

HIV Vaccine Approaches

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Advanced article

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An HIV (human immunodeficiency virus) vaccine is needed to combat the 1–2 million cases on new HIV infection each year. The goal of vaccination is to establish long-term immunological readiness that allows rapid protection against infectious disease. All current successful vaccines achieve this by inducing neutralising antibodies, which are effective against acute, cytopathic infections that could otherwise prove fatal. By contrast, potential vaccines against highly variable pathogens that establish persistent infections, such as HIV, have had limited success. There have been multiple attempts to induce effective immunity to HIV in humans, with only one approach showing partial (31%) efficacy. Several clues on a better path forward have, however, emerged from HIV vaccine trials in humans, studies of humans who naturally control HIV infection and studies in animal models. There has been an improved understanding of anti-HIV neutralising antibodies, which, if could be induced successfully, are likely to be highly effective. Antibodies that engage Fc receptors on innate immune cells (the so-called ADCC (antibody-dependent cellular cytotoxicity) antibodies) have emerged as a key component of successful vaccine strategies. New vaccine design and delivery strategies are now

entering large-scale human efficacy trials. These approaches offer real hope that a safe and effective HIV vaccine will eventually be developed to assist in the control of the HIV/AIDS (acquired immunodeficiency syndrome) pandemic.

Introduction

Human immunodeficiency virus (HIV) continues as a major global epidemic. In 2016, UNAIDS reported on the HIV/acquired immunodeficiency syndrome (AIDS) epidemic; at the end of 2015, 36.7 million people were infected with HIV (www.unaids.org). During that year, some 2.1 million new infections had occurred. The consequences of the epidemic stretch much further; there are currently more than 15 million orphans who have lost parents to AIDS and are left defenceless to poverty and street living. The vast majority of HIV-infected individuals live in the developing world with poor health care systems and limited resources. Despite this, treatment for HIV has been rolled out to a remarkable 17 million infected persons worldwide. Unfortunately, current treatments are not curative, and treatment must be taken lifelong with significant cost, side effects and the risk of developing drug resistance.

Global HIV infection rates remain unacceptably high and it will be difficult to treat our way out of the HIV epidemic – there is an urgent need to prevent more HIV infections (Johnston and Fauci, 2008). New prevention technologies, including pre-exposure prophylaxis (PreP) and vaginal microbicides, offer promise that new infections may be blunted; however, trials show that compliance with such strategies is often low (Abdool Karim *et al.*, 2010; Grant *et al.*, 2010; Van Damme *et al.*, 2012). Prevention of spread of HIV through vaccination is an urgent priority. To date, attempts to generate traditional vaccines have been disappointing, but as scientists learn more about the virus and the immune system with

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which it interacts, the prospect of new generation vaccines offers hope for the future.

Speed of Response: The Adaptive Immune System's Achilles Heel

The human immune system is a complex network of cells and molecules capable of mounting highly specific, potent protective responses against a broad range of invading pathogens. Nonetheless, infectious diseases are still a major problem, due in part to an inherent weakness of the immune system; the extended time taken to generate protective adaptive responses if innate immune mechanisms fail. This weakness is an unavoidable consequence of the principal dilemma that faces the immune system that infectious episodes cannot be predicted in advance. The adaptive immune system attempts to solve this problem by preparing for every eventuality; it generates billions of T and B lymphocytes, each with a unique antigen receptor, in the hope that a small fraction of these cells will recognise any particular infectious agent. However, the sheer number of receptors needed to provide the necessary breadth of antigen recognition dictates that each unique specificity can be represented on only very few lymphocytes, well below that needed for an effective immune response. Thus, pathogen-specific T and B cells must undergo massive expansion in the early days of infection before protective responses can be generated. This provides a window of opportunity for infection to take hold, generate symptoms and possibly lead to death. Vaccination attempts to limit this window of vulnerability by preparing the immune system in advance to respond faster and better when infection strikes. In the case of natural HIV infection, however, the virus establishes an effectively permanent infection by integrating successfully into the host genome. The long lag time for the development of adaptive immunity means that once HIV infection is established beyond the very first days of infection, it is very difficult to eliminate, described previously as 'too little too late' (Reynolds *et al.*, 2005).

Vaccines Induce Immunological Readiness

Vaccines are pathogen-specific antigenic preparations that attempt to stimulate protective immune responses and are one of the most powerful and cost-effective health interventions. Indeed, protective vaccines are a major success of modern medicine and were the driving force behind the birth of immunology as a scientific discipline. Since Edward Jenner's first attempts to protect against smallpox by the administration of cowpox in 1796, the goal of vaccination has been to prepare the immune system in advance to respond faster and better to infectious agents, minimising the time in which pathogens could cause death and disease. Vaccines aim to provide lifelong protection by inducing a prolonged state of immunological readiness. The precise mechanism by which this state is maintained is still the subject of debate (Ahmed and Gray, 1996; Zinkernagel and Hengartner, 2006), but it is fair to say that it capitalises on

the ability of the immune system to preserve useful 'memory' lymphocytes; essentially, an increased frequency of protective pathogen-specific cells that will respond rapidly and efficiently to reinfection. **See also: Immunological Memory.** Normally, this heightened state of readiness can only be reached by engaging pathogen in a full-blown infectious setting. Vaccination attempts to mimic these interactions, selecting useful lymphocytes for use in later immune responses, but importantly doing so in the absence of the very great dangers of disease. For HIV, as the virus rapidly establishes a permanent infection, there is potentially a need to have a large number of effective immune responses constantly ready at the site of possible infection – this poses a significant challenge faced by few if any other pathogens (Masopust and Picker, 2012).

