Open access • Journal Article • DOI:10.1038/ICB.2010.118

Human immunodeficiency virus-1 vaccine design: where do we go now?

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Published on: 01 Mar 2011 - Immunology and Cell Biology (Blackwell Publishing Ltd)

Topics: AIDS Vaccines, SAIDS Vaccines, Vaccination, Viral vector and Immunity

Immunology and Cell Biology (2011) 89, 367–374

www.nature.com/icb

REVIEW

Human immunodeficiency virus-1 vaccine design: where do we go now?

Danushka K Wijesundara, Ronald J Jackson, Ian A Ramshaw and Charani Ranasinghe

Numerous human immunodeficiency virus (HIV)-1 vaccines have been developed over the last three decades, but to date an effective HIV-1 vaccine that can be used for prophylactic or therapeutic purposes in humans has not been identified. The failures and limited successes of HIV-1 vaccines have highlighted the gaps in our knowledge with regard to fundamental immunity against HIV-1 and have provided insights for vaccine strategies that may be implemented for designing more effective HIV-1 vaccines in the future. Recent studies have shown that robust mucosal immunity, high avidity and polyfunctional T cells, and broadly neutralizing antibodies are important factors governing the induction of protective immunity against HIV-1. Furthermore, optimization of vaccine delivery methods for DNA or live viral vector-based vaccines, elucidating the immune responses of individuals who remain resistant to HIV-1 infections and also understanding the core immune responses mediating protection against simian immunodeficiency viruses (SIV) and HIV-1 in animal models following vaccination, are key aspects to be regarded for designing more effective HIV-1 vaccines in the future.
Immunology and Cell Biology (2011) **89,** 367–374; doi:10.1038/icb.2010.118; published online 19 October 2010

Keywords: HIV-1 vaccines; mucosal immunity; prime–boost vaccination; T-cell avidity; cytokines/chemokines

OVERVIEW

Since the identification of human immunodeficiency virus-1 (HIV-1) as the causative agent of the acquired immunodeficiency syndrome (AIDS) in 1983,[1] numerous different vaccines against this virus have been developed, albeit with much failure in human clinical trials. Yet, the development of an HIV-1 vaccine that may prevent acquisition or reduce transmission of this virus is deemed to be of extreme importance to minimize the expansion of this epidemic, which currently accounts for over 33 million infected individuals and over 25 million deaths worldwide. In this article, we discuss what we believe needs to be done for the future of HIV-1 vaccine development with respect to the lessons we have learnt from vaccine strategies that have failed and succeeded in animal models as well as in human clinical trials.

ADAPTIVE IMMUNITY AGAINST HIV-1

The success of most vaccines depends on the induction of adaptive immune responses, which in the context of HIV-1 infections and numerous other viral infections are crucial for either preventing or limiting infections. The successful prevention of HIV-1 infections of target cells (for example, cluster of differentiation (CD)4^+ T cells, dendritic cells, macrophages, and so on) is mainly reliant on the availability of HIV-1-neutralizing antibodies (Figure 1). Neutralizing antibodies bind to the viral surface envelope (*env*) protein, which is critical for mediating viral entry into target cells. This binding usually prevents HIV-1 from infecting target cells. Although HIV-1-neutralizing antibodies are known to occur following natural infection with this virus, these antibodies are largely ineffective owing to the capacity of HIV-1 to mutate rapidly and conceal antibody epitopes by means of extensive glycosylation and conformational masking of the *env* protein.[2–6] Given that simian immunodeficiency virus (SIV) can also be transmitted as a cell-associated form,[7] HIV-1 may also be transmitted in a similar manner in which case-neutralizing antibodies may be ineffective. Nonetheless, numerous research groups have identified monoclonal antibodies that bind to conserved regions of HIV-1 *env* protein, which have the potential to neutralize a broad spectrum of HIV-1 quasi-species.[8–14] The discovery of these broadly neutralizing antibodies have generated much enthusiasm and hope for the development of an HIV-1 vaccine that may successfully provide sterilizing immunity against this virus (see below).

If antibodies fail to prevent HIV-1 infection, then effector $CD8^+$ T cells, also known as cytotoxic T lymphocytes (CTLs), play a crucial role in limiting further infections. CTLs recognize viral peptide-human leukocyte antigen class I complexes presented on the surface of virus-infected cells, which triggers the cytolytic machinery of CTLs and apoptosis of virus-infected cells (Figure 1). Lysis of infected cells results in the release of immature virions, which are rapidly degraded in the extracellular milieu.[15] CTLs in HIV-1 infections as well as in SIV infections appear to play a crucial role in limiting replication of these viruses.[16–18] Individuals who remain resistant to HIV-1 infections, also

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Received 10 September 2010; accepted 13 September 2010; published online 19 October 2010

