Progress and obstacles in the development of an AIDS vaccine

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Abstract | Recent experimental observations suggest approaches to immunization that might finally result in at least a partially effective vaccine against infection with HIV-1. In particular, advances in our understanding of the contribution of vaccine-elicited cellular immunity to protecting memory $CD4^+$ T cells from virus-mediated destruction provide rational strategies for the development of this vaccine. This is therefore an ideal time to review our current understanding of HIV-1 and its control by the immune system, as well as the remaining problems that must be solved to facilitate the development of an effective vaccine against AIDS.

Five million new infections with HIV-1 occur each year[1]. AIDS has already ravaged sub-Saharan Africa, and is rapidly spreading through the Indian subcontinent and Southeast Asia (FIG. 1). Although antiretroviral therapy is available for infected individuals, these therapies are not curative. Moreover, antiretroviral therapies are prohibitively expensive for most people in the developing world[2]. The devastating consequences of this infection for both individuals and society make the development of an effective vaccine against HIV-1 an absolute priority.

However, the creation of an HIV-1 vaccine represents an unprecedented scientific challenge. The traditional approaches for creating effective antiviral vaccines have proved inadequate for making one against HIV-1. For example, vaccines based on live attenuated HIV-1 might mutate and regain their pathogenicity after inoculation into humans. Inactivated virus and protein-based vaccines have not generated antibodies that neutralize the genetically diverse viruses that are currently infecting human populations throughout the world. Moreover, these types of vaccines would not induce the cell-mediated immune responses that are needed to control the spread of HIV-1 in exposed individuals. A novel vaccine approach will be required to stop the spread of this virus.

In this Review article, I highlight aspects of the unique biology of HIV-1 and describe the non-human primate models used to evaluate prototype vaccines against AIDS. I detail our current understanding of how the immune system of an immunologically naive individual mounts a defence against this virus, and discuss recent observations that begin to define vaccine-associated correlates of immune protection against clinical disease progression. I then review the data indicating that traditional vaccine strategies will not provide protection against HIV-1, and highlight newly developed vaccine vectors that might provide more effective immunity against the virus. Finally, I outline some of the remaining problems that must be addressed if we are to succeed in making an effective AIDS vaccine. There is reason to be optimistic that new approaches will resolve these remaining problems and provide a path forward for the development of an effective vaccine.

HIV-1 and non-human primate models

Several unique characteristics of HIV-1 transmission and replication must be considered when developing strategies for vaccination to prevent infection by this virus. HIV-1 is transmitted both by sexual contact and haematogenously through contaminated needles and blood products, so the virus can initiate infection by crossing a mucosal barrier or by direct entry into a T cell or monocyte/macrophage lineage cell in the peripheral blood[3]. It is reasonable to assume, therefore, that a vaccine must elicit both mucosal and systemic immune responses to provide defence against infection (FIG. 2). Because HIV-1 can spread as either a cell-free or cell-associated virus, successful containment of the virus will require immune mechanisms that can act on both extracellular and intracellular virus particles[4]. Therefore, both humoral and cellular immune responses will probably be needed to contain its transmission.

Finally, and perhaps most importantly, HIV-1 has intrinsically inaccurate machinery for replication (FIG. 3). Every time a virus particle creates progeny particles,

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doi:10.1038/nri1959

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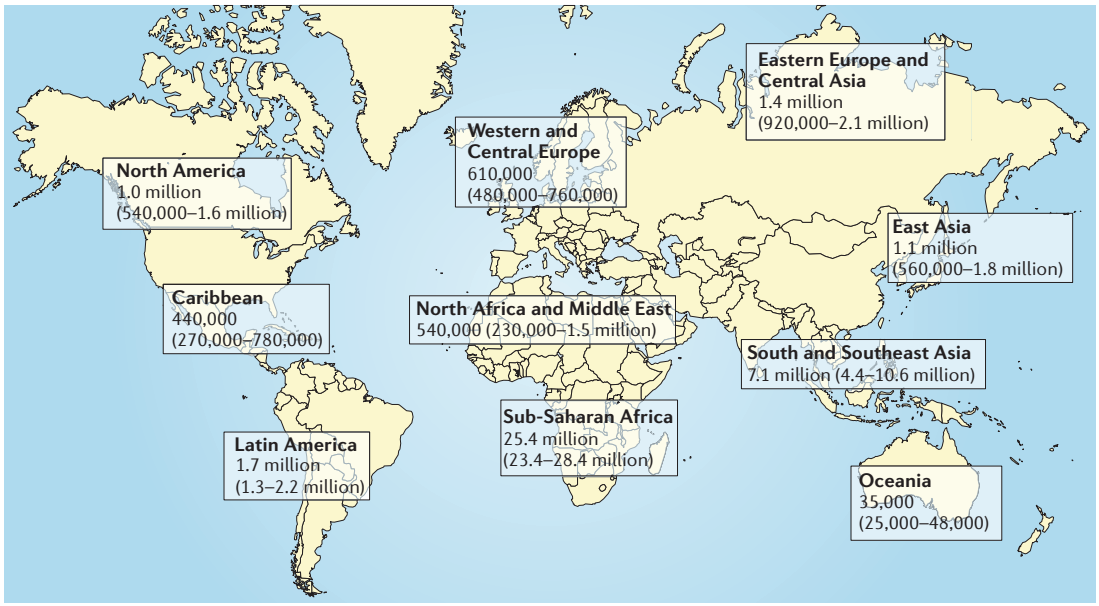

