lower setpoint viral load in some patient cohorts[29]. Thus, CD8+ T-cell responses that emerge during progressive HIV infection may be relevant candidates for augmentation through prior vaccination. Alternatively, it has been argued that vaccine augmentation of CD8+ T-cell responses directed against epitopes that are immunodominant during acute infection may be relatively more effective at controlling acute virus replication[73]. In the SIV/macaque model, macaques that express the Mamu A*01 MHC-class-I molecule mount dominant CD8+ T-cell responses against Tat_{28-35} (STPESANL) and $Gag_{181-189}$ (CTPYDINQM) epitopes during acute infection with SIV[25,67]. The acute Tat_{28-35} -specific CD8+ T-cell response rapidly selects for SIV variants that harbor mutant Tat_{28-35} epitopes that exhibit reduced binding affinity for the Mamu A*01 molecule[25]. Prime/boost immunization of macaques with vectors that encode SIV-Tat (or encode only the wild-type Mamu-A*01-restricted Tat_{28-35} epitope) elicited moderate levels of Tat-specific CD8+ T-cells, but had little effect on altering the kinetics of challenge virus (SIVmac239) replication[66]. Thus, vaccination against a mutable epitope (one which can harbor mutations that result in reduced binding affinity for its restricting MHC class I molecule, while not simultaneously compromising viral replicative capacity) may hold little benefit for use in prophylactic vaccination against AIDS.

In contrast, the Mamu A*01-restricted SIV $Gag_{181-189}$ (CTPYDINQM) epitope does not rapidly mutate despite an acute CD8+ T-cell response. However, this less mutable epitope may not be ideal for a vaccine either, as, in a vaccinated macaque, it has been documented that mutations arise that result in loss of epitope binding to the MamuA*01 molecule and correlate with loss of control of viral (SHIV89.6P) replication[74]. Thus, it will be important for future vaccine design to identify HIV epitopes that are targeted by CD8+ T-cells during acute infection that cannot easily sustain mutation to confer immunological escape without a concomitant drop in viral fitness[75].

An important corollary of these macaque studies is that immunodominance of a virus-specific-CD8+ T-cell response does not necessarily correlate with effective suppression of virus replication[66,74]. A similar finding that protective immunity does not necessarily correlate with the immunodominant hierarchy of CD8+ T-cell responses has also been observed in the murine LCMV model[76]. Whether any particular HIV antigen elicits a CD8+ T-cell

response that is effective at clearing infected target cells will likely be the outcome of a complex set of interactions[77] that includes: restriction of epitope presentation by inherited host MHC-class I alleles; efficiency of antigen processing; affinity of epitopes for binding their restricting MHC-class-I molecules[78]; sensitivity/responsiveness of CD8+ T-cells to epitope concentration (TCR avidity)[69]; conservation versus plasticity of an epitope (for balancing viral fitness versus immune recognition); and the overall immunological context in which a CD8+ T-cell response is generated – particularly, conditions of sufficient vs limiting CD4+ T-cell help. Greater understanding of these mechanisms and their favorable intersections should lead to the development of novel HIV immunogens and vaccine vectors that will then require efficient preclinical and clinical evaluation against current candidate HIV/AIDS vaccines.

Human clinical trials of candidate HIV/AIDS vaccines

Today, there are 24 ongoing human clinical trials of candidate HIV/AIDS vaccines and 16 additional trials are expected to begin through 2004 (Table 2)[79,80]. While nearly 10,000 HIV seronegative volunteers have participated in previous and ongoing phase I and phase II clinical trials to evaluate the safety and immunogenicity of candidate HIV vaccines[79,81], results from the world's first phase III efficacy trial of a candidate AIDS vaccine have only recently been reported. The results from this North American trial of VaxGen's AIDSVAX B/B, a formulation of recombinant (clade B) gp120 protein, disappointingly showed this vaccine to have no effect on reducing the rate of HIV infection within the overall trial population of 5,009 individuals that predominantly consisted of gay men, few women, and relatively few racial minorities[82]. While this study was not designed to assess racial or gender differences, *post hoc* statistical analyses of racial subsets within the study population suggested there was a significant vaccine-mediated reduction of HIV infection in non-white participants (Asian, black, other). However, this initial claim has since come under increased scrutiny as the scientific community has voiced concerns over proper statistical approaches (if any) to use for such subgroup analyses[83]. Nevertheless, results from an ongoing phase III trial of VaxGen's AIDSVAX B/E[79,84,85] in Thailand are expected to be reported in the latter half of 2003. If vaccine efficacy is not observed in the Thai trial (as it was not observed in the overall North American