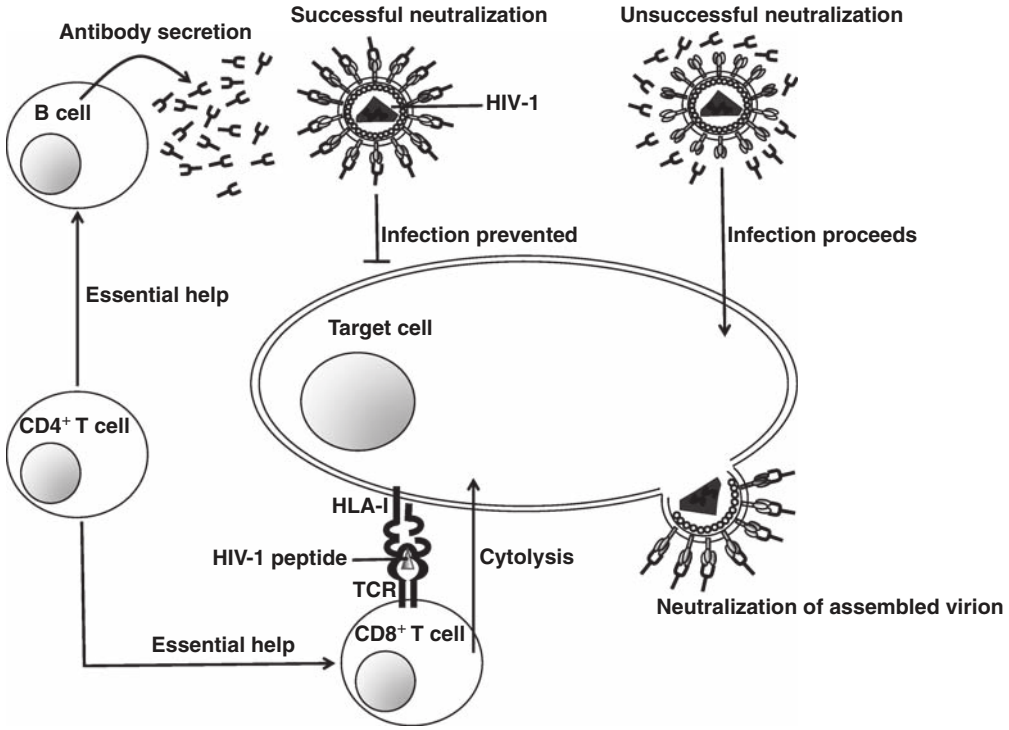

Figure 1 Key operations of adaptive immunity against HIV-1. $CD4^+$ T cells, $CD8^+$ T cells and B cells have all been known to play an important role in either preventing or limiting HIV-1 infection. B cells secrete antibodies with the potential to neutralize this virus. Successful neutralization requires antibodies to bind to the protruding viral envelope glycoproteins that are crucial for mediating viral entry into target cells. Failure to do so allows HIV-1 to infect target cells. Further infections with HIV-1 can be prevented or limited either via antibodies that can effectively neutralize newly assembled virions or via effector $CD8^+$ T cells that can mediate cytolysis of infected cells following T-cell receptor (TCR)-mediated recognition of HIV-1 peptide-human leukocyte antigen class I complexes. Effector $CD4^+$ T cells provide help for efficient mobilization of B-cell and $CD8^+$ T-cell-mediated immune responses against HIV-1.

referred to as elite controllers of HIV-1, despite being exposed to this virus exhibit enhanced HIV-specific CTL activity compared with more susceptible individuals.[19–22] It is believed that CTL activity may be sufficient to reduce HIV-1 viral set point to an extent (<1000 viral RNA copies per ml blood) that HIV-1 is no longer transmissible between individuals.[23–24] The effectiveness of CTLs in suppressing HIV-1 replication is primarily owing to the ability of CTLs to recognize HIV-1 epitopes (for example, HIV-1 *gag* protein) that are conserved within an HIV-1 subtype and across various different HIV-1 subtypes.[23] However, unlike antibodies CTLs do not have the potential to prevent HIV-1 infection of cells. Studies conducted in non-human primates suggest that viral escape of CTL recognition is also a concern with regard to CTL-mediated immunity against HIV-1 given that it has already been shown that SIV can mutate its immunodominant epitopes to escape CTL recognition.[25–26]

$CD4^+$ T cells are primary targets of HIV-1 infection and HIV-1-specific $CD4^+$ T cells appear to be particularly susceptible for HIV-1 infection.[27] Despite this, $CD4^+$ T-cell activation may yet be more favourable than detrimental to the HIV-1-infected host.[28] This is especially true given that $CD4^+$ T cells appear to provide essential help (for example, interleukin (IL)-21 secretion, sustaining CTL cytotoxicity and CTL mucosal homing) to CTLs responding against HIV-1.[29–30] The requirement of $CD4^+$ T cells for combating HIV-1 is still a controversial issue given that the presence of $CD4^+$ T cells at sites of HIV-1 infections may fuel virus replication, but $CD4^+$ T-cell help is known to be required for optimizing B-cell and CTL effector functions following infection with numerous pathogens including HIV-1.

