Human Vaccines & Immunotherapeutics

Therapeutic vaccines against HIV infection
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Resistance to medication, adverse effects in the medium-to-long-term and cost all place important limitations on lifelong adherence to combined antiretroviral therapy (cART). In this context, new therapeutic alternatives to cART for life in HIV-infected patients merit investigation. Some data suggest that strong T cell-mediated immunity to HIV can indeed limit virus replication and protect against CD4 depletion and disease progression. The combination of cART with immune therapy to restore and/or boost immune-specific responses to HIV has been proposed, the ultimate aim being to achieve a functional cure. In this scenario, new, induced, HIV-specific immune responses would be able to control viral replication to undetectable levels, mimicking the situation of the minority of patients who control viral replication without treatment and do not progress to AIDS. Classical approaches such as whole inactivated virus or recombinant protein initially proved useful as therapeutic vaccines. Overall, however, the ability of these early vaccines to increase HIV-specific responses was very limited and study results were discouraging, as no consistent immunogenicity was demonstrated and there was no clear impact on viral load. Recent years have seen the development of new approaches based on more innovative vectors such as DNA, recombinant virus or dendritic cells. Most clinical trials of these new vectors have demonstrated their ability to induce HIV-specific immune responses, although they show very limited efficacy in terms of controlling viral replication. However, some preliminary results suggest that dendritic cell-based vaccines are the most promising candidates. To improve the effectiveness of these vaccines, a better understanding of the mechanisms of protection, virological control and immune deterioration is required; without this knowledge, an efficacious therapeutic vaccine will remain elusive.

Introduction

Combined antiretroviral therapy (cART) has become one of the best tools to control HIV infection at both the individual and social level. Indeed, cART has achieved impressive reductions in morbidity and mortality among patients with advanced human immunodeficiency virus (HIV). However, despite these advances in infection control, the cost of medication, the mandatory high adherence to medication for life, side effects and the risk of development of resistance remain unsolved problems. Furthermore, the HIV epidemic has severely hampered economic growth in developing countries. Although the number of HIV-infected patients under treatment in these countries has increased steadily in recent years, and although the number of new HIV infections has fallen, the demand for treatment continues to rise. In fact, the number of new infections is still higher than the number of new patients on treatment. For economic, social, cultural and political reasons, widespread treatment is difficult to implement in these countries. Given this situation, there is a need for new therapeutic alternatives to cART for life in HIV-infected patients.

Two such alternatives which have been proposed are eradication and functional cure. There is one report of an HIV-infected patient in whom viral replication remained absent, despite discontinuation of antiretroviral therapy, after transplantation with CD34+CD38− stem cells. Recently published follow-ups of this patient strongly suggest that cure of HIV has been achieved. Intensification of cART, stem cell therapy, gene therapy and the elimination of latently infected cells are other alternatives to eradication which are currently being assessed. However, it is unlikely that therapeutic strategies leading to eradication will emerge as a valid alternative to cART, and in fact, there are no biological models to support this approach. Conversely, the concept of "functional cure," defined as control of HIV replication without cART, is based on the observation that a small proportion of HIV-infected patients (referred to as long-term non-progressors or elite controllers) are able to control HIV replication to below detectable levels, and without abnormalities in CD4 T-cell counts, for more than 25 y. It has been proposed that the strong HIV-specific immune responses observed in these patients could account for this natural "functional cure." Specifically, control of viral replication has been associated with HLA types B*57:01, B*27:05 and B*14, and it has been proposed that the viral peptides presented by these HLA molecules induce strong HIV-specific CD8 cytotoxic T lymphocytes (CTLs), together with strong CD4 T-cell help, in HIV-infected patients. Recent reports suggest that a strong T cell-mediated immunity to HIV can indeed limit virus replication and protect against CD4 depletion and disease progression. Here we will review the basis of the therapeutic vaccine strategy and the different candidates tested in human clinical trials.
Pathogenic Basis for a Therapeutic Vaccine Design