Figure 1 | **Prevalence of HIV-1 infections in different geographical regions worldwide.** HIV-1 is a worldwide epidemic, and sub-Saharan Africa, the Indian subcontinent and Southeast Asia are the areas most affected. Infections with HIV-1 clade B are found mainly in the Western hemisphere, whereas non-clade B infections predominate worldwide. Estimates of the number of infections are shown in the boxes. Data from REF. 1.

it must first undergo reverse transcription of its single-stranded RNA genome. This is an inherently inaccurate process, because it lacks proofreading mechanisms. Consequently, almost every newly infected cell contains a proviral genome whose sequence differs from that of the infecting virus by approximately one nucleotide per viral genome. This results in the creation of a swarm or 'quasi-species' of viruses that are antigenically heterogeneous[5]. Although HIV-1 isolates are extraordinarily diverse, analysis of their sequences has shown that the viruses cluster into distinct groups, referred to as subtypes or clades (FIG. 4). Most HIV-1 isolates found in the Western hemisphere and Europe are clade B viruses, whereas the majority of those in India are clade C viruses. Any strategy for an effective HIV-1 vaccine must take into consideration this antigenic diversity.

All vaccine strategies must be evaluated in animal models to select approaches for testing in humans. HIV-1 is a member of a family of primate lentiviruses that are species restricted in their ability to replicate, and so does not infect small laboratory animals. Therefore, mice cannot be used to assess the efficacy of HIV-1 vaccine prototypes. Nevertheless, several small laboratory animal models have been developed with the hope that they might be used to test HIV-1 vaccine approaches[6]. These models have been created using the tactic of reconstituting the immune systems of immune-incompetent mice with human lymphoid tissue. However, the use of such models has been limited, because the complex biology of dissemination of HIV-1 infection in humans is not reproduced in mice.

Furthermore, common isolates of HIV-1 do not replicate in monkeys. Although they can infect chimpanzees, these isolates replicate at levels that are too low to cause clinical disease[7]. Importantly, certain lentiviruses of non-human primates — known as simian immunodeficiency viruses (SIVs) — cause disease that has remarkable similarities to AIDS in humans when they infect Asian macaque monkey species[8]. These viruses have considerable sequence homology with HIV-1 and almost the same genomic organization. Certain SIV isolates infect activated $CD4^+$ memory T cells in macaques, causing progressive immunodeficiency and eventual death due to opportunistic infections and tumours (TABLE 1). Chimeric viruses, known as simian human immunodeficiency viruses (SHIVs), have been synthesized in the laboratory with an SIV backbone and various HIV-1 envelope proteins, and these viruses also cause AIDS in macaques[9]. These observations have been used to develop SIV- and SHIV-infected macaques, powerful model systems for exploring the potential use of vaccine strategies against AIDS.

Immune containment of HIV-1 spread

The kinetics of HIV-1 replication *in vivo* are characterized by an early burst of viral replication during the first weeks after infection[10]. Because the virus usually uses both surface-expressed CD4 and CC-chemokine receptor 5 (CCR5) to enter a cell, HIV-1 selectively infects and destroys memory $CD4^+$ T cells[11]. This early, intense viral replication ends abruptly as a consequence of the emergence of virus-specific immune responses and perhaps destruction of the target cells for the virus. However, the dampening of viral replication is incomplete, and ongoing, active HIV-1 replication can be demonstrated in almost all chronically infected individuals[12].

Antiviral antibodies do not seem to have a central role in controlling the spread of HIV-1 during either primary or chronic infection. Several observations have led to this conclusion. Although a neutralizing antibody response is

Lentiviruses
A genus of slow viruses, which are characterized by long incubation periods, of the *Retroviridae* family that includes HIV-1 and SIV.

Neutralizing antibody
An antibody that reacts with an infectious agent, usually a virus, and destroys or inhibits its infectiveness.

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generated in infected individuals, this response is weak and emerges long after the early partial control of HIV-1 replication that occurs during primary infection[13]. Moreover, monkeys depleted of B cells by infusion of CD20-specific antibody and then infected with SIV show normal virus clearance in the early stages of infection[14]. Interestingly, recent evidence indicates that immune pressure mediated by antibodies results in the selection of HIV-1 mutants that are not susceptible to antibody neutralization[15]. Therefore, a diversity of viral mutants are generated as HIV-1 replicates, and the viruses that have a replicative advantage are those that are not affected by the circulating antiviral antibodies. However, although this process of viral escape from autologous neutralization occurs, it does not seem to be associated with clinical deterioration in the infected individual. Furthermore, treatment of chronically infected humans with monoclonal antibodies that have HIV-1 neutralizing activity *in vitro* has very little effect on viral replication *in vivo*. This might be a consequence of the inability of neutralizing antibodies to affect established viral reservoirs in cells.