DESIGNING AN EFFECTIVE NEUTRALIZING ANTIBODY-BASED VACCINE AGAINST HIV-1

Neutralizing antibodies against HIV-1 bind to the *env* glycoproteins of the virus to prevent viral entry into cells as well as prevent the release of viral progeny from infected cells (Figure 1). Historically in experimental animal models, the presence of virus-neutralizing antibodies has been shown to correlate with protection against HIV and simian-human immunodeficiency virus (SHIV) infections in chimpanzees and macaques, respectively, which had prompted vaccinologists to design vaccines that have the potential to induce neutralizing antibodies against HIV-1 in humans.[31] For this purpose, numerous DNA vaccine vectors encoding recombinant *env* glycoprotein120 (gp120) have been tested in human clinical trials, but these vaccines were shown to have poor or modest efficacy in preventing HIV-1 infections.[32–34] This may primarily be attributed to the inability of these vaccines or vaccine strategies to induce high titres of neutralizing antibodies that can recognize the conserved CD4 binding region of the *env* gp120. Most antibodies in the course of a natural HIV-1 infection fail to mediate optimal virus neutralization owing to extensive glycosylation of neutralizing epitopes, resulting in epitope masking and owing to antigenic diversity of the *env* glycoproteins, resulting from the high error rate of the virus reverse transcriptase.[2] The presence of 12 different clades of HIV-1 with approximately 30% clade diversity (based on the amino-acid sequence) between the *env* proteins of the different clades has also made it difficult to develop a universal preventative HIV-1 vaccine.[2]

To circumvent these problems, numerous groups have attempted to characterize neutralizing antibodies with the ability to recognize *env*

epitopes conserved between HIV-1 subtypes and have broad neutralization capacity against the different circulating strains of HIV-1. Antibodies with the ability to cross-neutralize different subtypes of HIV-1 have been described.[8,10,13,14,35–39] Among these antibodies, VRC01 is reported to exhibit the most potent cross-neutralization capacity thus far with the ability to neutralize 90% of the currently circulating strains of HIV-1.[8] The salient features of VRC01 that makes it an attractive candidate for its implementation in a preventative HIV vaccine are: it has the ability to access and bind the conserved CD4 binding region of the *env* gp120; the nascent precursor of VRC01 is thought to be present at a reasonable frequency in humans and is not subject to clonal deletion unlike other broadly neutralizing antibodies (for example, 2G12, 2F5 and 4E10); and it is also able to tolerate multiple changes to its genomic sequence without significantly affecting its *env* gp120 neutralization capacity.[8]

Although the discovery of VRC01 is a landmark finding in HIV-1 research, immunogens that can efficiently elicit the production of this antibody and other broadly neutralizing antibodies have to be identified in a vaccine context. Given that many broadly neutralizing antibodies against HIV-1 undergo extensive affinity maturation and develop only after ~20–30 months post-infection,[40] it will be a difficult task to accomplish. Researchers must therefore strive to characterize other broadly neutralizing antibodies and immunogens that may elicit the production of such antibodies. This could be useful for two reasons. First, there may yet be broadly neutralizing antibodies that are easier to elicit than VRC01 in a vaccine context and/or have a better cross-neutralization capacity than VRC01. Second, incorporation of multiple immunogens that may induce the production of multiple broadly neutralizing antibodies following vaccination against HIV-1 may not only enhance the protective capacity, but also the breadth of neutralization across different HIV-1 subtypes. The use of the recently described high-throughput methods (that is, flow cytometry and single-cell polymerase chain reactions) have been thought to allow for the characterization of novel broadly neutralizing antibodies against HIV-1 within the next few years.[2] Despite the enormity of these challenges and given the failures of using recombinant env gp120 encoding vaccine vectors, development of vaccines that elicit broadly neutralizing antibodies appear to be the way forward in developing an effective preventative HIV-1 vaccine.