HIV-specific responses against HIV infection in antiretroviral naïve patients. The main question to be answered here is whether the immune system can contain viral replication without cART, even if only for limited periods. One major problem is that the types of immunological responses which are able to control viral replication are not known, although it does seem that the relevance of the different response types changes with the stage of HIV infection. In a study by Jaffe et al., the results of a preventive vaccine which suggest that protection against infection is mediated mainly by antibodies against env, whereas post-infection control of viral replication is associated with cellular responses against gag. Recent data reported by Picker et al. in relation to SIV-infected rhesus macaques suggest that the induction of effector memory T-cell responses (and not central memory T-cell responses) with a CMV-vectored vaccine is essential for control of viral replication.

Anti-HIV-1 CTL responses have been detected in all studied cases of acute HIV infection, and it is believed that they reduce the peak plasma viral load (PVL) to the stabilization level (set-point) of PVL which is reached at the end of the acute phase. It has therefore been hypothesized that CTL play an essential role in controlling viral replication during acute infection. However, it is not clear that neutralizing antibodies contribute to the control of viral replication during acute infection, since a delay in the development of these antibodies is observed which is not associated with changes in viral replication. Finally, and in contrast to CTL and neutralizing antibodies, a clonal depletion of HIV-specific CD4+ T cells occurs during acute HIV infection. It has been suggested that the lack of response of these cells is key to explaining the failure to control viral replication during this phase. In fact, massive infection and loss of memory CD4+ T cells occur in multiple tissues during acute infection, and HIV mainly infects HIV-specific CD4+ T cells.

Findings may provide a basis for explaining why a therapeutic vaccine-induced transient activation of HIV-specific CD4—but not CD8+—T cells might have a detrimental effect on HIV replication, and could offer important clues for the future design of therapeutic vaccines. Some data suggest that strong T cell-mediated immunity to HIV can indeed limit virus replication and protect against CD4 depletion and disease progression in chronic HIV-infected patients. Although in most infected patients replication leads to the progressive destruction of the immune system and evolves inevitably toward AIDS, a small number of immunologically "privileged" individuals, or long-term non-progressors (LTNP), have a potent and sustained response of anti-HIV-1 CTL, Th cells and neutralizing HIV-1 antibodies. This is associated with control of viral replication and the presence of very low or undetectable viral concentrations in plasma in the absence of ART. Direct data on the critical role of the CTL response in controlling viral replication have been obtained in both the infection model with macaques devoid of CD8+ T lymphocytes, and in the immunodeficient murine model. In addition, there is clear evidence from both human and animal models that a specific helper T response against HIV is crucial in obtaining an optimal, specific CTL response which can control viral replication. This is consistent with other reported data on chronic viral infections in murine models. Finally, studies in animal models have shown that high levels of neutralizing antibodies can block infection regardless of the route of exposure to the virus. It has also been reported that plasma virus continuously and rapidly evolves to escape neutralization, indicating that neutralizing antibodies exert a level of selective pressure. It is therefore possible that neutralizing antibody responses account for the extensive variation in the envelope gene observed in the early months after primary HIV infection.

Despite the relevance of HIV-specific immune responses in both the acute and chronic stages of infection these responses are not, in most patients, able to control viral replication to undetectable levels and prevent the infection from progressing to AIDS. Many authors hypothesize that selective escape from CD8+ T-cell and other immune responses is the main reason for the failure to control HIV replication. Some studies suggest that selective escape from CD8+ T-cell responses represents a major driving force of HIV-1 sequence diversity. Allen et al. assessed the relationship between viral evolution and adaptive CD8+ T-cell responses in four HIV-infected patients who were studied for five years after acute infection. Nearly two-thirds of the amino acid mutations identified in non-envelope antigens were attributable to CD8+ T-cell selective pressures. The preferential selection of individual residues for mutation and the repeated selection for identical mutations observed in this study suggest that there are significant biochemical constraints on viral evolution. In addition, some mutations induced by immune-mediated selective pressures have an impact on viral replication capacity. Allen et al. also found that an optimal, specific CTL response can control viral replication. This is consistent with other reported data on chronic viral infections in murine models. Finally, studies in animal models have shown that high levels of neutralizing antibodies can block infection regardless of the route of exposure to the virus. It has also been reported that plasma virus continuously and rapidly evolves to escape neutralization, indicating that neutralizing antibodies exert a level of selective pressure. It is therefore possible that neutralizing antibody responses account for the extensive variation in the envelope gene observed in the early months after primary HIV infection.