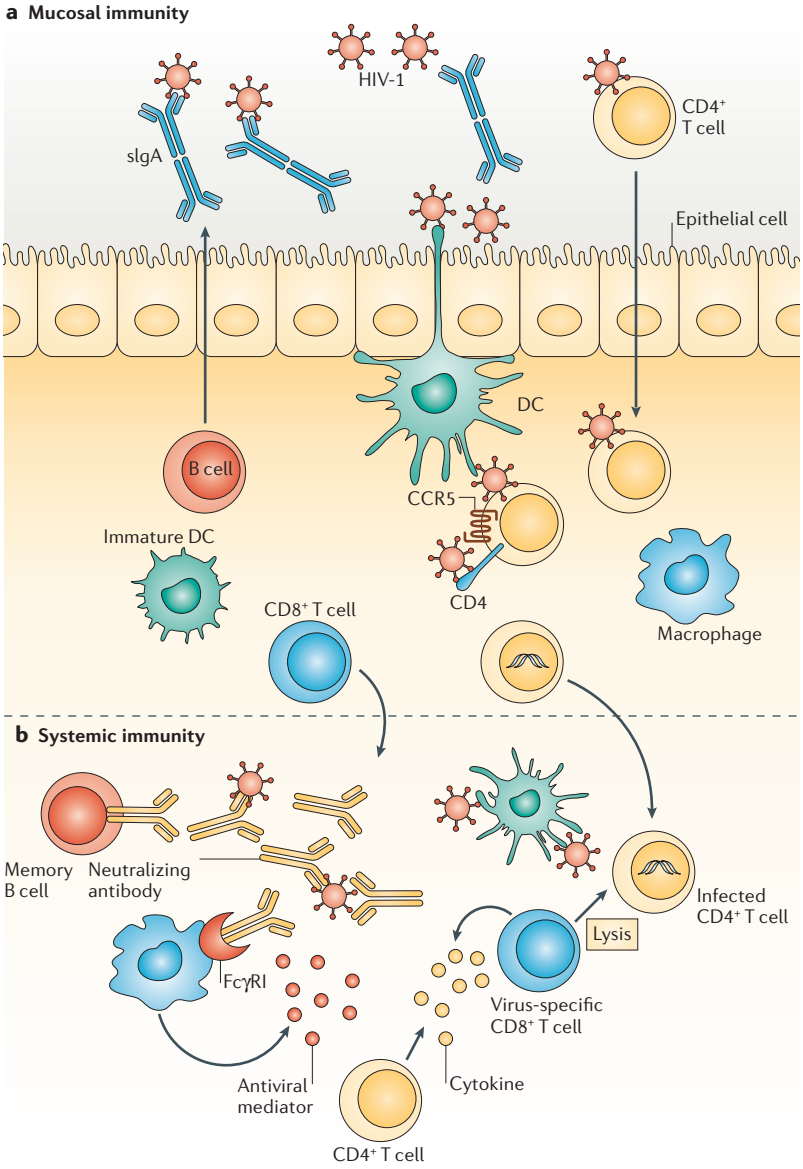

Figure 2 | **Immune responses following HIV-1 infection. a** | Infectious virions enter the host as both cell-associated and cell-free particles. Cell-free virus can be bound and neutralized by secretory IgA (sIgA) secreted by antibody-producing B cells at mucosal surfaces. Unbound particles can be taken up by dendritic cells (DCs) and transferred across a mucosal surface into the submucosal epithelium to infect $CD4^+$ T cells and macrophages. **b** | Adaptive immune responses are important in the control of HIV-1. Antibodies bind virions and neutralize them or, by binding to Fc receptors, they activate other cells that mediate antiviral activity. $CD8^+$ T cells produce cytokines and lyse infected $CD4^+$ T cells. $CD4^+$ T cells provide cytokines and co-stimulatory help to effector cells such as cytotoxic T lymphocytes. CCR5, CC-chemokine receptor 5; FcγRI, high-affinity Fc receptor for IgG.

Although neutralizing antibodies make at most a modest contribution to viral clearance, the immune control of HIV-1 does depend on cellular immunity. Data supporting this contention have been provided by various observations of both primary and chronic infection. $CD8^+$ T cells from an infected individual can inhibit HIV-1 or SIV replication in autologous $CD4^+$ T cells, an inhibition that is mediated by both direct cytotoxicity and the production of soluble antiviral mediators including β-chemokines, such as CC-chemokine ligand 3 (CCL3), CCL4 and CCL5 (REFS 16–18). The early containment of HIV-1 and SIV replication in acutely infected individuals is temporally associated with the emergence of a virus-specific $CD8^+$ cytotoxic T lymphocyte (CTL) response, and high levels of circulating CTLs are associated with good clinical status in chronically infected individuals[19–22]. Importantly, in monkeys depleted of $CD8^+$ T cells through the administration of a monoclonal CD8-specific antibody there is no control of SIV replication during primary infection, and the animals die after a rapidly progressive disease course[23,24]. Moreover, dominant-epitope-specific CTLs have been shown to select for HIV-1 and SIV variants that can escape immune recognition, and the emergence of these escape variants can be associated temporally with a dramatic increase in viral replication and clinical deterioration[25,26]. These observations suggest that an effective HIV-1 vaccine should generate a potent cellular immune response.

Evaluation of vaccine prototypes

Despite the considerable efforts that have gone into developing an HIV-1 vaccine, only one prototype immunogen has been evaluated for protective efficacy in human clinical trials. This vaccine, which consists of a recombinant form of the envelope glycoprotein gp120 administered with the adjuvant alum, was shown to confer protection against challenge by a highly attenuated HIV-1 isolate in chimpanzees[27]. However, the vaccine was not protective in humans[28]. Most investigators involved in HIV-1 vaccine development were not surprised by the outcome of this trial. The vaccine elicited a gp120-specific antibody response, but the antibody did not neutralize HIV-1 isolates that had recently been obtained from the blood of infected individuals[29–31]. Moreover, because a vaccine antigen of this type does not enter the MHC class I pathway after administration, it cannot induce a CTL response.