Table 2. *Current and anticipated clinical trials of HIV vaccines**

Phase	# Trials	Vaccine	Boost	Status**
III	1	gp120		Current
	1	rCanarypox	gp120	Pending
II	2	rCanarypox	gp120	Current
	1	rCanarypox	Lipopeptide	Q2 2003
	1	rAdenovirus		Q2 2003
	1	Plasmid DNA	rAdenovirus	Q4 2003
I	10	Plasmid DNA		Current
	2	Plasmid DNA		Q2 2003
	2†	Plasmid DNA	rMVA	Current
	3	Plasmid DNA (+ cytokine adjuvant)		Q2, Q4 2003; 2004
	2	Plasmid DNA (+ PLG adjuvant)	gp140	Q2, Q3 2003
	3	rCanarypox		Current
	1	rCanarypox	gp120	Current
	1	rCanarypox	Lipopeptide	Current
	2	rAdenovirus		Current, Q3 2003
	1	rVaccinia virus		Current
	1	Lipopeptide		Current
	1	NefTat fusion protein	gp120	Current
	1	r*Salmonella typhi*		Current
	1	rVEE		Q2 2003
	1	r*S. Cerevisiae*		Q2 2003
	1	rMVA	rFowlpox	Q3 2003
	1	Peptides		Q3 2003
	1	rVSV	rVSV	2004

*See www.iavi.org/trialsdb and www.hvtn.org for additional trial details and information updates
**Anticipated start dates for non-current trials
†Includes 1 I/II trial

dataset), this would further suggest that the racial subset outcomes of the North American trial results were an artifact. In any case, additional analyses, both statistical and biological, will likely be necessary to determine if any biological features (e.g. antibody titers) are protective factors that correlate with risk of HIV infection or clinical outcome following infection.

In addition to these phase III efficacy trials of gp120 protein vaccines that are designed to elicit only antibody responses, numerous other trials are underway to assess the safety and immunogenicity of recombinant viral, bacterial, yeast, and plasmid DNA vectors that are primarily designed to elicit CD8+ CTL responses, and in some cases, antibody responses as well. Among these, several phase-I and –II trials of rCanarypox vectors alone, or in combination with gp120 or lipopeptide booster immunizations, are currently underway or have been recently completed (Table 2)[79,80,86,87]. Results from the first phase II study of rCanarypox (ALVAC vCP205) showed that immunization with vCP205 alone resulted in the development of neutralizing antibodies against the homologous lab strain of HIV (MN) in 56% of vaccinees. This level was further increased to 94% when booster immunizations with gp120 were included in the vaccination regimen. However, previous phase I testing had shown such antibody responses to be very limited in their abilities to neutralize primary HIV isolates (1/9)[87]. In comparison to antibody responses, generation of CD8+ CTL responses against HIV antigens following vCP205 immunization was much less impressive, with only 33% of vaccinees exhibiting CD8+ CTLs at any time throughout the study[86]. Because of this limited ability of rCanarypox vectors to prime antiviral CTL responses[86], in addition to the lack of protection afforded by antibodies alone (as observed in VaxGen's North American efficacy trial), phase III studies of rCanarypox ± gp120 vaccine regimen will not be conducted in the US. However, the decision to conduct a rCanarypox ± gp120 efficacy trial in Thailand is pending.

Limitations of non-human primate models for pre-clinical evaluation of HIV/AIDS vaccines

On the other hand, optimism for advancing a number of other recombinant viral vectors and plasmid DNA immunogens into human clinical trials has followed from promising "proof-of-concept" pre-clinical evaluation of these vaccine modalities in non-human primate models of AIDS[88-94]. In these pre-clinical studies, rhesus macaques were vaccinated with DNA- and/or recombinant viral-vectors and were protected against disease following subsequent challenge with a pathogenic virus (SHIV89.6P). Among these studies, Amara, et al. reported immunization of macaques with a heterologous DNA-prime/rMVA-boost strategy that resulted in durable protection against disease following mucosal SHIV89.6P challenge[90]. Heterologous prime/boost regimens utilize different vaccine vectors for sequential immunizations (e.g. DNA followed by rMVA) to elicit high levels of cellular immunity[95]. The success of prime/boost approaches is due to an initial focusing of cellular immune responses on the vaccine antigen (prime), rather than on irrelevant vector-associated antigens, and the subsequent expansion of those responses by (booster) immunization with recombinant viruses that express large amounts of the vaccine antigen and that engender pro-inflammatory responses[96]. In prime/boost experiments analogous to those of Amara, et al., Shiver, et al. reported protection against disease following intravenous SHIV89.6P challenge of macaques that had been immunized with a single SIV gene product (Gag) via heterologous prime/boost with recombinant plasmid DNA and MVA- or adenovirus-vectors[91]. Alternatively, Barouch, et al. achieved protection against intravenous SHIV89.6P challenge by immunization of macaques with plasmid-encoded antigens (SIV-Gag + SHIV89.6P-Env) in the presence of a cytokine adjuvant (IL2-Ig) that was administered as recombinant protein or expressed from recombinant plasmid DNA[88].