In summary, it seems clear that both cellular and humoral immune responses are critical for correct control of HIV infection. Viral escape and functional defects in these immune responses are
likely to be the main causes of sustained viral replication in HIV infection, and constitute the two main problems that a therapeutic vaccine should solve.

**Influence of cART on HIV-specific immune responses.** The benefit of cART on the immune system is obtained through an increase in the absolute number of circulating naive CD4+ T lymphocytes, a concomitant reduction in the number of T lymphocytes with activation markers, and restoration of the response to memory antigens. However, despite the clinical effects of cART, the therapy is unable to eradicate the infection, even if it were administered for more than 60 y.56,57 This is mainly due to the fact that therapy cannot eliminate latent HIV-1 in the form of integrated proviral DNA, as well as to the existence of low levels of viral replication, which makes even cell-to-cell infection possible.58,59 Furthermore, cART is incapable of restoring the immune-specific response to HIV,43 and, in fact, it leads to a fall in the specific CTL response due to the lack of antigenic exposure.60 Some reports have shown that the helper proliferative response to HIV p24 Ag presented by some cART patients does not reflect an improvement in the immune phenotype or function of CD8+ or CD4+ cells, but, in fact, is secondary to the small increases in viremia typically observed in patients taking cART.61 This would explain the rapid "rebound" of viral load that occurs within days or weeks of suspending cART, even after several years of effective therapy.62-64 This rebound occurs even if cART is initiated in very early-stage HIV-infected patients, in whom the immune system is theoretically still well preserved (decidual CD4+ T lymphocytes above 500 cells/mL; PVL, 5,000–10,000 copies/mL). Similarly, these viral dynamics occur even when immune restoration is practically complete in terms of the homeostasis of T lymphocytes and their subpopulations, as well as in terms of the capacity for response to polyclonal stimuli and memory antigens with cART.65-67 Taken together these data suggest that the first challenge for a therapeutic vaccine is to demonstrate that it is possible to induce de novo HIV-specific responses that are able to control viral replication, albeit only partially. If this is the case, it would be a proof of concept that would justify further investigation of different immunotherapies.

**The Therapeutic Vaccines**

Objectives of a therapeutic vaccine. The main objective of a therapeutic vaccine is to induce or increase immune responses against HIV infection using a planned exposure to HIV viral antigens. In the ideal scenario, these responses would control viral replication to an undetectable level, mimicking the situation of the minority of patients who control viral replication without treatment and do not progress to AIDS. This has been termed "functional cure" and it is observed in most other infections (e.g., herpes or tuberculosis). Previous research has suggested that partial control of viral replication could be enough to delay the initiation of cART in antiretroviral naive patients or to offer "drug holidays" in already-treated patients. However, the current knowledge of HIV pathogenesis precludes this "partial remission" of infection from being a conceivable clinical objective. Even antiretroviral naive patients with a normal CD4+ T-cell count could be at higher risk of suffering non-AIDS events (cancer, cardiovascular or neurological events) than the general population.75,76 In addition, it seems that the early initiation of antiretroviral therapy significantly improves survival, as compared with deferred therapy.77 Furthermore, the discontinuation of antiretroviral therapy in order to reduce drug-related adverse effects and to improve the patient’s quality of life has been shown to be associated with increased morbidity and mortality.78-80 It has been suggested that an intense activation of virus rebound after ART interruption would explain this increase in the rates of AIDS-defining and non-AIDS-defining malignancies or cardiovascular events among patients who discontinued cART. Untreated HIV infection is characterized by a profound and continuous state of immune activation manifested by an increased turnover of B and T lymphocytes, natural killer cells and a high level of pro-inflammatory cytokines such as IL-7 and TNF-α. Another relevant feature is the elevation of activated CD8+ T-cells expressing the DR/CD38+ phenotype, which is considered as a surrogate marker of disease progression.81-83 Systemic immune activation could lead, first, to exhaustion of the immune system, including lymph node fibrosis,17,45 retention of effector T cells in lymph nodes,70,79,84 clonal exhaustion and thymic dysfunction. In addition, it could contribute to the spreading of HIV infection by generating more targets for HIV, thereby allowing ongoing HIV replication.85 In summary, partial control of viral replication remains deleterious for HIV-infected patients, and the objective of a therapeutic vaccine should be complete remission of infection. Nonetheless, if a therapeutic vaccine were shown to be capable of partially controlling viral load this would be a proof of concept justifying further investigation of new candidates.