THE IMPORTANCE OF T-CELL AVIDITY (QUALITY) IN PROTECTION AGAINST HIV-1

Given the difficulties associated with vaccine-based induction of protective neutralizing antibody responses against HIV-1, many researchers have focused their attention in T-cell-based vaccines against HIV-1. As mentioned previously, T-cell immunity plays an integral role in controlling HIV-1 replication and in the ability of elite controllers to regularly resist infection with this virus. A correlate of T-cell mediated protection against HIV-1 has not yet been defined, but it appears that 'magnitude' or the number of interferon (IFN)-γ-producing HIV-specific T cells is clearly not an effective measure of protective immunity.[41] Studies conducted in animal models as well as in patients infected with HIV-1 suggest that T-cell quality is an important parameter in defining correlates of T-cell-mediated protection against HIV-1.[40,42–44] In the recent years, avidity, rates of clonal turnover and the ability of T cells to secrete multiple (polyfunctional) anti-viral cytokines (for example, IFN-γ, IL-2 and tumour necrosis factor (TNF)) have been identified as important factors in defining the quality of a T-cell response against HIV-1.[45]

T-cell avidity is defined as the ability of T cells to respond to a given peptide-major histocompatibility complex. High-avidity $CD8^+$ T cells can better control viral replication and require lower amounts of cognate peptide-major histocompatibility complex for triggering effector functions than low-avidity $CD8^+$ T cells in response to viral infections *in vivo*.[46–47] High-avidity HIV-specific $CD8^+$ T cells are polyfunctional in nature and have high clonal turnover rates.[42] The presence of high-avidity HIV-specific $CD8^+$ T cells have been shown to correlate with enhanced ability of HIV-1-infected patients to control HIV-1 replication.[42] The importance of high-avidity HIV-specific $CD8^+$ T cells is also highlighted by the fact that the loss of high-avidity HIV-specific $CD8^+$ T cells is a clinical feature of HIV-1 disease progression to chronic phase infections in HIV-1-infected individuals.[44] Furthermore, the presence of high-avidity SIV-specific $CD8^+$ T cells have also been shown to enhance the capacity of non-human primates to more efficiently control SIV replication and delay the establishment of peak viraemia.[43]

Given the importance of T-cell avidity for controlling HIV-1 replication and disease progression, there is a great need to understand the determinants that may influence T-cell avidity in order to develop HIV-1 vaccines that may induce the production of optimal numbers of high-avidity HIV-specific T cells. So far, immunization route, cytokine milieu, immunodominance and antigen density have been identified as key determinants that may influence avidity of $CD8^+$ T cells.[41,43,48–50] As will be discussed later, route of delivery of vaccines may significantly contribute to T-cell avidity with vaccines delivered into the mucosa (for example, intrarectal and intranasal immunisation) having better efficacy in enhancing the development of high-avidity antigen-specific $CD8^+$ T cells than delivery of vaccines into systemic compartments.[41,43] The development of high-avidity antigen-specific $CD8^+$ T cells is also dependent on the presentation of immunodominant peptides by dendritic cells during T-cell priming.[49] $CD8^+$ T cells that respond to immunodominant epitopes are characteristically of high avidity.[49] Although this would suggest that incorporation of HIV-1-immunodominant epitopes in vaccines is a strategy for generating optimal numbers of high-avidity HIV-specific T cells, the caveat in this instance is that HIV-1 escape variants may evolve that can potentially abrogate the efficacy of the vaccine-induced T-cell immunity. Therefore, it is important to characterize immunodominant epitopes that when mutated detrimentally affect viral fitness; as such epitopes will be most useful for vaccination purposes given that these epitopes can be used to target a broader range of HIV-1 isolates. The density or quantity of antigen presented during T-cell priming can significantly impact $CD8^+$ T-cell avidity. For instance, presentation of lower antigen densities during T-cell priming facilitate the development of high-avidity antigen-specific memory $CD8^+$ T cells, which suggests that there is an inverse correlation between $CD8^+$ T-cell avidity and antigen density during T-cell priming.[50] Ideally, a vaccine will express low levels of the T-cell immunogen of interest, but the expression of this immunogen will also need to be sufficient to facilitate the development of substantial number of antigen-specific T cells.

The ability of T cells to secrete/produce certain cytokines also appears to have an influence on $CD8^+$ T-cell avidity. T cells have the potential to secrete multiple type-1, type-2 or both type-1 and type-2 cytokines during differentiation.[51] Hence, it has been difficult to identify certain subsets of T cells that may be protective against HIV-1 with respect to cytokine production. We have found that HIV-specific $CD8^+$ T cells that produce type-2 cytokines such as IL-4 and IL-13 are of low avidity and lower production of these cytokines correlates with enhancement of $CD8^+$ T-cell avidity.[48] Others have found that $CD8^+$ T cells that produce type-1 cytokines such as IL-2 are of higher avidity.[52–53] It also appears that high-avidity T cells have

