Therapeutic vaccination against HIV: current progress and future possibilities

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ABSTRACT

Although effective in reducing mortality, current antiretroviral therapy for HIV infection involves complex and expensive drug regimens that are toxic and difficult to take. Eradication of HIV reservoirs is not possible with existing therapies. The concept of therapeutic vaccination has been investigated to increase the potency and breadth of anti-HIV immune responses in order to delay or reduce antiretroviral therapy use. A variety of approaches targeted to both cell- and antibody-mediated immunity have been developed, including whole inactivated HIV-1, protein subunits and synthetic peptides, DNA vaccines and a number of viral vectors expressing HIV-1. These investigations have occurred in the absence of a clear understanding of disease pathogenesis or the correlates of protective immunity. At this time, there is no licensed therapeutic vaccine for any viral disease, including HIV; however, this review will consider recent progress in the field and summarize the challenges faced in the development of a therapeutic HIV vaccine.

INTRODUCTION

Current treatment for HIV infection relies upon long-term chronic use of HAART [highly active ART (antiretroviral therapy)] [1]. Although effective in controlling HIV replication and delaying HIV-related morbidity and mortality in infected individuals, these regimens are not without cost [2]. Effective use requires strict adherence to often complex regimens that significantly impact upon daily life through special dietary and scheduling requirements. Even minor breakdowns in adherence are likely to result in the development of drug-resistant HIV that can have a negative impact upon the efficacy of sequential ARTs (antiretroviral therapies) [3].

In addition, it is now clear that even short-term use of these products is associated with significant increases in plasma lipids, glucose intolerance, bone mineral metabolism and alterations in body shape, and together these are known as the ‘HIV-associated lipodystrophy syndrome’ [4–8]. Over the long term, the first two of these changes may increase an individual’s risk of cardiovascular disease, diabetes and fracture [9], although the latter can be stigmatizing for the patient with a subsequent impact upon adherence [7]. At the other end of the spectrum, many infected individuals around the world are unable to access ART due to resource constraints. Improvements in some regimens, such as fixed dose combinations and once-daily dosing, will only be of benefit for a fraction of the total time that an individual is treated. Alternative treatment strategies that might reduce or delay the need for complex, toxic and expensive ART are required for long-term management of HIV infection. As a result, the concept of therapeutic vaccination has been investigated throughout the past two decades. This review...
will consider recent progress in the field and summarize the challenges faced in the development of a therapeutic HIV vaccine.

**CHALLENGES FOR THERAPEUTIC VACCINATION**

The ultimate goal of a therapeutic vaccine would be to generate sufficient anti-HIV immune responses to allow the infection to be completely cleared either alone or in combination with other novel therapies [10–13]. However, a more attainable, but nonetheless meaningful, objective may be to generate sufficient immune responses to allow individuals to cease using antiretroviral drugs or to defer their use significantly [14].

The concept of prophylactic vaccination involves stimulation of the immune system with exogenous antigen to induce immune responses to protect from a particular infection. However, HIV-infected individuals have already developed an immune response that has failed to prevent infection and also fails to prevent subsequent infection with a different strain [15,16]. Therapeutic vaccination assumes that the natural history of disease can be modified by enhancing existing or generating new anti-HIV immune responses. There is much circulating antigen in peripheral blood of an infected individual [17] and, therefore, it seems unlikely that the amount of antigen is insufficient for induction of effective immune responses. Presentation of exogenous antigen may either boost an existing immune response or induce a qualitatively different immune response from that induced by natural infection. These modified responses are hypothesized further to result in better viral control and therefore slow disease progression [18,19].

**Cell-mediated immunity in HIV infection**

Despite extensive efforts, the correlates of viral control in HIV disease are still not fully understood, although current dogma proposes that cell-mediated immunity plays a significant, if not central, role in the control of viral replication [20–24] (Figure 1). The onset of HIV-specific CD8+ T-cell responses at primary infection correlates with viral control [25,26], and depletion of these cells from SIV (simian immunodeficiency virus)-infected macaques either at primary infection or early chronic infection results in increased viral load and disease progression [27,28]. Despite vigorous CD8+ CTL (cytotoxic T-cell) responses directed at the virus, the majority of individuals fail to clear the infection. The selective pressure exerted by these CTLs forces viral-escape mutations in HIV [20,29–35], including escape through epitope mutation in which the virus varies its sequence at epitopes to avoid the CD8+ T-cell response complexes [36–40].