A second objective has recently been proposed for therapeutic vaccines. Based on data related to the eradication of HIV in the "Berlin patient86,87 it has been suggested that the combination of cART intensification with a vaccine able to induce effective HIV-specific responses could purge the HIV reservoir. This strategy, together with others using different drugs or gene therapy, is already being assessed in clinical trials.88 Types of therapeutic vaccines. Most of the therapeutic vaccines tested have previously been used as preventive candidates. Classical approaches such as whole inactivated virus (REMUNE) or recombinant protein (gp120) were initially shown to be useful. In general, however, the capacity of these early vaccines to increase HIV-specific responses was very limited and study results were discouraging, as no consistent immunogenicity was demonstrated and there was no clear impact on viral load.79,80,85-87 Recent years have seen the development of new approaches based on more innovative vectors such as DNA, recombinant virus or dendritic cells. Most of the clinical trials involving these new vectors have been exploratory and with a low number of patients. In fact, in randomized studies no therapeutic vaccine has yet to demonstrate lasting and potent effectiveness in terms of controlling viral load, improving CD4+ T-cell count or delaying clinical progression. These weak results have precluded further development. Although a great number of candidates have been
Table 1. Therapeutic vaccine clinical trials

<table>
<thead>
<tr>
<th>References</th>
<th>Immunogen</th>
<th>Virological responses</th>
<th>Immunogenicity responses</th>
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<tbody>
<tr>
<td>Whole inactivated vaccine</td>
<td></td>
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</tr>
<tr>
<td>87–90</td>
<td>Remune inactivated whole virus</td>
<td>No clinical benefits</td>
<td>Induction of gag-specific helper responses</td>
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**Subunit vaccines**

<table>
<thead>
<tr>
<th>References</th>
<th>Immunogen</th>
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<th>Immunogenicity responses</th>
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<tbody>
<tr>
<td>91–100</td>
<td>Recombinant envelope protein</td>
<td>No clinical benefits</td>
<td>Improvement in specific lymphocyte proliferation</td>
</tr>
<tr>
<td>101–103</td>
<td>HIV p24-like peptides (Vacc-4x)</td>
<td>No clinical benefits</td>
<td>Induction of HIV-associated specific responses in 90% of patients</td>
</tr>
<tr>
<td>104–106</td>
<td>Tat protein</td>
<td>Not assessed</td>
<td>Induction of Tat-specific T helper cell responses Consistent decrease in cellular and biochemical activation markers Persistent increases in regulatory T cells Stable increases in the percentage and absolute numbers of both CD4 T cells and B lymphocytes Reduced percentage of CD8 T cells and NK lymphocytes</td>
</tr>
<tr>
<td>107</td>
<td>A combination of Nef-Tat fusion protein and envelope glycoprotein gp120 formulated with AS02A adjuvant</td>
<td>Not assessed</td>
<td>Induction of strong gp120-specific CD4+ T-cell responses. Induction of HIV-1-specific CD4+ T cells and CD8+ T-cell proliferation</td>
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**Subunit vaccines using DNA as a vector**