the capacity to secrete multiple type-1 cytokines. We have found that high-avidity HIV-specific $CD8^+$ T cells secrete both TNF-α and IFN-γ (Ranasinghe *et al.*, manuscript in preparation). Using multi-parameter flow cytometry, Almeida *et al.*[42] have shown that high-avidity HIV-specific $CD8^+$ T cells in HIV-1-infected patients produce IFN-γ, TNF-α, CD107a and macrophage inflammatory protein-1β. This was also found to correlate with efficient control of HIV-1 replication in these patients. Similar findings have been observed with respect to SIV infections in non-human primates. Hansen *et al.*[54] have shown that vaccine vectors that elicit SIV-specific $CD4^+$ and $CD8^+$ T cells that produce TNF-α and IFN-γ among other factors (for example, CD107) contribute to resistance against SIV infections in rhesus macaques. Collectively, it appears that T cells' capacity to secrete at least IFN-γ and TNF-α is likely to define high-avidity T cells and a correlate of T-cell-mediated protection against HIV-1, but this has not yet been thoroughly confirmed with respect to HIV-1 infections.

RECOMBINANT DNA OR LIVE VIRUS VECTOR-BASED VACCINE STRATEGIES AGAINST HIV-1

The pitfalls

DNA vaccines emerged in the early 1990s and were first tested in humans for vaccination against HIV-1.[55] DNA vaccines became popular, as they possessed numerous advantages over other vaccines. The main advantages of DNA vaccines were that they were cheap and easy to produce and allowed for a prolonged immune response to develop against encoded antigens in vaccinated hosts. However, vaccination based solely on the use of DNA vaccines have failed to induce robust immune responses against HIV-1 in humans, which has mainly been attributed to the low dose of vaccine used for vaccination (that is, need repeated immunizations with over 5–10 mg of recombinant DNA per immunization to induce robust immunity in vaccinated host) or insufficient uptake of DNA vectors by cells in vaccinated hosts.[56]

The range of live viral vectors such as avian poxvirus (for example, fowlpox virus and canarypox virus), mammalian poxvirus vectors (for example, modified vaccinia Ankara) and adenovirus virus vectors (for example, adenovirus serotype 5 (Ad5)) have been used for vaccination against HIV-1 in humans (see www.iavi.org). The incorporation of HIV antigens to live viral vectors allows for high-level expression of HIV antigens *in vivo* owing to the ability of these viruses to replicate in infected cells. Live virus vectors can efficiently potentiate immune responses against encoded antigens owing to their ability to infect immature dendritic cells and stimulate innate immune responses.[57] Despite these attractive features, the use of a recombinant live viral vector as a stand-alone vaccine against HIV-1 in humans have been met with failure and sometimes puzzling outcomes, which was highlighted in the recently concluded Step vaccine trial.

The Step vaccine trial tested the ability of a recombinant Ad5 vector encoding HIV-1 *gag*, *pol* and *nef* proteins to mediate protection against HIV-1.[58] This trial was mainly prompted from the success of using a recombinant Ad5 vector encoding SIV *gag* to induce protective SIV-specific $CD8^+$ T-cell responses against SHIV in rhesus macaques.[59] In the Step trial, individuals receiving the recombinant Ad5 vaccine were found to be more susceptible to HIV infection than individuals receiving the placebo vaccine. The presence of pre-existing immunity (antibodies) against Ad5 appeared to have enhanced the susceptibility of vaccinated individuals to HIV-1, but immunologically irrelevant factors such as being uncircumcized were also found to enhance the susceptibility of vaccinated individuals to HIV-1 in these trials. Hence, the Step trial raised more questions than answers with regard to the safety and efficacy of using live viral vectors (for example, Ad5) for vaccination against HIV-1.

Heterologous prime–boost vaccine strategies against HIV-1

Heterologous prime–boost vaccine strategies against HIV-1 have also been implemented to counter the shortcomings of using either a DNA vector or a live viral vector as a stand-alone vaccine against HIV-1. Since its inception in 1997,[60] heterologous prime–boost vaccination has been implemented successfully in numerous studies with animal models to induce robust immune responses against HIV and SIV antigens with protective effects in some instances.[43,48,61–62] During heterologous prime–boost immunization, antigens of interest are encoded in a recombinant DNA or live viral vector to be used for priming the immune system and then boosting is carried out using a second recombinant vector encoding the same antigen to amplify the number of antigen-specific lymphocytes.[56] The caveat in using live viral vectors in this instance is not to use genetically related viral vectors for the prime and the boost in order to minimize the development of anti-vector immunity.[56]