CD8+ T-cell-mediated epitope-specific anti-HIV responses can be detected prior to and even after disease progression [41]. Despite this apparently robust CTL response, the overwhelming majority of infected individuals progress to AIDS if left untreated, although viral control in certain long-term non-progressors appears to correlate with the presence of CD8+ T-cells with retained proliferative capacity [24,42,43]. In more detailed analyses, the accepted measures of CD8+ T-cell responses have failed to show either a correlation with disease state or disease progression [41], and it has been suggested that the quality and breadth of CD8+ T-cell immune response is as important as the magnitude. Defects of CD8+ T-cell function have been described in HIV-infected individuals [44–47], and the antigen-specific CD8+ T-cell response is skewed to the effector rather than central memory.
phenotype. The exact cause of these qualitative defects is unclear, but may be attributed to either the loss of T-cell help and/or the lack of antigen clearance resulting in prolonged stimulation and subsequent generation of aberrant relatively ineffective CTL responses.

In addition, HIV-specific CD4+ T-cell responses are impaired from early infection, when the preferential loss of HIV-specific CD4+ T-cell proliferative responses occurs in most individuals, whereas short-term production of cytokines such as IFN (interferon)-γ is comparatively well maintained [48]. Maintenance of proliferative CD4+ T-cell responses in long-term non-progressors or by various therapeutic interventions initiated during primary infection has, in certain studies, correlated with viral control [22,49], and there is less evidence for viral escape from CD4+ T-cells to recognize processed viral antigens [51,52]. The mechanism of this is still unclear, although down-regulation has been attributed to at least three different HIV-1 proteins: Env, Nef and Tat [51,53,54].

Humoral immunity in HIV infection

The contribution of humoral and innate immunity in controlling the pathogenesis of HIV disease still remains unclear, although it has been reported that the only HIV antigen that is relevant to humoral immunity is the envelope glycoprotein [55]. Neutralizing antibodies to epitopes of the envelope protein appear several weeks, months or even up to a year after seroconversion [21,56], and autologous neutralizing antibodies in sera were never collected at the same time as the relevant virus was isolated [57]. Generally, neutralizing antibodies are isolate-specific and not broadly cross-reactive, although sera from long-term non-progressors were more able to neutralize a primary isolate compared with progressors or short-term non-progressors [58]. Unless broadly neutralizing antibodies are stimulated, escape from neutralizing antibodies may occur [59]. Indeed, several cross-reactive neutralizing antibodies to epitopes of gp120 or gp41 have been isolated, suggesting that at least some envelope epitopes may be broadly conserved [60–64].

The degree of antigenic diversity between subtypes and lineages of HIV-1 is greater than any other virus investigated [55] and, within a given individual, the sequences of replicating virus can differ by as much as 10% [65]. For this reason, it is plausible that the conserved epitopes of gp160, to which broadly neutralizing antibodies have been shown to be directed, should be a potential target of a humoral-targeted therapeutic vaccine. Similarly, a CTL-based vaccine must be targeted to conserved viral sequences to be relevant across many viral variants. Ideally, a single vaccine or a combination of vaccines that elicit both humoral and cellular immune responses would be the ultimate aim to treat HIV infection.

Vaccine approaches for the treatment of HIV infection

Initially, approaches that had been used to produce earlier preventative vaccines were applied in the development of HIV vaccines. The immunogenicity of many of the previous globally important vaccines was never tested in animal models prior to testing in humans, although non-human primates have been used in the preclinical development stages of both prophylactic and therapeutic HIV vaccines.