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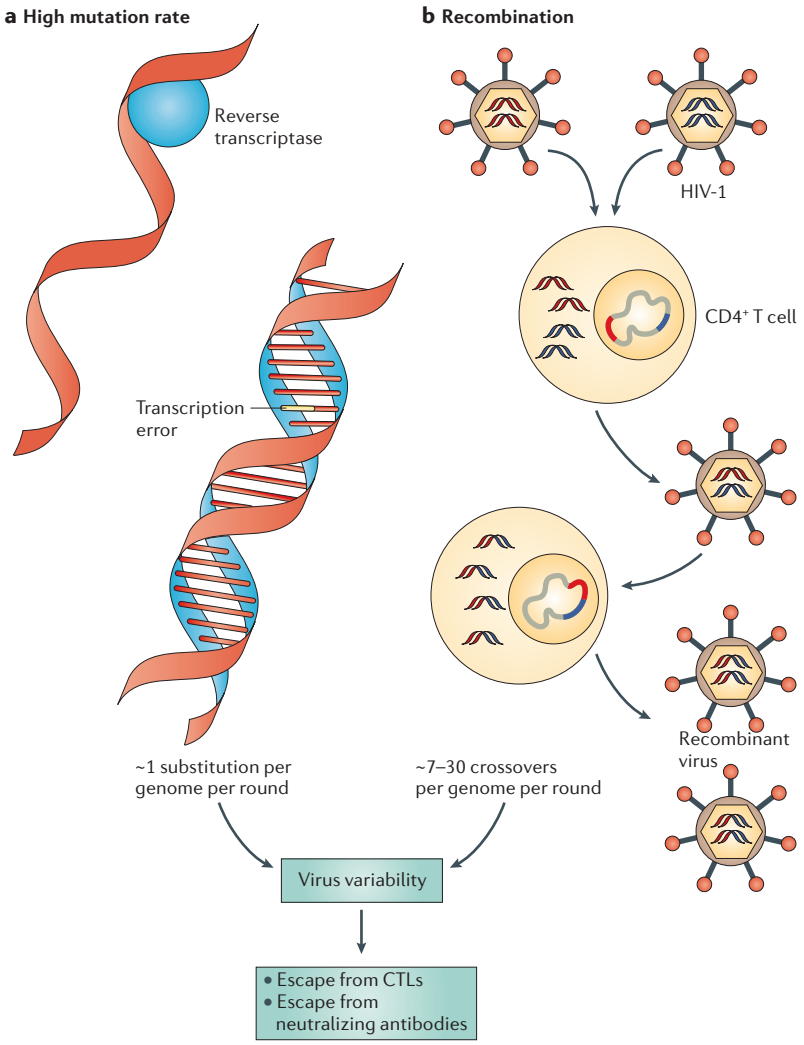

Figure 3 | **Molecular mechanisms of HIV-1 genetic variation. a** | The viral reverse transcriptase is highly error prone, resulting in each new virion encoding approximately one new mutation. **b** | Viral recombination in CD4+ T cells can also generate HIV-1 genetic variation. When two HIV-1 virions with different genetic sequences enter the same cell, they can both integrate and produce viral RNA. Homologous recombination or packaging of RNA from different parent viruses leads to the creation of entirely new HIV-1 genomes. The mechanisms illustrated in **a** and **b** both contribute to HIV-1 genetic variability and, therefore, to the potential of the virus to escape host immune responses. CTLs, cytotoxic T lymphocytes. Figure modified, with permission, from REF. 92 © (2006) International AIDS Vaccine Initiative.

Another HIV-1 vaccine efficacy trial is currently underway to evaluate this same subunit immunogen in combination with a recombinant viral vector using a prime–boost regimen (TABLE 2). The vector being assessed in this clinical trial is a canarypox virus expressing HIV-1 genes. Although the canarypox virus has been shown to express inserted genes *in vitro* in human cells, it does not replicate efficiently in these cells. It is hoped that, *in vivo*, this vector will express HIV-1 transgenes at a high enough level in humans to elicit a CTL response but will have no associated toxicity because it does not replicate. There is, however, also little optimism associated with this trial because of the low level of immunogenicity of the vector in humans observed in earlier clinical studies, which indicated that less than 20% of normal volunteers generated CD8+ T-cell responses against the vaccine[32].

Although these advanced-phase human clinical trials for candidate vaccines have aroused little optimism, studies in non-human primates suggest that it should be possible to create an effective HIV-1 vaccine. Pretreatment of rhesus monkeys with SIV-specific immunoglobulin results in a reduction of viral replication following challenge with SIV[33]. Infusion into rhesus monkeys of monoclonal antibodies that neutralize HIV-1 isolates *in vitro* has been shown to block the subsequent acquisition of primate lentivirus infection that is initiated by either intravenous or mucosal viral exposure[34,35]. Confidence that these findings might be translated into an effective antibody-based vaccine for HIV-1 is tempered by the observation that protection against infection in this type of experimental model has required levels of circulating antibody that are too high to be induced by routine vaccination strategies[36,37]. Moreover, we remain unable to create a subunit immunogen that elicits broadly neutralizing antibodies. It is possible, however, that the rapid mobilization of a vaccine-elicited memory B-cell population soon after exposure to HIV-1 might result in an early, robust antibody response that could eliminate or partially contain the replicating virus.

Studies in non-human primates have also shown that vaccine-elicited cellular immune responses, even in the absence of neutralizing antibodies, can confer significant protection against the clinical consequences of a lentivirus infection. Monkeys immunized with plasmid DNA, a live recombinant viral vector or a combination of the two develop cellular immune responses that do not protect against infection, but do slow the rate of clinical disease progression after infection[38–42]. In comparison with control vaccinated animals, these experimentally vaccinated monkeys show a more rapid expansion of memory CTLs, a consequent reduction in peak and chronic levels of viral replication, a delay in the loss of CD4+ T cells, and prolonged survival.