Heterologous prime–boost vaccination against HIV-1 was used in the recently concluded human HIV-1 clinical trial in Thailand, which was the only vaccination strategy reported thus far to provide some protective effect (vaccine efficacy of 31.2%) against HIV-1 in humans.[34] Trial participants in this instance were given priming injections with ALVAC-HIV (recombinant canarypox vector vaccine) and booster injections with AIDSVAX B/E (recombinant gp120 subunit vaccine).[34] Prime–boost strategy was used in this trial owing to the failure of these vaccine vectors when used alone for vaccination against HIV-1 to confer protective effects in previous HIV-1 human clinical trials.[31–32] The observed protective effect or vaccine efficacy in this trial was significantly higher in individuals deemed to be of low/medium risk compared with individuals being in high risk of exposure to HIV-1.[34] Hence, the overall vaccine efficacy in this instance appears to be more reflective of the low/medium-risk groups. The vaccines used in this trial induced extremely low $CD8^+$ T-cell responses among the vaccinated individuals,[34] which may have contributed to the inability of the vaccinated individuals, who were infected with HIV-1 following vaccination, to efficiently control HIV-1 viraemia.

The fact that a protective effect was observed in this trial showed promise that optimization of prime–boost vaccine strategy can be more effective than using recombinant HIV-1 vaccine vectors alone in a none prime–boost manner for vaccination against HIV-1 in humans. We have identified certain key elements in mouse models with respect to the use of live virus vectors (fowlpox and vaccinia viruses) for heterologous prime–boost vaccination against HIV-1, which when optimized could be used to generate optimal high-quality (that is, avidity) $CD8^+$ T-cell responses against HIV-1.

The dose and order of the vaccine vectors used for priming and boosting plays a significant role in the number of responding antigen-specific $CD8^+$ T cells that develop against HIV-1. For instance, priming with recombinant fowlpox virus encoding HIV antigens and boosting with recombinant vaccinia virus encoding the same HIV antigens yielded more HIV-specific IFN-γ-secreting $CD8^+$ T cells than priming with recombinant vaccinia virus encoding HIV antigens and boosting with recombinant fowlpox virus encoding HIV antigens.[63] The route of vaccine delivery used for heterologous prime–boost vaccination against HIV-1 significantly influences the quality (that is, avidity) and the magnitude of the response that develops against HIV-1.[41] We have found that mucosal (intranasal) delivery as opposed to systemic (intramuscular) delivery of vaccine vectors

against HIV-1 significantly enhances the number of high-avidity HIV-specific $CD8^+$ T cells that develop.[41] Belyakov *et al.*[43] also have reported that mucosal (intrarectal vaccine delivery) prime–boost vaccination against SHIV in rhesus macaques enhances the avidity of SHIV-specific $CD8^+$ T cells. However, we have also found that the magnitude of the HIV-specific $CD8^+$ T cells that develop following purely mucosal immunization against HIV-1 is far less than the magnitude of HIV-specific $CD8^+$ T cells that develop following purely systemic immunization against HIV-1.[41] This effect was related to the cytokine milieu induced following prime–boost vaccination against HIV-1.[48] Systemic immunization against HIV-1 appeared to enhance the *in vivo* IL-4 and IL-13 production by $CD8^+$ T cells compared with mucosal immunization against HIV-1.[48] IL-4 and/or IL-13 were also found to function directly in reducing avidity of HIV-specific $CD8^+$ T cells.[48] Finally, co-expression of molecular adjuvants together with vaccine antigens such as chemokine (c–c– motif) ligand 5 (CCL5), CD40 ligand, IL-15, IL-12, IFN-α/β and toll-like receptor ligands (for example, CpG motifs) can also be used to enhance the number and survival of lymphocytes responding against a given pathogen including HIV-1.[25,45,64–67] However, we have found that many of these molecular adjuvants are unable to enhance the avidity of $CD8^+$ T cells (C Ranasinghe and IA Ramshaw, unpublished observations).

Summary of the lessons learnt from using recombinant DNA or live virus vector-based vaccine strategies against HIV-1