HIV-1 only infects a few species of non-human primates and there has been no report of progression to AIDS [66], with one exception [67]. Early work was carried out in HIV-infected chimpanzees and has shown some enhancement of HIV-specific immune responses using gp120-depleted inactivated HIV-1 [68] and DNA vaccines [69,70]. However, it proved difficult to examine the effect of these vaccines, due to the general lack of clinical progression to immunodeficiency in this animal model and the prohibitive cost of these experiments.

Therefore different animal models of HIV were sought, the first of which was SIV-infected monkeys. SIV is from the same primate lentivirus family as HIV and, although it has only approx. 40% nucleotide homology with HIV, it has a similar biological character and genomic organization, but differences in the progression to disease. SHIVs (simian/human immunodeficiency chimaeric viruses) were then designed that infect certain non-human primates leading to rapid development of immunodeficiency similar to AIDS. Using non-human primate models, such as the SIVmac239-, SIVmac251- and SHIV-infected rhesus macaques, many important insights have been achieved into various aspects of HIV infection. These models have proven useful in preclinical studies prior to progression to human therapeutic vaccine clinical trials.

More than 40 candidate therapeutic vaccines have progressed into human testing since HIV was identified as the aetiologic agent of AIDS (Table 1). Most candidates have been safe and well-tolerated, and many are immunogenic by standard tests, but none have yet shown clinical benefit warranting license either as a prophylactic or therapeutic vaccine. Since early initiatives, there has been significant technological advancement and the different types of approaches are discussed below.

Whole inactivated HIV-1

After preclinical safety and immunogenicity testing in HIV-infected chimpanzees [68], the whole inactivated HIV-1 immunogen called Remune (Immune Response) was tested in an open-label Phase I trial of chronically
HIV-infected human subjects [71]. Remune is composed of purified inactivated virions encoding HIV-1 clade A envelope and clade G gag, stripped of surface gp120 and emulsified in incomplete Freund’s adjuvant. Remune was able to increase anti-p24 antibody responses in nine out of 23 (39%) recipients and induce HIV-specific delayed-type hypersensitivity responses in 12 of 25 (48%) recipients. Long-term follow-up showed that those with the latter response had clinically better outcomes in terms of temporal loss of CD4+ T-cells, incidence of opportunistic infections and time to death. Further studies confirmed the immunogenicity in the presence [72–74] and absence [75] of ART. However, a large multi-centre Phase III clinical endpoint trial in 2527 HIV-infected individuals was stopped due to the failure of Remune to improve clinical outcome and time to disease progression [76]. These results call into question the validity of the observed laboratory markers as correlates of immunity.

### Protein subunits and peptides

Vaccines developed to produce sterilizing immunity in other viral illnesses include purified recombinant hepatitis B surface antigen for hepatitis B virus and the purified haemagglutinin proteins for influenza A and B. A range of candidate vaccines comprising subunit components of whole killed virus formulations were developed and the first of these, purified or recombinant HIV envelope subunit proteins (gp120 and gp160), were also designed and tested with the aim of stimulating neutralizing antibodies against HIV-1 as a treatment. In an open-label Phase I study, rgp160 (recombinant HIV-1 gag rgp160; VaxSyn HIV-1; MicroGeneSys) was administered to 30 ART-naïve individuals with primary HIV infection. Of the vaccine recipients, 63% demonstrated gp160-specific immune responses and the majority of these responders commenced the trial with > 600 CD4+ T-cells/mm³ [77]. However, the lack of a placebo group in this trial limited the interpretation of results.

The safety and immunogenicity of rgp160 was tested in early clinical studies [78–80], although the clinical efficacy was not tested due to constraints in experimental design. When tested in a large multicentre randomized placebo-controlled clinical trial in 278 ART-naïve subjects, a rgp160, VaxSyn, failed to demonstrate any clinical benefit in terms of CD4+ T-cell count, viral load, time to ART or time to death [81] and these results were confirmed in subsequent trials [75,82]. In addition, rgp160 was demonstrated to be less beneficial for CD4+ T-cell counts and HIV viral load measurements than zidovudine monotherapy in this patient group [83]. Other recombinant Env proteins have been shown to be safe and some immunogenic by standard laboratory parameters, although no candidate vaccine has significantly delayed disease progression in any patient group [84,85].