The results of recent studies have begun to delineate the mechanisms underlying this prolonged survival of vaccinated, challenged monkeys. Because memory CD4+ T cells express CCR5, the co-receptor for transmitted HIV-1 and SIV isolates, there is a rapid and dramatic loss of this lymphocyte subpopulation during the first days after infection as the virus targets these cells for destruction[11,43,44]. By contributing to the control of early replication, vaccine-induced CD8+ and CD4+ T-cell responses in rhesus monkeys provide some degree of protection against the destruction of these memory CD4+ T cells[45,46]. This protection confers a survival advantage, because the immune systems of the monkeys are better able to cope with potential opportunistic infections and malignancies.

Although the level of protection afforded by a CTL-based vaccine is not ideal, the use of such a vaccine in human populations could have several important benefits. The prolonged survival of vaccinated individuals who subsequently become infected with HIV-1 would be an enormous advantage, particularly in resource-poor

Prime–boost vaccination
When a single application of a vaccine is insufficient, repeated immunizations are performed using either the same vaccine preparation (homologous prime–boost) or different vaccine preparations (heterologous prime–boost) to stimulate a better immune response. Prior exposure to one strain diverts the antibody response to shared epitopes of a second strain after exposure.

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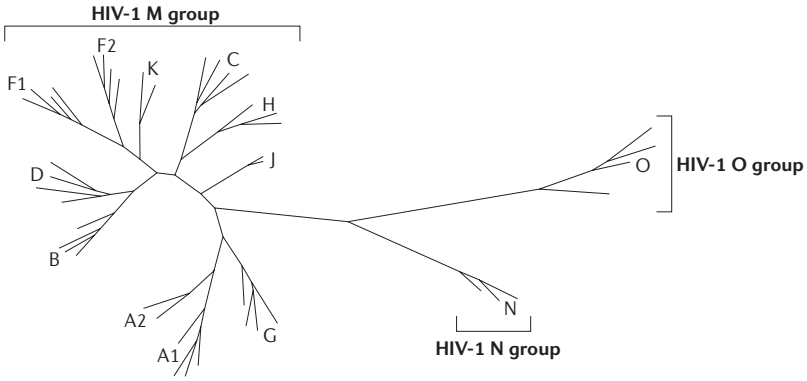

Figure 4 | **Genetic diversity of HIV-1 isolates.** This figure depicts a neighbour-joining tree, based on a full genome sequence alignment with a few representatives of each of the M group major HIV-1 subtypes (also known as clades): A, B, C, D, F, G, H, J and K. In addition, N and O groups are shown; these groups seem to have arisen from separate introductions of the virus to humans from chimpanzees. Forty-four sequences are included in this tree. Some subtypes (such as clade K) are very rare, and only two full-length genome sequences are available. There is no subtype I, and no-full length sequence for clade E. The tree was drawn using the Treemaker tool at the Los Alamos HIV-1 database.

areas of the world where access to antiretroviral drugs remains limited. Furthermore, if vaccinated individuals who become infected with HIV-1 maintain low levels of viral replication, the speed of viral spread from infected to uninfected people might slow[47]. This is because low levels of HIV-1 replication in an individual should be associated with low levels of virus in their secretions, which, in turn, should be associated with inefficient viral transmission. In fact, levels of HIV-1 viraemia in an infected individual have been shown to correspond to the likelihood of that individual transmitting the virus[48]. Therefore, a vaccine of this type could have benefits for both the individual and the population as a whole.

Nevertheless, accumulating evidence from non-human primate studies indicates that the duration of protection associated with a CTL-based vaccine might be limited. Vaccine-related containment of primate lentivirus replication can be abruptly lost in monkeys after viral challenge, with a consequent dramatic clinical deterioration[49,50]. In these monkeys, the change in clinical status was associated with the acquisition by the infecting virus of a mutation in a dominant CTL epitope that allowed the virus to escape CTL control. The mutant viruses rapidly became the predominant viral quasi-species in the infected monkeys. These findings suggest that vaccine-elicited dominant-epitope-specific CTLs limit lentivirus replication, and that the immune pressure on the virus that is mediated by these CTLs selects for mutations that allow the virus to escape recognition by this cellular immune response.

However, although it is reasonable to assume that such events occur regularly during ongoing viral replication, protection might last for a relatively long period of time after infection. If viral replication is initially contained at a very low level, it could take a long time for escape variant viruses to be generated, as mutations will emerge in a viral population at a rate proportional to the rate of ongoing viral replication. Moreover, if CTLs are generated to several virus epitopes, escape of the virus at all of those epitopes might be required for the virus to evade T-cell recognition and effective immune control. A CTL-based vaccine strategy might therefore be a viable approach to vaccine control of HIV-1 despite the phenomenon of viral escape from CTL recognition[51]. Bolstered by these rationales, two CTL-based vaccines are currently moving into advanced-phase clinical testing: a recombinant adenovirus-based vaccine, and a plasmid DNA prime and recombinant adenovirus boost-based vaccine (TABLE 2).