Overall, the failures of DNA or live virus vector-based vaccine approaches tested against HIV-1 in human clinical trials have highlighted certain key issues that need to be overcome for developing better recombinant DNA or live virus vector-based vaccine approaches against HIV-1. The repeated failures of using stand-alone DNA or live virus vector-based vaccine approaches against HIV-1 would suggest that more focus needs to be given to optimizing prime–boost vaccine strategies against HIV-1, especially given the recent success of using a prime–boost vaccination strategy against HIV-1 in Thailand. However, it will be of great benefit for researchers to identify the factors that have contributed to the failures of using stand-alone DNA or live virus vector vaccines against HIV-1. For instance, the factors that contributed to the failure of the Step trial is currently not known and will need to be evaluated for developing safer and more effective live viral vector vaccines against HIV-1 in the future. More studies need to be conducted to evaluate the safety and efficacy of using viral vectors for priming and boosting against HIV-1, rather than focusing too heavily on the conventional DNA vector prime/live viral vector boost strategies. We have found that the use of heterologous viral vectors encoding HIV antigens in a prime–boost manner can be used to generate robust and high-avidity HIV-specific $CD8^+$ T-cell responses in mouse models.[41,48] Numerous DNA or live virus vector-based vaccine approaches against HIV-1 in human clinical trials have been successful in inducing neutralizing antibody and HIV-specific $CD8^+$ T-cell responses against HIV-1, but these responses have been inefficient in preventing HIV infections or controlling HIV-1 viraemia in vaccine recipients. This highlights our lack of knowledge with regard to the correlates of protection against HIV-1 and also the inability of the immunological assays (for example, antibody neutralization assays, IFN-γ enzyme-linked immunospot assays, and so on) used to assess HIV-1 vaccine efficacy to reliably predict the protective capacity of the vaccines already tested in humans. Hence, alternative immunological assays (for example, CTL assays) need to be developed and used in future human clinical trials. The use of SIV and SHIV challenge systems in non-human primates also require further validation given that they have not been able to correctly predict the protective capacity of HIV-1 vaccines tested in humans (for example, the Step trial). Lastly, as discussed above, we believe that researchers should devote more efforts into optimization of prime–boost vaccination regimens against HIV-1 before conducting further HIV-1 clinical trials in humans.

IMPORTANCE OF MUCOSAL IMMUNITY AND MUCOSAL DELIVERY OF VACCINES AGAINST HIV-1

All HIV-1 prophylactic vaccines that have been tested in human clinical trials have been delivered using the systemic route (for example, intramuscular), but they have been associated with poor outcomes. In most instances, host immune responses initially encounter HIV-1 at mucosal surfaces given that most incidences of HIV-1 transmission occur during sexual exposure of the genitorectal mucosa. HIV-1 replication and $CD4^+$ T-cell depletion resulting from HIV-1 infection of $CD4^+$ T cells appear to be most prolific in the gut mucosa.[68] This results in the disruption of the gut mucosa causing the release of microbial products from the gastrointestinal tract that induces chronic activation of immune responses.[69] Therefore, the presence of mucosal immune responses against HIV-1 before infection is believed to be crucial for enhancing the ability of an individual to limit systemic dissemination of this virus and chronic phase disease progression.

Following transmission, most ($\sim$80%) primary HIV-1 infections appear to occur by a single virus, also referred to as the founder virus.[70] The establishment of a pool of virus quasi-species is believed to occur much later following founder virus infections.[40] Hence, the chances of controlling HIV-1 infections may be greatly enhanced if an HIV-1 vaccine successfully induces robust and high-quality (that is, avidity) immune responses against this virus at mucosal surfaces early on following initial exposure. Others and we have found that mucosal delivery (for example, intranasal, intrarectal and intradermal) of vaccine vectors can significantly enhance the avidity of $CD8^+$ T cells and protective efficacy against HIV-1 as well as against SIV and SHIV.[43–44,48,62,71] Although not yet clearly shown, vaccines that can induce the production of HIV-1-neutralizing antibodies at mucosal surfaces may potently prevent HIV-1 infections, especially given that there is a greater chance that these antibodies mediate effective neutralization of the founder virus, rather than the various virus quasi-species that arise later on in infection.

In some studies, systemic delivery of HIV-1 vaccines have been known to induce HIV-specific immune responses at the mucosa and mediate protection in animal models,[72–73] but our findings in mouse models suggest that systemic delivery of HIV-1 vaccines can compromise the quality or avidity of the HIV-specific immune responses depending on the cytokine milieu induced following vaccination.[48] Our studies have shown that combined mucosal prime/systemic boost delivery of HIV-1 vaccines has the potential of inducing robust and high-quality (that is, avidity) mucosal immunity at local as well as distal mucosa.[41] Mucosal delivery of HIV-1 vaccines may also better mimic natural route of HIV-1 infection than systemic delivery of HIV-1 vaccines given that HIV-1 transmission naturally occurs via the mucosa. Mucosal delivery of vaccines that mimic the natural route of infection have been reported to induce protective immune responses against viruses such as influenza virus infections.[74–75] Collectively, these studies show that mucosal vaccines exhibit strong potential in inducing protective immune responses, but mucosal delivery (that is, oral or nasal) of HIV-1 vaccines have not yet been evaluated for safety and efficacy in human clinical trials. Furthermore, the effectiveness of mucosal immune responses cannot be evaluated using immune measurements in the blood or systemic compartments, and hence

there is an urgent need to develop novel biomarkers that may effectively measure mucosal immune responses in humans following HIV-1 vaccination.