Other HIV proteins, such as the regulatory protein Tat that is expressed early and plays a crucial role in the life cycle of HIV by facilitating viral spread and playing a part in immunosuppression [86], have been employed. Tat has shown some promise as a candidate vaccine antigen [87] and clinical trials are currently ongoing. Recombinant Gag subunits (combinations of both p17 and p24) have been developed that self-assemble into VLPs (“virus-like particles”). A fusion protein was created from p17/p24 and a yeast retrotransposon gene expressing p1, a protein capable of inducing self-assembly into a VLP [88]. When administered to seronegative volunteers, p24-VLP was able to induce production of anti-p24 antibodies [89], although there was no evidence of any immunological or clinical benefit in HIV-infected individuals [90–92].

A number of synthetic peptides aimed at targeting the immune response to dominant CTL epitopes or HIV-neutralizing determinants have been tested in humans. Synthetic peptide vaccines designed to contain combinations of T-helper epitopes from the C4 region of HIV-1MN gp120 and T-helper/CTL/neutralizing epitopes from the V3 loop from several HIV-1 strains were tested in a pilot Phase I-controlled trial in eight HIV-infected patients [93]. Increased neutralizing antibody titres and lymphoproliferative responses resulted, although no improvement of viral load or CD4+ T-cell count was observed. In other studies, a peptide-based vaccine candidate was designed to induce cellular immunity consisting of highly conserved regions of HIV-1 p24 recognized by long-term non-progressors [22]. When examined in several trials in HIV-infected volunteers in the absence or presence of HAART, some Gag-specific responses were observed, although the clinical impact of these was unclear [94,95].

Peptide vaccines have also been linked to lipids in an attempt to induce cellular immunity by delivery direct to cell membranes and, thus, the immune system. There have been a number of promising results in trials of these lipopeptides on their own [96,97], as adjuvants to aid in

Table I  Approaches to therapeutic vaccination for HIV infection

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Description</th>
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<tr>
<td>Whole Inactivated HIV</td>
<td>Remune</td>
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<tr>
<td>Protein subunits and peptides</td>
<td>Purified recombinant proteins, eg gp120, gp160, Tat.</td>
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<td></td>
<td>Viral-like particles</td>
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<td></td>
<td>Synthetic peptides, eg. V3 loop, multivalent Gag peptides</td>
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<td></td>
<td>Lipopeptides, eg. Lipo-ET</td>
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<tr>
<td>DNA Plasmids</td>
<td>Contain various HIV-1 genes, eg. env, nef, rev, tat.</td>
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<tr>
<td>Viral vectors</td>
<td>Prionus vectors, eg. casasangop, footpox, vacinia, modified vaccinia Ankara</td>
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<td>Adenoviruses</td>
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<td>Adeno-associated viruses</td>
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<tr>
<td>Cytodendritic cell based</td>
<td>Synthetic HIV peptide pulsed dendritic cells</td>
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protein subunit vaccine delivery [98] and to boost other therapeutic or prophylactic vaccine candidates [99].

DNA vaccines

Promising advances in recombinant techniques have led to the development of a variety of DNA and viral vectors that have been modified to facilitate the expression of xenogeneic genes. Such vectors encode HIV proteins to facilitate their delivery to cells, where expression and intracellular processing can occur by normal cell mechanisms in a manner mimicking natural HIV infection. A wide range of DNA plasmids encoding exogenous proteins have been developed in many different disease settings, and DNA vaccination has been shown to induce both cellular and humoral immune responses, but without the pathogenesis fears of live vectors.