Vaccine-elicited neutralizing antibody responses

Although neutralizing antibodies do not have a pivotal role in early HIV-1 clearance, a vaccine-elicited neutralizing antibody response might confer substantial protection. For a vaccine to prevent infection by a diverse range of HIV-1 isolates, it must induce antibodies that neutralize genetically disparate HIV-1 isolates. However, the creation of a subunit immunogen that can elicit such an immune response has proved difficult. Some of the subunit envelope immunogens evaluated so far generate antibodies that block infection by HIV-1 isolates that have envelope proteins similar in sequence to those used in the vaccine. They also induce antibodies that have activity against a small subset of HIV-1 isolates that are particularly sensitive to neutralization. However, these vaccine-elicited antibodies do not neutralize most circulating HIV-1 strains[52–54]. Nevertheless,

Table 1 | **Characteristics of HIV-1, SIV and SHIV-89.6P infection**

Features of infection*	HIV-1	SIV‡	SHIV-89.6P
Usual co-receptor	CCR5	CCR5	CXCR4
Main target cell	Memory $CD4^+$ T cells	Memory $CD4^+$ T cells	Naive $CD4^+$ T cells
Time to peak viral load	2 weeks	2 weeks	2 weeks
Setpoint viral load (RNA copies per 1 ml of blood)	10^5	10^7	10^6
Median survival time (without treatment)	11 years	3 years	200 days
Time from infection to autologous neutralizing antibody response	3 months to 1 year	3–6 months	6–12 weeks (in those that seroconvert)

*HIV-1 infection of humans; SIV and SHIV-89.6P infection of Indian-origin rhesus monkeys. ‡SIV mac239/25. CCR5, CC-chemokine receptor 5; CXCR4, CXC-chemokine receptor 4; SHIV, simian human immunodeficiency virus.

Immunogenicity
The ability to provoke overt immune responses.

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Table 2 | **HIV vaccines that are currently in large and mid-sized clinical trials for immunogenicity and efficacy**

Vaccine candidate	Antigen (HIV-1 clade)	Manufacturer	Trial start date	Question being addressed
Prime with canarypox vector expressing HIV-1 genes	*env* (B, E), *gag/pol* (B)	Sanofi-Pasteur	October 2003	Will a gp120 protein vaccine that did not confer protection when used alone be useful in combination with a live, recombinant pox vector prime?
Boost with gp120 protein	gp120 (B, E)	Vaxgen		
Replication-defective adenovirus serotype 5 expressing HIV-1 genes	*gag*, *pol*, *nef* (B)	Merck	December 2004	Will an adenovirus-based vector vaccine confer a clinical benefit in individuals who become infected after vaccination?
Prime with plasmid DNA encoding HIV-1 genes	*gag*, *pol*, *nef* (B), *env* (A, B, C)	Vical, VRC	September 2005	Will a prime–boost strategy using DNA- and adenovirus-based vaccines encoding envelope proteins from three HIV-1 clades, as well as viral structural proteins, confer a benefit?
Boost with replication-defective adenovirus serotype 5 expressing HIV-1 genes	*gag*, *pol* (B), *env* (A, B, C)	GenVec, VRC		

Further information on ongoing trials of preventative AIDS vaccines can be found in the 2006 International AIDS Vaccine Initiative report. *env*, envelope; *gag*, group-specific antigen; gp120, glycoprotein 120; *nef*, negative factor; *pol*, polymerase; VRC, Vaccine Research Center, National Institutes of Health, Maryland, USA.

it should be possible to configure an immunogen that will elicit a broadly neutralizing antibody response. This is illustrated by the demonstration that monoclonal antibodies can be generated that neutralize diverse HIV-1 isolates[55,56].

At present, several strategies are being explored to create an effective subunit envelope immunogen. One of these approaches involves generating novel envelope-specific monoclonal antibodies that can neutralize a diverse range of HIV-1 isolates[57]. By mapping the recognition specificities of these antibodies, domains of the virus will be identified that can serve as targets for broadly neutralizing antibodies. The underlying assumption in this strategy is that if a monoclonal antibody that neutralizes the virus can be generated, an immunogen can be configured to elicit an antibody with the same specificity and activity. However, it was recently reported that at least some of the monoclonal antibodies with broad neutralizing activity have polyspecific reactivity with autoantigens, and the production of these antibodies might be regulated by tolerance mechanisms[58]. In fact, two of the most broadly reactive HIV-1 envelope-specific monoclonal antibodies are polyspecific autoantibodies that are reactive with the host phospholipid cardiolipin. It should be noted, however, that some investigators feel that this apparent crossreactivity might reflect the hydrophobicity of the combining sites of these antibodies[59]. This raises questions about the feasibility of generating antibodies in healthy individuals with these envelope specificities.

Another approach being explored to solve the difficult problem of inducing broadly neutralizing HIV-1-specific antibodies is to gain a more in-depth understanding of the structure of the HIV-1 envelope. It is hoped that this will lead to the development of approaches for creating immunogens that approximate the native envelope glycoprotein in its biologically relevant three-dimensional conformations. Progress in this area has been slow because of the technical difficulties associated with determining the structure of this flexible, heavily glycosylated protein. So far, structures of monomeric HIV-1 gp120 bound to CD4 and unbound monomeric SIV gp120 have been determined[60,61]. However, the envelope glycoprotein of HIV-1 comprises two components, gp120 and gp41, the transmembrane part. Moreover, it exists on the virus as a trimeric molecule, and undergoes conformational changes as the virus fuses to the outer membrane of a cell during the infection process[62]. The structures of these biologically relevant molecules remain to be elucidated.

Although considerable effort is being made to define conserved neutralizing epitopes of the HIV-1 envelope and to determine the structure of the pre-fusion envelope trimer, novel envelope immunogens continue to be created and evaluated as potential vaccine immunogens. These include baculovirus-expressed particles that express the envelope glycoprotein on their surface, chemically stabilized envelope trimers, and domains of the membrane-proximal region of the envelope expressed in membrane fragments.