It is not currently known to what extent these protective effects, observed under optimal experimental conditions, can be translated into relevant protection of humans against AIDS. Both the relevance of the SHIV89.6P challenge model in predicting potential protective efficacy of vaccines against HIV and the use of homologous antigens in the vaccines and challenge viruses are caveats that deserve careful consideration when evaluating such reports of vaccination 'successes' (Table 3)[88-94,97]. The limitation of the SHIV89.6P challenge is that this virus' extraordinary pathogenicity in naive macaques may, paradoxically, render the virus extraordinarily susceptible to control by very modest levels of cellular and/or humoral immunity[97]. In naive macaques, SHIV89.6P infection results in consistently high levels of setpoint viremia, rapid and near complete depletion of CD4+ T-cells, and rapid progression to simian AIDS[98,99]. It

Table 3. *Limitations of non-human primate challenge models to predict efficacy of HIV/AIDS vaccines: i) homologous antigens in both vaccine and challenge virus, and ii) relevance of particular challenge virus strain as model of HIV infection*

	1º HIV isolate	*SIVmac239*	*SHIV89.6P*
Antibody neutralization:	Resistant	Resistant	Susceptible
CD4+ T-cell depletion:	Gradual	Gradual	Rapid
Co-receptor usage:	CCR5	CCR5	CXCR4

differs from typical primary HIV-1 isolates in terms of co-receptor usage (CXCR4 versus CCR5)[100], magnitude and rapidity of depletion of CD4+ T-cells, and sensitivity to antibody neutralization[98,101]. It has been argued that attenuation of SHIV89.6P's acute depletion of CD4+ T-cells by even modest levels of vaccine-induced (or -primed) cellular or humoral immunity may provide a critical window of time in which additional antiviral immune responses, especially neutralizing antibody responses, may mature and contribute to subsequent control of infection[97]. Because SHIV89.6P is more susceptible to antibody neutralization than are most primary HIV-1 isolates, the overall mechanisms of vaccine-elicited protection against disease caused by SHIV89.6P infection of macaques may have little relevance for predicting vaccine efficacy against HIV/AIDS[97].

However, some experimental vaccines, including rMVA vectors and DNA/rMVA prime/boost regimens, have been evaluated for their abilities to elicit protection of macaques against challenge with alternative pathogenic viruses (SIVmac239, SIVsmE660) that may model HIV infection more precisely than does SHIV89.6P (i.e. are more resistant to antibody neutralization and do not cause abnormally rapid loss of CD4+ T-cell counts) (Table 3)[102-106]. In these studies, prior immunization of macaques with SIV antigens generally resulted in reduced viral loads and increased time of survival following homologous challenge, but was unable to prevent the ultimate loss of CD4+ T-cells and progression to AIDS[102-104]. While phase III clinical trials will ultimately be required to prove efficacy of candidate HIV vaccines in humans, concordance of protection results from non-human primate studies that employ >1 challenge virus may help to prioritize which vaccine concepts should be advanced through phase I and II clinical trials.

Another caveat to the macaque studies is that many vaccines that have been evaluated have employed immunogens which are homologous (or nearly so) to those present in the challenge virus[88-93]. The breadth of cellular immune responses elicited by vaccines that share such extensive genetic identity with challenge viruses may grossly overestimate the degree of protection that any 'real-world' HIV vaccine may be expected to elicit against diverse, circulating field strains of HIV. Recent reports of HIV superinfection[107,108] have raised new concerns about the ability of CTL-based vaccines to protect against AIDS[109]. In one case, a patient undergoing STI was reported to exhibit broad CD8+ T-cell responses that were controlling replication of the primary virus at the time of intra-(B)-clade superinfection[108]. It is possible that progressive CD4+ T-cell impairment, resulting from continued low-level replication of HIV, led to a tenuous immune control of the initial HIV strain that was unbalanced following superinfection. Future studies to determine how commonly cross-clade and intra-clade HIV superinfection occurs will be required to put such case studies into context and to gauge the effectiveness of cellular immunity in protecting against HIV infection. For now, the sobering documentation of HIV superinfection, in the presence of CD8+ T-cell responses that recognized multiple epitopes common to both the primary and superinfecting virus strain, begs the question of what will constitute a protective cellular immune response against HIV.

Given all the caveats and complexities in AIDS vaccine development detailed above, we are now in possession of substantially more immunogenic vaccine strategies, much higher resolution quantitative immunological assays, and a significantly improved understanding of the host immune response to AIDS virus infection. With carefully designed pre-clinical studies to define the most favorable vaccine regimens, and their expeditious advancement into clinical evaluations in humans, our road to a safe and effective AIDS vaccine can now hopefully be traversed in a more rapid and more direct manner.

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