Early studies in HIV-infected chimpanzees demonstrated that immunization with DNA vaccines expressing HIV-1 gp160 and rev led to enhanced virus-specific immune responses [69,70]. The first human trial of a DNA vaccine for HIV was an open label dose-escalating trial of DNA vaccine expressing HIV-1MN env gp160 and rev genes [100]. Fifteen asymptomatic HIV-infected ART-naïve individuals received intramuscular injections of 30, 100 or 300 µg of DNA at 10 week intervals. The vaccine was well-tolerated but there was no change in CD4+ T-cell count or plasma viral load, although there were marginal increases in anti-gp160 CTL activity in a small number of volunteers. With the suggestion that virus replication must be suppressed for the immune system to be able to mount an immune response to a potential vaccine, this same group vaccinated 13 stable asymptomatic HIV-infected patients on HAART with a combination of the env/rev DNA vaccine and a similar one expressing HIV-1MN gag/pol genes. In this double-blind placebo-controlled dose-ranging clinical study [101], there was no change in T-cell counts with vaccination, but eight of 13 vaccinees showed positive IFN-γ ELISpot (enzyme-linked immunospot) responses. Interestingly, vaccination appeared to exert significant control over the transient increases in viral load, known as viral blips, compared with the placebo group.

Other groups have used DNA plasmids encoding HIV-1 nef, tat or env genes (100 µg) in asymptomatic HIV-infected individuals, which were found to be safe, but only moderately immunogenic [102,103]. It is now known that these doses of DNA vaccine are relatively small and efforts are being made to augment plasmid expression using co-expressed cytokine [104,105] and to improve DNA delivery through the use of physical techniques, such as gene gun injection and lipophilic, cationic and targeted delivery systems [106,107].

Viral vectors

A variety of viruses have been used to construct live and infectious recombinant vectors, including adenoviruses (adenovirus subtype 5 vaccines are currently in clinical trials in HIV-seropositive and -seronegative individuals) [108], adeno-associated viruses [109] and the poxviruses (canarypox, fowlpox, vaccinia and MVA (modified vaccinia Ankara)). The latter are attractive candidates, as their replication is limited to the cytoplasm (minimizing the risk of viral integration into the host genome). In addition, they are able to accommodate large foreign genes while maintaining genome stability [110] and can stimulate both cell-mediated and antibody immunity of relatively long duration [111–113].

A Phase I safety and immunogenicity trial was carried out in ten chronically HIV-infected individuals using a MVA vector expressing HIV-1LAI nef genes [114]. Three subcutaneous injections over a 16 week period were well tolerated and able to induce new nef-specific CD4+, but not CD8+, T-cell responses in most vaccine recipients. The clinical benefits remain unclear and investigations are ongoing.

The most widely explored poxvirus vectors for therapeutic HIV vaccination are the ALVAC recombinant canarypox virus vectors, which undergo restricted replication in human cells and are safe and well-tolerated in uninfected volunteers [115,116]. A Phase I/II double-blind randomized clinical trial of ALVAC expressing HIV-1MN gp160 in 20 ART-naïve HIV-infected volunteers demonstrated the safety of this vaccine, although failed to enhance HIV-specific humoral or cellular immune responses [117]. Further ALVAC vectors were developed to express multiple HIV-1 genes and a later trial used four vaccinations of vCP1452 (an ALVAC vector encoding HIV-1MN gp120, HIV-1LAI gp41 and a fragment of pol with protease activity) plus recombinant gp160 in 15 HIV-infected volunteers treated early since primary HIV infection with HAART [118]. The patients remained on therapy and 93% of vaccine recipients had significantly increased titres of anti-gp120 or anti-p24 antibodies (although not necessarily neutralizing), 64% had increased HIV-specific lymphoproliferative responses and 79% had augmented CD8+ T-cell responses (the majority to more than one antigen).

Therapeutic immunization and STI (‘structured treatment interruption’)