Mucosal immune responses in HIV-1 infection

Because much of the HIV-1 epidemic is fuelled by sexual transmission of the virus, most investigators assume that an effective vaccine must elicit a mucosal immune response that will block the transmission of the virus at a mucosal surface. It is important to recognize that there are caveats associated with this assumption. First, it has still not been proved that HIV-1 crosses an intact mucosal surface to initiate infection. Epidemiological studies have clearly shown that the likelihood of HIV-1 acquisition is increased in individuals with genital ulcers, suggesting that sexual transmission of the virus might occur by haematogenous seeding[63–65]. Furthermore, it remains possible that many HIV-1 transmission events occur through microvascular tears in mucosal tissue rather than across intact mucosa. Therefore, it has not been proved beyond doubt that sexual transmission of HIV-1 occurs across an intact mucosal surface.

Second, it is well established that vaccine-induced systemic immune responses can confer effective protection against mucosally transmitted viruses. A clear example of this phenomenon is the protection provided by the Salk poliovirus vaccine[66]. This inactivated-virus vaccine induces a systemic antibody response that does not block the transmission of the virus. Rather, it diminishes poliovirus replication systemically after mucosal

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transmission has occurred, blocking seeding of the central nervous system with the virus. This highly effective vaccine therefore aborts the natural course of a mucosally transmitted poliovirus infection without acting at the site of transmission.

Accepting these possible caveats, considerable effort is being focused on inducing both cellular and antibody responses that might contribute to containing the virus at a mucosal surface. At least some parenterally administered live recombinant vectors have been shown to elicit cellular immune responses at mucosal surfaces in non-human primates[67]. Studies are being pursued to determine whether mucosal delivery of vectors, such as the gene-deleted adenoviruses, can actually enhance the mucosal cellular and humoral immune responses elicited by those vaccines. Novel live vector systems that are specifically designed to induce mucosal immunity are also being explored. Foremost among these strategies is the use of live recombinant *Mycobacteria* spp. and enteric bacteria such as *Salmonella* spp. These are non-pathogenic recombinant microorganisms that can be administered at a mucosal surface and should, therefore, specifically elicit mucosal cellular and humoral immune responses. Whether strategies specifically designed to generate potent mucosal immune responses can confer protection against sexually transmitted HIV-1 remains an open question.

Vaccine strategies for dealing with viral diversity

Mutations rapidly accumulate in HIV-1 as it replicates such that circulating strains of the virus can differ from one another by up to 20% in the more conserved proteins and by as much as 35% in the envelope proteins[68,69]. Moreover, primary HIV-1 isolates have no main neutralizing determinants, and each of these isolates can have a distinct target for the neutralizing antibody response. The implications of the sequence diversity of HIV-1 are also problematic for virus-specific $CD8^+$ T-cell responses. Certainly there can be some antigenic crossreactivity between isolates of HIV-1 in the cellular immune response, but single amino-acid substitutions can sometimes abrogate $CD8^+$ T-cell recognition of a dominant epitope of the virus[70–72]. HIV-1 vaccines that are based on a single sequence of the virus might therefore elicit immune responses that are too focused and type-specific to provide effective protection against the quasi-species of genetically diverse HIV-1 isolates that exist in a human population. As a result, a vaccine strategy for HIV-1 must take into account the extreme sequence diversity of this virus.

One vaccine strategy being developed to deal with HIV-1 genetic variation involves the use of immunogens that incorporate a combination of variant genes or proteins from representative viruses. The rationale behind this approach is that immune responses to a particular portion of the virus might diversify after exposure to many related variant proteins, leading to the generation of memory T and B cells that can recognize sequences related to but distinct from those used to elicit the memory cell populations. The immunogens moving forward into advanced-phase human clinical testing by the Vaccine Research Center at the National Institutes of Health in Maryland, USA, include three distinct envelope constructs, encoding representative proteins from clade A, B and C viruses[73] (TABLE 2). Preliminary immunological evaluation of immune responses to the HIV-1 envelope induced by this vaccination regimen suggests that the cellular and humoral immune responses do not interfere with one another[74]. However, although these vaccine-elicited immune responses have greater breadth than those elicited by envelope immunogens using a single gene sequence, the breadth of these responses has not proved to be greater than would be expected on the basis of the additive properties of the immunogens.

Other approaches to dealing with the immunological problems resulting from the genetic diversity of HIV-1 isolates are also being evaluated. Several of these make use of hypothetical viral gene sequences that are generated by phylogenetic reconstruction. These strategies are based on the data that have been accumulated to document the diversity of HIV-1 gene sequences worldwide. Consensus sequences for each HIV-1 gene have been designed that encode the most common amino acid at each position of each viral protein. Ancestral sequences have also been constructed to approximate progenitor viral gene sequences[75]. In both of these approaches, the sequences of these hypothetical genes are closer to the sequences of currently circulating viruses than most currently circulating viruses are to each other. Importantly, envelope proteins that have been constructed on the basis of these types of hypothetical sequence have proved to be functional in several *in vitro* assay systems[69]. These envelope proteins have been shown to bind to both CD4 and chemokine receptors, and to bind several of the monoclonal antibodies that recognize conserved neutralizing determinants of HIV-1. It remains to be determined whether vaccines that are based on these hypothetical genes elicit immune responses with greater breadth than vaccines that incorporate single, naturally occurring viral genes.

Vaccine-elicited immune responses

Should we be able to create immunogens that induce both broadly neutralizing antibodies and broadly reactive CTLs, the magnitude, durability and quality of the immune responses that we can generate with currently available technologies might still not be adequate to provide effective protection against the virus. In fact, studies have suggested that the level of circulating neutralizing antibody needed to prevent infection of macaques with a primate lentivirus are much higher than can be induced with traditional vaccine modalities[32,33]. Moreover, the magnitude of the CTL responses that have been generated in human volunteers so far is not nearly as great as the vaccine-elicited responses in macaques that have controlled primate lentivirus spread[76,77].