LIVE ATTENUATED SIV VACCINES AND THERAPEUTIC VACCINES AGAINST HIV-1

The use of live attenuated SIV vaccines has been extremely successful in protecting non-human primates against homologous SIV challenge.[76] Live attenuated SIV strains have been created through deletion of SIV genes (that is, deletion of *nef*), deletion of certain regions of SIV genes (that is, variable (V)1–V2 region of *env*) and through the treatment of anti-retroviral drugs immediately following administration of wild-type SIV.[76–79] The mechanisms by which live attenuated SIV vaccines mediate protection is not known, but a recent study has shown that intrarectal immunization of macaques with *nef* gene deleted SIV (mac239 strain) induces robust anti-viral mucosal immunity.[80] This study using *ex vivo* viral replication assays showed that mucosal (lung) $CD8^+$ T cells rather than systemic (peripheral blood mononuclear cells) $CD8^+$ T cells were significantly more efficient in suppressing SIV replication. This further highlights the importance of generating mucosal immune responses from vaccines against HIV-1. Although application of live attenuated HIV-1 vaccines in humans may not be practical owing to the obvious risks involved, more studies examining the mechanisms as to why live attenuated SIV vaccines are protective will shed light into developing more effective HIV-1 vaccines.

Although currently the use of anti-retroviral drugs remains the most successful treatment for reducing HIV-1 virus levels and prolonging survival of HIV-1-infected individuals, the use of therapeutic vaccines to augment the beneficial effects of anti-retroviral therapy have received considerable attention. Given that over 33 million people are infected with HIV-1 globally, the development of effective therapeutic vaccines that may achieve this purpose is extremely beneficial. In a recent report, Schooley *et al.*[81] have shown that HIV-1-infected individuals undergoing anti-retroviral therapy after vaccination with a recombinant replication-deficient Ad5 vector-expressing *gag* had a modest reduction in HIV-1 viral levels compared with individuals undergoing anti-retroviral therapy following placebo vaccination. The vaccine recipients in this instance were also shown to have augmented anti-*gag* T-cell responses compared with placebo recipients. The use of recombinant fowlpox virus co-expressing HIV *gag*/*pol* and IFN-γ for vaccination of HIV-1-infected individuals in Australia have yielded similar results with vaccine recipients having a log reduction in HIV-1 virus levels compared with placebo recipients over the course of the study.[82] However, this study was based on a small sample size (n=25) and the results of this study will require further validation from a larger population of HIV-1-infected individuals. These studies highlight promising outcomes for application of therapeutic vaccines for suppressing HIV-1 disease progression in HIV-1-infected individuals.

CONCLUDING REMARKS

The search for the elusive HIV-1 vaccine has been met with much disappointment for nearly three decades, but the recent success of the human HIV-1 clinical trials in Thailand offers optimism that a protective HIV-1 vaccine is not in the realms of impossibility. The pitfalls and failures of HIV-1 vaccines tested in human clinical trials have also taught researchers some valuable lessons with regard to the gaps in the knowledge of protective immunity against HIV-1 and also vaccine approaches that may be more effective in future human clinical trials. In Table 1, we have summarized some key research areas that were discussed throughout this article and that need further investigation for designing more efficacious vaccines against HIV-1. We believe that developing a thorough understanding of the factors highlighted in Table 1 will facilitate the design of more effective HIV-1 vaccines in the future.

Table 1 Summary of key factors that need further investigation in HIV-1 research for the design of more effective HIV-1 vaccines in the future

Research focus	*Required future research*
Immunity against HIV-1	Identify immune correlates of protection against HIV-1 that may be used to reliably predict HIV-1 vaccine efficacy in humans and animal models Define immune mechanisms that allow elite controllers of HIV-1 infection to effectively resist HIV-1 infections
Antibody-based HIV-1 vaccines	Characterize more broadly neutralizing antibodies that would occur in the course of natural infection against HIV-1 Characterize and test immunogens that may elicit the production of broadly neutralizing antibodies in a vaccine context
DNA or live viral vector HIV-1 vaccines	Clearly understand the factors that have contributed to the failures of these vaccines in humans (for example, the Step trial) Identify strategies to generate optimal high-quality (for example, avidity) immunity when using prime-boost vaccines against HIV-1 (for example, using mucosal immunization route as opposed to systemic immunization route for vaccination)
Mucosal HIV-1 vaccines	Test mucosal delivery of candidate HIV-1 vaccines in human clinical trials for effectiveness and safety Identify effective methods that can be used to measure mucosal immune responses in humans following vaccination against HIV-1 Identify novel biomarkers to measure mucosal immune responses
Therapeutic HIV-1 vaccines	Develop and test more therapeutic HIV-1 vaccines that may alleviate HIV-1 disease progression in infected patients when used alone or in conjunction with anti-retroviral therapy
Live attenuated SIV vaccines	Define the mechanisms that have successfully allowed these vaccines to induce protective immune responses against SIV

Abbreviations: HIV-1, human immunodeficiency virus; SIV, simian immunodeficiency viruses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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