STI is a process where all ART is ceased for one or more specified periods of time, generally in patients treated during or shortly after primary infection. During this interruption, HIV-infected individuals generally experience some degree of plasma viral load recrudescence [119–121], even after as little as 2 weeks off therapy [122]. In HIV vaccine research, these STI episodes can be used to illustrate whether vaccine recipients experience any benefit following cessation of ART. The time to re-initiation may be based upon pre-defined cycles, plasma viral load/CD4+ T-cell measurements or other relevant parameters. In one such example, 11 vCP1452/rgp160
HIV-infected individuals, having initiated HAART (during or shortly after primary HIV infection) and maintained control of virus replication, were randomized to diluent alone ($n=12$) or rFPV expressing HIV gag/pol and IFN-$\gamma$ (rFPV-gag/pol-IFN-$\gamma$; $n=12$). Intramuscular injection of vaccine ($5 \times 10^7$ pfu/ml) occurred at weeks 0, 4 and 12 and HAART was maintained until week 52. Those subjects consenting to receive a fourth vaccination discontinued HAART 1 week later. The primary end point was time-weighted mean change from baseline plasma HIV viral load. Following a fourth immunization and treatment interruption, rFPV-gag/pol-IFN-$\gamma$ recipients experienced an approx. 1.0 log greater reduction in plasma HIV viral load compared with placebo recipients.

In the Quest trial, individuals with primary HIV infection received 76 weeks of HAART and then were randomized to HAART alone, HAART + vCP1452 or HAART + vCP1452 + Remune. In all groups, HAART was discontinued at week 24 and patients were followed up for a subsequent 24 week period and the primary end point was proportion of patients with a viral load $<1000$ copies/ml at week 48 without re-initiation of ART. No patient reached this end point. Early published results suggest that the combination of vCP1452 + Remune was immunogenic, boosting HIV-1 Gag-specific CD4$^+$ and CD8$^+$ T-cell IFN-$\gamma$ ELISpot responses [124], confirming the work of others [118], although this did not translate into any significant clinical benefit [125].

rFPV (recombinant fowlpox virus) vectors, modified to deliver a combination of gag and pol regions of the HIV-1 genome without or with an immuno-enhancing cytokine gene IFN-$\gamma$ ($5 \times 10^7$ pfu (plaque-forming units)/ml), were examined in a multicentre randomized double-blind placebo-controlled trial [126]. Intramuscular injection of vaccines in 35 HIV-1-infected individuals treated with HAART around the time of their primary infection occurred at weeks 0, 4 and 12 and patients remained on therapy up to 52 weeks. In an extension to this study, 25 of these subjects consented to receive a subsequent vaccination and discontinued therapy 1 week later, with follow-up continuing to 20 weeks (Figure 2). Although safe, neither of the candidate vaccine constructs appeared to possess detectable T-cell-mediated anti-HIV immunogenic properties at any time following immunization. However, following further vaccination and STI, recipients of rFPV expressing HIV gag/pol + IFN-$\gamma$ (rFPV-gag/pol-IFN-$\gamma$) experienced a 0.8 log reduction in plasma viral load compared with recipients of rFPV expressing HIV gag/pol only or placebo. Intracellular expression and resultant biological activity of IFN-$\gamma$ may be driving such activity. Other measures of viral load change also suggest a superior but statistically non-significant viral load outcome for recipients of this vaccine construct.

In another encouraging approach, investigators gave intramuscular injections of a combination of an ALVAC-HIV canary pox vector (vCP1433) and lipopeptide adjuvant (Lipo-6T) at weeks 0, 4, 8 and 12, followed by three cycles of subcutaneous IL-2 (interleukin-2) at weeks 16, 24 and 32 in HIV-infected individuals treated for at least 12 months with HAART [99]. At week 16, vaccination with vCP1433/Lipo-6T significantly increased the breadth of existing immune responses and stimulated new anti-HIV lymphoproliferative responses compared with those receiving ART alone. At week 36 after IL-2 administration, IFN-$\gamma$ ELISpot anti-HIV responses significantly increased, with at least three times the number of vaccine recipients recognizing three or more HIV-1 peptide pools compared with controls. All patients ceased ART at week 40 and, after 12 weeks of STI, significantly more vaccine recipients had maintained virological control, as defined by predetermined parameters. The median time to re-initiation of ART was 42 days in the vaccine recipients compared with 29 days in the control group ($P=0.009$). The effects of the pCP1433/Lipo-6T on viral load set point following cessation of ART may be confounded by combination with the cytokine IL-2, but
this study suggests that the control of HIV replication is possible with such a vaccine strategy in the absence of ART.