Vaccines Must Induce Appropriate Immune Responses

The goal of vaccination is to initiate long-term protective immune responses. The optimal control of infection commonly involves both the cell-mediated (T cell) and humoral (antibody) arms of the immune response. Protective immunity can be distilled into two main areas: the induction of protective CD4+ and CD8+ T cells and the induction of B cells capable of generating neutralising antibodies. These key elements combine to neutralise invading pathogens before they establish an infectious foothold and allow the rapid expansion and mobilisation to appropriate anatomical locations of large numbers of pathogen-specific lymphocytes that subsequently regulate all elements of a coordinated immune response. However, the vaccine-induced generation of an appropriate balance between these key elements is not always straightforward, as effective immunity against different pathogens commonly requires specific immune effectors. Thus, modern research into vaccination seeks to promote appropriate and specific combinations of immune responses targeted to particular pathogens, especially those against which the more basic approaches of vaccination have so far failed. An understanding of precise immune responses that 'correlate with protective immunity' allows the vaccine field to move forward in a rational direction. For HIV, despite decades of research, there is still a need to more precisely define protective immune responses (Corey *et al.*, 2015).

Vaccination Success and Failure: Lessons to Be Learned

Successful vaccines are now used to immunise children against a variety of diseases that include smallpox, polio, measles, mumps, rubella, influenza, chickenpox, diphtheria, tetanus, pertussis, hepatitis B and rabies. Nonetheless, despite concerted efforts, vaccines remain unavailable for tuberculosis, leprosy, malaria, hepatitis C, leishmania, dengue fever and, of course, HIV/AIDS. Key questions are as follows: What distinguishes or links these two sets of diseases? And what can we learn for future vaccine development?

Diseases against which successful vaccines are available are invariably acute, cytopathic and often lethal infections that generate an abundance of antigen. Protection in all cases is due to establishment of high titres of specific neutralising antibody that prevent significant infection. Interestingly, natural immunisation through passage of maternal neutralising antibodies across the placenta and via breast milk is thought to attenuate these acute childhood infections if exposed too early in early infancy, helping the child's immune system to establish protective responses of its own. These types of infection are relatively easily imitated by current vaccination protocols which are often over 90% effective.

By contrast, diseases such as HIV/AIDS against which vaccine development has been unsuccessful tend to be persistent infections that evade or subvert adaptive responses. In natural infection, these are usually better controlled by activated T cells, especially cytotoxic T cells (CTLs), although neutralising antibodies are also involved. However, prolonged CTL function can result in exhaustion of these cells, limiting their capacity to control infection. Persistent infections do not kill the host quickly and coexist with low-grade immune responses for years.

Chronic viral pathogens are often capable of rapid variation, evading any protective responses that are generated (Ha *et al.*, 2008). HIV-1, in particular, is extraordinarily diverse. Within a single infected individual, up to 10^9 variants are produced daily (Pang *et al.*, 1992). The plasticity of HIV means that virtually every type of immune response directed against HIV has been shown to be subject to mutational escape from recognition over time (Chung *et al.*, 2011; Ferrari *et al.*, 2011). Indeed, the difficulties of the immune system in dealing with HIV/AIDS and other chronic infections are reflected in our failure, so far, to devise successful vaccine strategies against them.

Through infecting CD4 T cells, HIV also causes substantial immune dysfunction that limits the host immune response. HIV-specific CD4 T cells are even more highly susceptible to infection and dysfunction, rendering their central role in coordinating both cellular and humoral immunity weakened (Douek *et al.*, 2002). Successful vaccines for HIV will need to block the establishment of infection in CD4 T cells before these cells die and become dysfunctional.

Common Vaccine Strategies That Have Failed to Make an Impact of HIV Immunity

Critically important considerations directly impact on the success of a vaccine. These condense to several key issues. First the vaccine preparation must be immunogenic; it must induce pathogen-targeted protective immunity that avoids or breaks tolerogenic and inhibitory mechanisms. Unfortunately, the HIV envelope, a key target of humoral immunity, is relatively weakly immunogenic. Second, the vaccine should induce immunity at appropriate anatomical sites that ideally reflect the likely route of pathogen entry. Immunity at mucosal sites is particularly relevant for HIV as sexual contact is the major route of infection, and the gut-associated lymphoid tissue (GALT) is a major site