Although live recombinant virus vectors continue to show promise as a viable approach for eliciting cellular immune responses to HIV-1, the magnitude of the

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responses in human volunteers immunized with such vaccines has been substantially lower than that seen in non-human primates. This reduced immunogenicity might be a consequence of pre-existing vector-specific immunity. For example, adenovirus serotype 5 (Ad5)-specific immunity induced by naturally occurring adenovirus infections probably reduces the immunogenicity of immunogens based on gene-deleted Ad5 vectors[78]. Ad5-directed immunity does not interfere with the immunogenicity of the Ad5-based vectors in macaques because non-human primates are infected with a distinct family of adenoviruses with different serological profiles. To circumvent the problem of pre-existing Ad5-specific immunity, adenovirus vectors are being developed for which there should be no pre-existing immunity in human vaccine recipients. These vectors include selected human adenoviruses, such as adenovirus serotype 35, that rarely cause natural infections in humans. Adenoviruses that infect non-human primate species, including selected chimpanzee adenovirus isolates, are also being developed as potential vaccine vectors[79,80]. In addition, chimeric adenovirus vectors are being constructed that circumvent susceptibility to pre-existing neutralizing antibodies against the vector but maintain high levels of immunogenicity[81,82].

In light of the concern that the durability of vaccine-elicited cellular immune responses will be important for an HIV-1 vaccine, several vectors are being explored that should persist in vaccinees. The assumption underlying this approach is that microorganisms that are never fully cleared in infected individuals should continue to make these proteins and therefore perpetuate an immune response. The most promising of the persisting vectors are those constructed with mycobacteria, including *Mycobacterium bovis* bacillus Calmette–Guérin (BCG)[83]. However, it should be noted that emerging data indicate that the maximal generation of memory T cells might occur when antigen does not persist[84].

Plasmid DNA constructs have proved to be effective immunogens in mice for eliciting cellular immune responses and for priming antibody responses. It is now clear, however, that DNA vaccines are less immunogenic in non-human primates than they are in mice, and even less immunogenic in humans than in non-human primates. Therefore, strategies are being aggressively pursued to improve the immunogenicity of plasmid DNA vaccines. These strategies include formulating the plasmid DNAs with liposomes or polymers to increase their *in vivo* expression as well as protect them from rapid degradation[85–88]. Novel delivery technologies are also being explored, including the use of *in vivo* electroporation to increase the efficiency of cellular transfection with DNA[89]. In addition, the results of some studies have shown that co-administration of plasmid DNA immunogens with plasmids encoding cytokines has the potential to increase vaccine-elicited cellular immune responses[90]. The best studied of these approaches includes the use of cytokines that increase the clonal expansion of antigen-specific T cells, such as interleukin-2 and interleukin-15, and cytokines that attract and induce the maturation of antigen-presenting cells, including granulocyte/macrophage colony-stimulating factor and β-chemokines.

It remains unclear whether qualitative aspects of vaccine-elicited cellular immune responses or particular viral protein specificities of those cellular responses will prove important in protecting against viral replication and disease progression. The increasingly sophisticated use of polychromatic flow cytometry will allow the assessment of the functional capabilities of vaccine-induced T-cell populations[91]. It will be important to determine whether the production of certain cytokines or cytotoxic mediators by vaccine-elicited T cells is associated with particularly effective control of virus replication. Furthermore, although there is a consensus that it will be desirable to generate immune responses to several viral structural gene products, it is not yet clear whether cellular immune responses to some of the early viral regulatory proteins such as Tat (transcriptional transactivator) and Nef (negative factor) will confer a substantial additional benefit to vaccinees.

Finally, there is considerable focus on developing technologies that will augment humoral immune responses to subunit HIV-1 envelope immunogens. This is a particularly daunting challenge, because it will be important to deliver such immunogens in a manner that will not alter their natural structural conformations. The technologies receiving the most attention at present include oil and water emulsions and CpG-motif-containing adjuvants that signal through Toll-like receptor 9.

Concluding remarks

Data from non-human primate studies indicate that existing technologies should allow for the creation of HIV-1 vaccines that preserve memory $CD4^+$ T-cell populations and, as a result, attenuate the clinical course of infection. The ongoing advanced-phase clinical testing of recombinant adenovirus-based immunogens and prime–boost immunization strategies using plasmid DNA followed by recombinant adenovirus vaccines will test this possibility (TABLE 2). Importantly, several recent advances promise to improve the protection conferred by immunization. The magnitude and durability of vaccine-elicited immune responses should be dramatically improved by advances in increasing the immunogenicity of plasmid DNA immunogens as well as in the development of new generations of viral vectors that can escape recognition by pre-existing vector-specific immunity. A greater breadth of immune recognition should be achieved using consensus gene sequences in these novel vectors. Moreover, investigations into the strategies for eliciting broadly neutralizing antibodies and mucosal immune responses should further improve the efficacy of HIV-1 vaccines. Although the creation of an effective HIV-1 vaccine remains an enormous challenge, continuing progress in all of these areas provides reason to be optimistic about our ultimate ability to control the spread of AIDS.

Liposomes
A lipid vesicle that encapsulates vaccines in a lipid bilayer membrane and facilitates their delivery.

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Acknowledgements
The author thanks J. Mascola and B. Hahn for their careful review of this manuscript and B. Korber for providing Figure 4. This work was supported by the US National Institutes of Health.

Competing interests statement
The author declares no competing financial interests.

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