Chronically HIV-1-infected patients on HAART (not necessarily initiated at primary infection) were vaccinated with Remune prior to two rounds of STI [127]. No differences were found in plasma viral load during the first STI, although there was a lower viral load set point during the second STI in Remune compared with placebo recipients. There was also no difference in CD4+ T-cell count, a commonly used surrogate marker for immune competency, which is similar to other therapeutic vaccine/STI studies [123,126]. It is believed that CD4+ T-cell competency in addition to cell number is important in the control of viremia [128,129].

Lower plasma HIV viral load rebound was also demonstrated in SIVmac251-infected macaques treated during primary infection with ART and immunized with NYVAC (a recombinant attenuated canarypox virus vaccine), compared with placebo [130,131]. Importantly, in this macaque cohort, cell-mediated immune responses were only induced in animals with viral load that had been well suppressed on therapy. Such shorter and repeated rounds of STI may precipitate these responses and have well suppressed on therapy. Such shorter and repeated rounds of STI may precipitate these responses and have been demonstrated to enhance CD4+ T-cell proliferative and CD8+ T-cell responses with plasma HIV viral load rebound [127,132]. Indeed autologous wild-type virus was used as a vaccine in macaques with acute SIVmac251 infection and, after repeated STI, control of viremia without ART was demonstrated [122], although not if ART was commenced late in infection [133].

It has been hypothesized that during STI the increase in autologous viral antigen in the peripheral blood after an extended period of viral suppression may induce HIV-specific memory T-cell responses following STI. This concept is known as ‘autovaccination’ and has been suggested for patients treated early in infection with ART. Thus far, STI has not been successful in chronically infected patients and is not yet recommended for use outside the clinical trial setting [134–136]. During time off treatment, viral rebound occurs with concomitant activation and turnover of CD4+ T-helper populations that are generally in low numbers during effective ART-mediated viral suppression [137]. Although the time to treatment is not predictive of viral load set point [138], the nadir CD4+ T-cell count appears to be predictive of the length of time that patients take off treatment when re-initiation is defined by predetermined parameters [139].

**Dendritic-cell-based vaccines**

It has been shown that the functional capacities of antigen-presenting cells, such as dendritic cells that are instrumental in the induction of the anti-HIV immune response, decline with time of HIV infection [48,128,140,141]. A number of studies have investigated the ability of dendritic cells pulsed *ex vivo* with a range of HIV peptides to present antigen to T-cells both *in vitro* [142,143] and to a very small number of HIV-infected individuals [144] without approaching clinical benefit. In a hu-PBL-SCID (human peripheral blood leucocyte-reconstituted severe combined immunodeficient) mouse model, autologous dendritic cells pulsed *in vitro* with chemically inactivated virus have been shown to elicit human CD8+ T-cell responses and a strong protective antiviral immunity [145,146]. Subsequently, whole conformationally intact SIV was administered early in infection via monocyte-derived dendritic cells, leading to enhanced T-cell responses and reduced viral loads in Chinese rhesus monkeys infected with SIVmac251 [147]. In a recent study [148], 18 chronically HIV-infected patients not taking ART, but with a 6 month history of stable viral loads, were vaccinated with the same dendritic cell-based vaccine. At 1 year post-vaccination, plasma viral loads had decreased by more than 5-fold and this viral load suppression correlated positively with generation of HIV-1-specific secretion of IL-2 and IFN-γ in CD4+ T-cells and Gag-specific perforin expression in CD8+ T-cells. Such a strategy in which autologous monocyte-derived dendritic cells pulsed with autologous inactivated whole HIV-1 induce effective HIV-1-specific T-cell responses associated with sustained viral suppression may be difficult on a larger scale, but shows great promise and investigations are continuing.

**PROBLEMS REMAINING TO BE OVERCOME**

To evaluate the efficacy of a therapeutic vaccine strategy, the aim is to measure the specific anti-HIV immune responses in a variety of assay systems. Ultimately, in the absence of established surrogates of HIV disease, it is necessary to establish how the observed immune responses translate into improved clinical outcomes. Disappointingly, many immunogenic candidates appear not to exert any effects on clinical outcomes relevant to the natural history of HIV disease.