of replication. Site-specific immunity can be achieved by administering the vaccine orally or through mucosal, intramuscular or intravenous inoculation. This allows distinct antigen-presenting cells to prime local T- and B-cell responses, influencing the subsequent compartmentalisation and nature of the induced immune response. There is a growing interest in the role that 'resident-memory T cells' may have in protective immunity at mucosal surfaces (Tan *et al.*, 2016b). Indeed, there is a relative paucity of studies effectively inducing mucosal immunity to HIV (Tan *et al.*, 2016a). Third, the vaccine must represent the global extent of expected pathogen diversity. For HIV/AIDS, vaccines for specific geographical regions may also be a consideration, but as yet there is no evidence to suggest that this might be a feasible strategy. The global diversity of HIV is strikingly large, and many strategies concentrate on subtypes of HIV that are especially common, such as subtype C in southern Africa. Finally, the vaccine must provide continuous and sufficient presentation of antigen in secondary lymphoid organs that allow long-term protective responses to become established. This may be particularly relevant for HIV/AIDS as highly exposed, persistently seronegative Kenyan sex workers displayed potent virus-specific CTL responses which waned when their constant exposure to HIV stopped, often resulting in the recurrence of susceptibility to HIV infection (Kaul *et al.*, 2001).

With the earlier considerations in mind, a myriad of vaccination strategies have been derived that range from passive transfer of antibodies through to the postgenomic vaccines based on modelling protein structure, many of which are being explored to combat HIV/AIDS. Early vaccines were pathogen derived; heat or chemically inactivated pathogens were used in the Salk polio vaccine and for cholera, influenza and plague. These are relatively safe but require adjuvants and stimulate poor cell-mediated responses owing to limited MHC (major histocompatibility complex) class-I presentation. Other vaccines, for example, against measles, mumps and rubella, and the Sabin polio vaccine, use live attenuated pathogens with greatly decreased virulence. These preparations have the advantage of entering both the class-I and class-II pathways of antigen presentation inducing both cell mediated and humoral immunity. However, reversion to more virulent forms is always a dangerous possibility, preventing the use of this strategy against rapidly mutating pathogens such as HIV. For HIV, reversion to virulence has been documented in both animal models and humans who have naturally acquired attenuated strains (Learmont *et al.*, 1999). As technology has progressed, subunit vaccines have become more prominent and are a more appropriate strategy for HIV. Examples have included toxoid-derived vaccines (e.g. against tetanus and diphtheria), vaccines derived from capsular polysaccharides, either alone or conjugated to a protein carrier (e.g. against bacterial meningitis) and vaccines that use recombinant DNA (deoxyribonucleic acid) technology (e.g. against hepatitis B virus – HBV). These are generally low risk but invariably require the correct choice of adjuvant to ensure sufficient immunogenicity and often fail to stimulate a full range of immune responses because, like killed vaccines, they have limited access to appropriate pathways of antigen presentation. Envelope protein approaches alone have to date not induced neutralising antibodies and earlier approaches were not protective in human efficacy trials (Table 1).

Table 1 Completed and initiated efficacy trials of human HIV-1 vaccine trials

Trial (year published)	Type of vaccine	Total number	Number of vaccine recipients	Number of placebo recipients	Number of vaccine recipients	Number of infections placebo group	Number of infections vaccine group	Percentage of efficacy	Comment	References
<i>Completed trials</i>										
Vaxgen B/E (2006)	Gp120 protein (two stains, one subtype B, one subtype E) in Alum	2527	1267	1260	106	105	None	None	Thai study	Pitisuttithum <i>et al.</i> (2006)
Vaxgen B/B (2005)	Gp120 protein (two stains, both subtype B) in Alum	5403	3598	1805	241	127	None	None	Primarily US-based	Flynn <i>et al.</i> (2005)
STEP (2008)	Adenovirus type 5 vector (three vectors, one expressing Gag, one Nef and one Pol)	3000	1494	1506	19	11	None	None	Primarily US-based. Stopped early when more recipients in vaccine arm acquired HIV than placebo arm	Buchbinder <i>et al.</i> (2008)
Phambili (2011)	Adenovirus type 5 vector (three vectors, one expressing Gag, one Nef and one Pol)	801	400	401	34	28	None	None	Primarily South Africa. Stopped early when STEP study results announced	Gray <i>et al.</i> (2011)
RV144 (2009)	(i) Canarypox vector prime subtype AE (expressing Gag/Pol/Env) (ii) Gp120 protein boost (two strains, one subtype B, one subtype E) in Alum adjuvant	16 395	8197	8198	51	74	31.2	31.2	Thai study. Efficacy correlated with non-neutralising Ab responses	Rerks-Ngarm <i>et al.</i> (2009)
HVTN 505 (2013)	(i) DNA vaccine prime (six plasmids expressing gag, pol, nef and three env strains) (ii) Adenovirus type 5 vector boost (four vectors, one expressing Gag/pol and three expressing Envs)	2494	1250	1244	41	30	None	None	Stopped early for futility	Hammer <i>et al.</i> (2013)
<i>Initiated</i>										
HVTN702 (started 2016)	(i) Canarypox vector prime subtype C (expressing Gag/Pol/Env) (ii) Gp120 protein boost (two subtype C strains) in MF59 adjuvant	5400	planned							
HVTN703/704 (started 2016)	Passive immunotherapy with 8 weekly VRC01 monoclonal antibody	5400	planned							

Modern Vaccine Strategies

More modern vaccines are now under development and aim to generate immunity beyond that induced by the pathogen itself, especially when tackling pathogens likely to establish persistent infections (Wack and Rappuoli, 2005). Antigens can now be selected and engineered to provide enhanced immunodominant epitopes for both T- and B-cell activations. **See also: Immunogenicity.** There is a growing appreciation of pathways forward to induce neutralising antibodies (McCoy and Burton, 2017). An emergent effort is underway to study and induce antibodies with strong and broad Fc-mediated functions (Kramski *et al.*, 2013). More efficient methods of vaccine delivery, such as advanced uses of DNA vaccines, RNA (ribonucleic acid) vaccines and a broad suite of recombinant vector vaccines (e.g. vaccinia, canarypox, cytomegalovirus (CMV) and adenovirus vectors), aim to maximise the induction of potent T- and B-cell responses (Sofra *et al.*, 2009; Tacken *et al.*, 2007).

A Strategic Approach: Correlates of Protection for HIV/AIDS

Identifying protective immune responses in natural HIV infection may suggest logical strategies for vaccine development. Unfortunately, not all observed responses confer protection, but those that are associated with control of viraemia are likely to be important. The typical course of HIV infection is shown in **Figure 1**. Approximately 3 weeks after initial exposure, virus (often $>10^7$ copies mL^{-1}) appears in the blood. At this time,

adaptive immune responses are initially evident which coincide with a drop in viral load of approximately 10- to 100-fold. After approximately 6 months, a stable level of blood-borne virus is reached, known as the viral set point, which correlates well with subsequent disease progression. The CD4⁺ T-cell population then slowly declines until viral replication can no longer be contained, viral loads increase, and as CD4 T cells fall below critical levels, AIDS develops. On average, this occurs 8–10 years after primary infection.

Neutralising antibodies

Virtually, all successful human vaccines primarily induce neutralising antibodies that can transfer immunity by passive immunisation. **See also: Antibodies.** However, the immense diversity of HIV poses serious questions to whether long-term effective antibody-mediated neutralisation will ever be possible. HIV-1 is classified into three groups: M, O and N. The M group, which is responsible for the current pandemic, is further divided into 10 genetically defined subtypes or clades (Robertson *et al.*, 2000). Unfortunately, the HIV viral envelope (Env), which mediates viral entry and is the principal target for neutralising antibodies, differs by up to 35% between clades and 20% within clades (Gaschen *et al.*, 2002).

Env is a trimeric protein with each monomer consisting of a transmembrane gp41 subunit attached to a surface gp120 subunit. The gp120 glycoprotein is made up of variable (V1–V5) regions interspersed with conserved (C1–C5) regions. In the native conformation, the core regions containing the conserved functional domains for CD4, and either CXCR4 or CCR5, receptor binding are shielded by the V1/V2 and V3 variable domains, respectively,

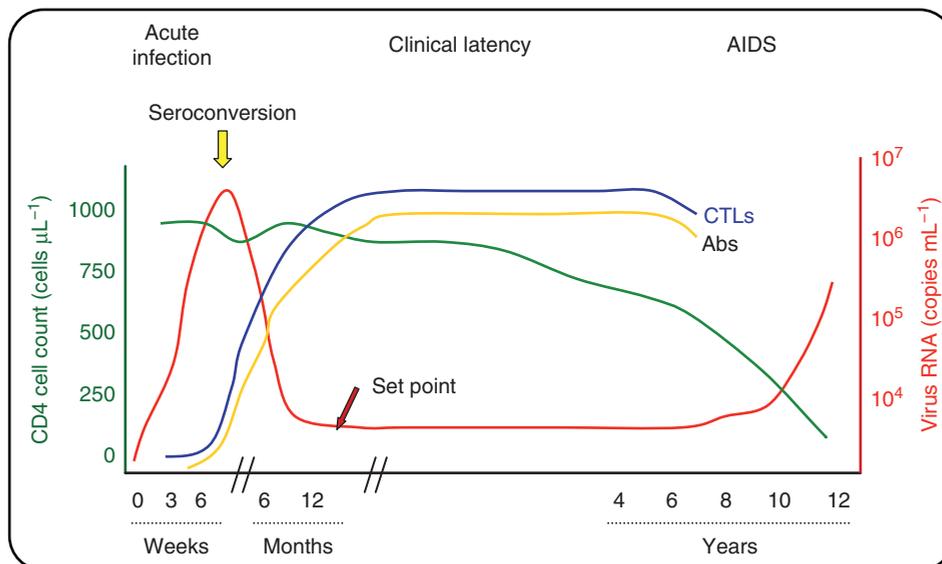


Figure 1 The course of HIV infection. After initial exposure, viral load reaches a peak at 3–4 weeks. CD4⁺ T cells decline but recover quickly on initiation of adaptive immune responses. This response includes cytotoxic T cells which probably control viral load. Antibodies to the viral envelope are also detected but are non-neutralising. These antibodies are capable of complement-mediated attack but their role in controlling viraemia is unclear. Viral load continues to decline until it reaches a 'set point'; a good correlate of subsequent disease progression. Neutralising antibodies cannot be detected until 3–6 months after infection. AIDS normally develops in untreated patients after 8–10 years.