Many candidate therapeutic vaccines have generated significantly augmented anti-HIV-specific immune responses *in vitro* and in animal model assessments that have not subsequently been found when tested in humans. There are many differences between the SIV/SHIV models in monkeys and humans with respect to the route of and dose involved in infection, the viral tropism and also the replicative capacity of the viruses in their respective hosts. In fact, the biology of the SIV-infected monkey and pathogenic SHIV macaque models is very different from that of HIV-infected humans [149,150].

Numerous recent conference reports have emphasized the apparent disconnection between human and primate models of therapeutic vaccination. Such observations are
supported by recent data from natural history studies in which the magnitude of IFN-γ ELISpot responses was not different between long-term non-progressors and other groups of individuals with HIV infection, including those with late stage disease [41]. In a recent study of macaques vaccinated with a live attenuated SHIV(89.6) construct and then challenged by pathogenic SIVmac239, in which an exhaustive range of assessments of immune response were performed, one of the few predictors of viral control post-challenge was endogenous IFN-α expression by PBMC (peripheral blood mononuclear cells) post-immunization [151].

When tested in humans, several agents have induced CD4+ lymphoproliferative and IFN-γ-induced responses by ELISpot [76,77,79,123], although this did not translate into clinical benefit. In fact, with a few exceptions in small-scale proof of concept trials [99,126,148], therapeutic vaccine strategies have so far failed to significantly increase control of viral load and there is no evidence for them impacting upon disease progression [76,91]. In the majority of these reports, there is no clear correlation between the measurement of anti-HIV T-cell immune responses (on standard tests) and control of viral replication.

The immune correlates of viral control in the natural history of HIV disease are unclear and, consequently, the required immune responses to therapeutic vaccination remain elusive. This may be due in part to the limitations of current assays, which concentrate on specificity and frequency of class I-restricted CD8+ cytotoxic T-cells, rather than their antiviral function [152]. The efficacy of CTL antiviral function is the result of a composite of cellular and virological features and the commonly used measurements to test vaccine immunogenicity, such as IFN-γ ELISpot, ICCS (intracellular cytokine staining) and CTL assays, may not necessarily reflect function. It has been suggested that production of a broader range of cytokines, such as IL-2, TNF-α (tumour necrosis factor-α) and TGF-β (transforming growth factor-β), may be examined in ELISpot and ICCS, and that the stimulatory antigens constructed from consensus sequences may be replaced with antigen-presenting cells loaded with whole inactivated virus [153,154]. The HIV antigens used in previous assays may not correlate with those circulating in vivo and patient-specific antigen may be more appropriate, but this would be logistically challenging and prohibitively expensive in large trials [155,156]. Such assays may emulate in vivo conditions more completely and may clarify the connection between experimental correlates and viral control. In addition, assays to predict the in vivo containment of virus following therapeutic vaccination would be useful, particularly in the absence of ART. As such, we may be measuring the wrong parameters and may even be sampling the wrong body compartments, given that most trials sample only peripheral blood, rather than lymphoid tissue.

CONCLUSIONS

Therapeutic vaccines could be of particular value in some countries of the developing world where the provision of long-term ARTs to large numbers of infected persons is severely constrained. In such countries, a vaccine that could reduce the need for antiretroviral use may contribute towards a significant reduction in the burden of disease. In other settings, vaccines specifically designed to treat multidrug-resistant strains may hold great promise. Therapeutic vaccines that reduce viral load may lead to reduced transmission and therefore to improved public health outcomes. In general terms, safe and effective therapeutic vaccines for HIV might reduce the lifetime costs of long-term exposure to ART.

However, a number of major problems persist, including how to utilize animal models in preclinical studies to predict human outcomes and how to measure the efficacy of such HIV vaccine candidates in humans. Despite these challenges, there have been advances with a varied selection of candidates in the past few years. At this time, the field is progressing well, hopefully towards a successful therapeutic HIV vaccine.

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