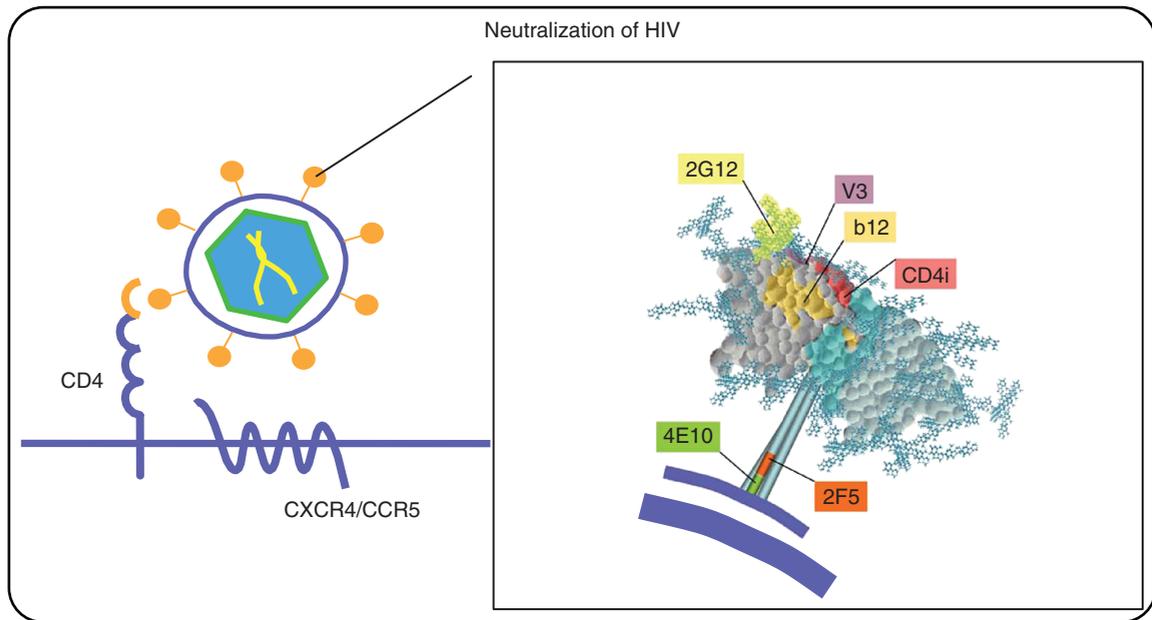


Figure 2 Neutralising antibodies bind to HIV Env. HIV Env binds to CD4 and a coreceptor (usually either CXCR4 or CCR5) to gain entry to target cells. A schematic enlargement of the envelope glycoprotein is shown. The Env glycoprotein contains epitopes for broadly neutralising monoclonal antibodies b12, 4E10 and 2F5 as well as those induced after CD4 binding. The variable regions are shielded with carbohydrate moieties shown. A monoclonal antibody 12G5 that recognises carbohydrate domains is also broadly neutralising.

and can mutate considerably with minimal cost (Kwong *et al.*, 1998). Furthermore, these domains are heavily glycosylated by host glycan moieties, rendering them in part an immuno-silent shield (Chen *et al.*, 2005). Indeed, removal of some glycosylation sites allows potent antibody-mediated neutralisation (Reitter and Desrosiers, 1998). There is, however, a growing appreciation that conserved glycosylations can sometimes be corecognised by broadly neutralising antibodies that offers a potential avenue of attack (Moldt *et al.*, 2012).

Despite this seemingly impenetrable armour, neutralising antibodies are induced in natural infection of HIV, although they are usually undetectable in blood until 3–6 months after the initial control of viraemia (Aasa-Chapman *et al.*, 2004; Richman *et al.*, 2003; Wei *et al.*, 2003). Nonetheless, viruses that are exquisitely sensitive to neutralisation replicate *in vivo* alongside neutralisation-resistant viruses suggesting that other forces play a role in the maintenance of a neutralisation-sensitive phenotype. Thus, a vaccine strategy that induces a broad range of neutralising antibodies, or specific neutralising antibodies that can recognise a broad range of HIV strains, may be viable. Indeed, despite the extraordinary diversity of HIV virtually, all patient-derived HIV-1 isolates bind to CD4 and a chemokine receptor (usually CCR5 or CXCR4), suggesting that the exploitation of this conserved functional constraint may ultimately prove crucial.

Passive immunisation of macaques with neutralising antibodies has demonstrated protection to simian immunodeficiency virus (SIV) after mucosal and intravenous challenge (Mascola *et al.*, 1999). Similarly, passive immunisation of patients on a ‘drug holiday’ delays the return of replicating virus to the blood (Trkola *et al.*, 2005). Thus, with respect to a vaccine, it is hoped that the

presence of neutralising antibodies at the time of exposure will prevent the establishment of productive HIV infection.

The characterisation of neutralising antibodies from HIV-infected humans can guide us not only to the possible neutralisation targets but importantly to those that are immunogenic *in vivo*. A number of human monoclonal antibodies (mabs) that can neutralise a broad number of HIV-1 variants have been generated (Figure 2). As might have been predicted, one of these, b12, blocks CD4 binding and can neutralise approximately 50% of variants. A vaccine strategy that exploits this epitope would need to include enough Env proteins with CD4-binding site conformational variants to induce 100% neutralisation. Considering the breadth of b12 reactivity, this may be feasible.

Two further mabs, 2F5 and 4E10, that bind to gp41 can neutralise approximately 80–100% of variants probably by inhibiting the fusion of virus with the target cell membrane. However, their activity is only modest. More potent antibodies that bind this site have been identified in recent years with remarkable breadth, such as the 10E8 monoclonal (Huang *et al.*, 2012).

Two potent human neutralising mabs were generated in 2009 from a Clade A-infected patient. The mabs, PG9 and PG16, recognise a novel epitope that is preferentially expressed on the trimeric Env spanning both conserved and variable regions (Walker *et al.*, 2009). The epitope is less exposed after CD4 binding suggesting that it may target a domain exposed on the native virion.

Recent years have seen a large increase in the number of broadly neutralising anti-HIV antibodies (bNAbs) identified, including the VRC01 class of antibodies that block the CD4-binding site (Zhou *et al.*, 2010). These bNAbs can protect

from infection in animal models and have greater potency and breadth than the earlier bNAbs outlined earlier. Several new sites of vulnerability on the Env surface have been identified as targets of bNAbs. Passive transfer studies in infected subjects show robust suppression of viremia, although as the bNAb concentration wanes, resistant virus emerges (Lynch *et al.*, 2015). Combination approaches using potent bNAbs targeting multiple will likely improve the effectiveness of this approach (Caskey *et al.*, 2017).

To assess whether these bNAbs can protect humans from HIV infection, a large passive transfer efficacy study is underway with the VRC01 bNAb. This involves regular infusions of the VRC01 monoclonal antibody over 2 years in 5400 subjects (Table 1). Although a relatively expensive and cumbersome approach at present, the cost of production and half-life of the bNAbs will likely improve. In addition, gene-therapy-based strategies using adeno-associated viruses for the bNAbs have been explored with considerable promise in animal models (Gardner *et al.*, 2015).

In summary, functional sites involved in the process of viral entry are promising targets for the generation of neutralising antibodies, but it is likely that any vaccine will need to induce a broad range of specificities to be effective.

HIV-specific T-cell responses

HIV-specific CTLs are detected relatively early after initial exposure, often reaching 10% of total CTL numbers. These responses are maintained throughout infection and are thought to control viraemia (Borrow *et al.*, 1997). Depletion of CD8+ CTLs using anti-CD8 antibodies in monkeys infected with SIV results in increased viral replication, whereas reintroduction of CTLs re-establishes control of viral load. Indeed, their critical role in viraemia control is evident in the strong selective pressure that continually drives HIV escape from CTL responses. **See also: Immune Evasion by Viruses**

By contrast, the contribution of CD4+ T cells to control viraemia is not as clear cut (McMichael, 2006). The maintenance of HIV-specific CD4 T cells correlates with improved CD8 T-cell responses. However, as activated CD4 T cells are a primary target of HIV infection, the presence of large numbers of activated CD4 T cells at the site of mucosal infections may be undesirable. Indeed, there has been speculation that the failed adenovirus vector-based HIV vaccine efficacy trials (STEP and Phambili trials, see Table 1) may have failed partly because of activated adenovirus-specific CD4 T cells at mucosal sites (Benlahrech *et al.*, 2009).

Cohorts of long-term survivors

Unfortunately, most HIV-infected patients fail to make adequate immune responses and eventually succumb to AIDS. Thus, finding correlates of protection in these individuals is problematic. However, a small number of patients, known as long-term non-progressors (LTNPs), have an unusually mild disease course and can control viral replication and maintain CD4+ T-cell counts for longer than 10 years. Studies on these cohorts are likely to reveal the factors, which include immune effectors, most suited to controlling HIV infection.

Investigations of LTNPs have indicated that T-cell effector function is a critical factor, rather than total HIV-specific T-cell

numbers (Pantaleo and Koup, 2004). **See also: An Overview of Cytokine Regulation of Inflammation and Immunity.** For example, CD4+ T cells in LTNPs appear to secrete a range of cytokines (termed 'polyfunctionality'), including interleukin 2 (IL-2) and interferon γ (IFN γ), whereas they tend to produce only IFN γ in patients that display normal disease course (Boaz *et al.*, 2002). Similarly, CD8+ CTLs appear to recognise multiple epitopes and produce IL-2 in individuals that display greater protection (Addo *et al.*, 2003; Betts *et al.*, 2006). Indeed, the presence of polyfunctional HIV-specific CD8+ CTLs in the mucosal-associated lymphoid tissue (MALT) is associated with slower HIV disease progression (Critchfield *et al.*, 2007).

Despite these promising initial observations, there is, as yet, no consensus as to the most critical factors in this small but interesting group of individuals. Apart from T-cell responses and the role of high-titre neutralising antibodies, many other factors will be involved, especially considering the heterogeneous nature of this population of patients. Nonetheless, the study of these individuals promises much for the future. Indeed, more homogeneous cohorts are continually being identified. For example, among LTNPs, there are individuals who maintain extremely low viral loads, often below the level of detection. These are known as Elite Controllers (ECs) (Bailey *et al.*, 2006; Betts *et al.*, 2006; Lambotte *et al.*, 2005), the preliminary characterisation of which has revealed strong HIV-specific CD4+ and CD8+ T-cell responses as well as Fc-functional antibody responses.

In summary, it appears that viraemia can be controlled through neutralising antibodies, Fc-functional antibodies and polyfunctional CD8+ T-cell responses. The fact that these exert a strong selective pressure on HIV is evident by the continual viral escape observed in most individuals, which is correlated with increased viral load and disease progression. Nonetheless, observations from LTNPs also indicate that these immune effectors can control virus levels for extended periods of time. This encourages vaccine developers, who aim to induce these potent immune responses before initial exposure, thus preventing HIV infection from taking hold.

Existing Vaccination Strategies for HIV

A wide suite of vaccines have been evaluated in clinical trials, six of which were phase III efficacy trials that have been completed (Table 1). HIV vaccine approaches have utilised various vaccine designs and strategies including using virus-like particles (VLPs), replication competent vectors, recombinant proteins, DNA vaccines, subunit vaccines and prime-boost vaccine combinations. A comprehensive review of these is not within the scope of this article, but the principles of a selected few are worth discussion.

Virus-like particles

VLPs are noninfectious viral particles without genomes. These core structures self-assemble and can display HIV Env or other immunogenic antigens on their surface. VLPs can be produced from many types of virus and a number of expression systems

such as baculovirus, vaccinia, adenovirus, yeast or directly from plasmids (Doan *et al.*, 2005). Once purified, VLPs are used directly for immunisation and have several advantageous characteristics; they are relatively immunogenic; they do not replicate or contain the HIV genome, so do not pose the safety risks of live attenuated viruses and they are treated *in vivo* by the immune system similar to natural virions.

Hepatitis B VLPs were shown to be taken up by dendritic cells resulting in antigen presentation to both CD4+ and CD8+ T cells, and the generation of neutralising antibodies similar to that observed for natural infection (Pumpens and Grens, 2001). Indeed, because HIV Env is presented on such particles as a trimeric protein that closely mimics native virus structure, effectively neutralising antibodies should be elicited. However, these vaccines have encountered two main problems; first they appear less immunogenic than replication competent vectors in humans. And second, the inclusion of enough VLPs from multiple strains to cover HIV variability has proved challenging and no VLP strategies are approaching efficacy trials.

Replication competent vectors

Unlike VLPs, replication competent vectors replicate *in vivo* and efficiently enter both the MHC-I and MHC-II presentation pathways in a similar manner to that seen for natural infection. Such vectors include those derived from vaccinia, modified vaccinia Ankara, avianpoxviruses such as canarypox and adenovirus. The latter appeared promising, as an HIV adenovirus serotype-5 (Ad5) vector proved highly immunogenic in nonhuman primates (Shiver *et al.*, 2002). However, although such vaccines do elicit T-cell responses in human volunteers, preexisting immunity to adenovirus, which causes the common cold, can significantly decrease immunogenicity (Barouch *et al.*, 2004). Indeed, the recent STEP vaccine trial that used this technology had to be prematurely halted. This phase III trial with over 3000 volunteers from the United States and South Africa, funded by the National Institute of Allergy and Infectious Diseases (NIAID) in conjunction with Merck, involved the Merck v250 vaccine of three injections of Ad5 carrying the HIV *gag/pol, nef* and *env* genes. Unfortunately, there was a trend towards more HIV infections in the vaccine group rather than protection. Those at greater risk had higher immunity to the Ad5 vector, although the reason why remains unclear. It has been suggested that CD4 T-cell responses to the adenovirus vector may have permitted a more robust initial infection (Benlahrech *et al.*, 2009). Subsequent analyses did show that the CTL responses induced by this Ad5 vector vaccine did impart immune pressure on the infecting strains as escape variants were selected for; however, this was insufficient to prevent or control infection (Rolland *et al.*, 2011). Adenovirus vectors generated from rarer subtypes or other primates, where antiadenovirus antibodies are not present commonly in humans, are currently being studied in early phase human trials (Clutton *et al.*, 2014).

A promising vector-based approach in recent years has been attenuated CMV vectors. Picker and colleagues have shown that these vectors can prevent disease in approximately 50% of macaques (Hansen *et al.*, 2011). The protection appears to be mediated by CTLs that are constantly highly activated ('effector memory' CTLs) so that they can control virus replication

very early and eventually eliminate it. Furthermore, the CTLs are in general not restricted by classical MHC-I molecules, overcoming some of the issues around the MCH I restriction of useful anti-HIV CTL responses (Hansen *et al.*, 2013). These vectors are entering early human trials, and the results are eagerly anticipated.

Recombinant proteins

The first AIDS vaccine trial, VaxGen's AIDSVax, found that immunisation with recombinant monomeric Env gp120 did not protect volunteers from subsequent HIV infection (Pitisuttithum *et al.*, 2006). Although neutralising antibodies were induced, they neutralised only the autologous virus. Nonetheless, vaccines using recombinant proteins are still the subject of intense investigation, particularly with novel 'scaffolding' approaches that utilise immunogenic proteins from bacteria to present immunogenic regions of HIV proteins in desired conformations. Similar to inactivated pathogens, recombinant protein and subunit vaccines usually require the correct choice of adjuvants to enhance appropriate immune responses. There is intense interest in mimicking the conserved sites on Env that bind neutralising antibodies. Some of these approaches are being tested in advanced mouse models at present (Sok *et al.*, 2016).

Plasmid DNA strategies

A promising vaccination strategy over the years has been the intramuscular injection of immunogen-encoding plasmid DNA (Kutzler and Weiner, 2008). Although not fully understood, this safe, cheap and stable method of immunisation mimics many of the characteristics of viral infection and induces good cell-mediated and humoral immune responses. The strategy is amenable to extensive manipulation to achieve desired outcomes. It is thought that muscle-resident dendritic cells take up the DNA and begin to express the encoded proteins in a physiologically relevant form. DNA-based vaccines are now undergoing clinical trials to combat diseases such as malaria, influenza and HIV/AIDS. However, although these studies have showed promise in mice, studies have indicated that the low level of expression in human cells may be problematic and advanced expression systems are likely to be required (Wyatt *et al.*, 2004). To this end, studies that use *in vivo* electroporation and other strategies to enhance uptake of the DNA and expression of the immunogenic material show greater promise (Mpendo *et al.*, 2015). As the DNA vectors generally only express the HIV antigens encoded and not other viral antigens (and thus antivector immunity is limited), DNA vectors are commonly used to prime the immune response in prime-boost combinations.

Prime-boost vaccine combinations

Combined modality HIV vaccines have been able to induce better immune responses than single modality vaccines alone. Common prime-boost combinations studied are DNA vaccine primes following by live vector boosts or alternatively live vector primes followed by Env protein boosts. A phase III trial of a DNA prime-adenovirus A Phase III vaccine trial in Thailand

(the RV144 trial) using a prime-boost strategy showed promise that a vaccine against HIV may be close (Rerks-Ngarm *et al.*, 2009). A live recombinant vaccine based on canarypox containing three HIV genes (*env*, *gag* and *pol*, Clade AE prevalent in Thailand) was used to prime the immune system followed by a boost with recombinant HIV Env gp120 subunits (Clade B and A/E) produced by VaxGen. The vaccines were studied in over 16 000 people in Thailand over 6 years, a remarkable effort. Protection was only moderate with approximately 31% less infections in the vaccinated group, which is not sufficient to pursue licensing the vaccine. Nonetheless, it provides a rationale that a more effective vaccine against HIV may in time be possible. Studies of the correlates of immunity in this trial were informative. The vaccine did not induce strong neutralising antibody responses nor CTL responses. Non-neutralising antibodies were strongly induced and serum IgG antibodies directed to the V1 and V2 loops of Env correlated with protection (Haynes *et al.*, 2012). Interestingly, strong IgA antibodies in serum were associated with worse protection. It has been suggested that IgA antibodies may be able to block IgG antibodies from binding Env and mediating innate immune cell activation through their Fc component (Tomaras and Haynes, 2014). Indeed, Fc-mediated function such as ADCC (antibody-dependent cellular cytotoxicity) may be a strong correlate of protection by this type of vaccine in the absence of neutralising antibodies and CTL responses. A subclass of IgG antibodies called IgG3, which mediates the strongest Fc-mediated functions, has also been implicated in the protection observed in the landmark RV144 trial (Chung *et al.*, 2014, 2015).

Hopes for the Future

Despite early promise that a vaccine for HIV would be an easily attainable target, initial attempts using conventional strategies proved disappointing. The problems in developing an effective vaccine are several. First, high-titre neutralising antibodies at early stages of infection coupled with prolonged and potent T cell-mediated immune responses are difficult to achieve with conventional vaccination strategies. Second, the high mutation rate of HIV allows immune evasion of even the best immune responses. And third, methods of inducing mucosal-directed immunity, which may best counteract HIV infection, are still being optimised. Nonetheless, recent studies of cohorts of long-term survivors are revealing the protective immune responses that must be mimicked by potential vaccine candidates. The landmark Thai RV144 trial showed that the protection of humans from HIV is possible and has strongly pointed to key immune responses that, in the absence of neutralising antibodies, can provide at least partial protection. New vaccine approaches have entered human efficacy trials (Table 1). In addition, evermore sophisticated vaccine vectors are being developed that aim to promote immunity over and beyond that seen for natural infection and directed to locations that represent likely routes of virus entry. The combination of these approaches should generate novel data in the coming years and provide real hope that the global HIV/AIDS epidemic can at last be defeated with the aid of vaccination.

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