<table>
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<tr>
<th>References</th>
<th>Immunogen</th>
<th>Virological responses</th>
<th>Immunogenicity responses</th>
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<tr>
<td>111, 112</td>
<td>DNA constructs encoding the nef, rev or tat regulatory genes of HIV-1</td>
<td>No clinical benefits</td>
<td>Induction of HIV-1-specific cellular responses</td>
</tr>
<tr>
<td>113</td>
<td>DNA encoding env/rev and gag/pol genes</td>
<td>Reduction of viral blips</td>
<td>Poor capacity to boost virus-specific CD4 and CD8 T-cell responses</td>
</tr>
<tr>
<td>114</td>
<td>DNA encoding HLA, an immunogen comprising HIV-1 clade A p24/p17 fused to a string of cytotoxic T-cell epitopes</td>
<td>Not assessed</td>
<td>Poor capacity to boost virus-specific CD4 and CD8 T-cell responses</td>
</tr>
<tr>
<td>115, 116</td>
<td>DermaVir, a single plasmid DNA (pDNA) immunogen expressing 15 HIV antigens formulated in a nanoparticle developed for topical application</td>
<td>No clinical benefits</td>
<td>Induction of HIV-specific immune responses</td>
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**Viral vector vaccines**

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<tr>
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<th>Immunogen</th>
<th>Virological responses</th>
<th>Immunogenicity responses</th>
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<tr>
<td>124</td>
<td>ALVAC-HIV vCP1452 (expresses HIV-1 env and gag gene products and nef and pol gene products encompassing known human HLA-A2-restricted cytotoxic T cell (CTL) epitopes from these genes) with Remune</td>
<td>No clinical benefits</td>
<td>Significantly increased HIV-1-specific CD4+ and CD8+ T-cell responses</td>
</tr>
<tr>
<td>77, 125</td>
<td>ALVAC-HIV vCP1452 and recombinant gp160</td>
<td>No clinical benefits</td>
<td>Significant increases in anti-gp120 or anti-p24 antibody titers, transient augmentation of T-cell proliferation responses to gp160 and/or p24. Increased HIV-1-specific CD4 and CD8 T-cell responses</td>
</tr>
<tr>
<td>129</td>
<td>ALVAC-HIV vCP1452</td>
<td>No clinical benefits</td>
<td>Increased virus production following treatment interruption</td>
</tr>
<tr>
<td>25, 130</td>
<td>ALVAC vCP1452</td>
<td>Increased virus production</td>
<td>Induction of HIV-specific cells, not correlated with viral load rebound</td>
</tr>
<tr>
<td>131</td>
<td>ALVAC vCP1452 with Remune</td>
<td>Tended to delay viral load rebound Associated with a longer time to meet pre-set criteria to restart ART</td>
<td>Induction of HIV-specific cells, not correlated with viral load rebound</td>
</tr>
<tr>
<td>126, 132</td>
<td>ALVAC-HIV vCP1433 (expresses several HIV genes for gp120, gp41, 55 polyprotein, for a portion of pol encoding the protease and for genes expressing cytotoxic T lymphocyte peptides from pol and nef) with Lipo-5T vaccine (a mixture of the tetanus toxoid TT-830–843 class II-restricted universal CD4 epitope combined with some peptides of Gag, Nef and Pol) and followed by three cycles of subcutaneous interleukin-2</td>
<td>24% of theVac-IL-2 group lowered their viral set-point compared with 5% of controls</td>
<td>Vaccine-elicited immunological responses predictive of virological control</td>
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used in clinical trials, this review will focus on those summarized in Table 1.

Remune. The Remune vaccine initially assayed considerable expectation. This is a vaccine of an inactivated whole virus in which the envelope protein has been removed during the process of inactivation, which is performed by synthesis and formulated with incomplete Freund adjuvant. The vaccine stems from a virus originally obtained in Zaire and contains a type-A envelope and type-G gag. It has been administered to more than 3,000 people with an antiviral-controlled virus. The results showed that it was able to induce gag-specific helper responses which were sometimes very potent, but did not suggest a capacity for immunological control of viral replication.92,93 The best and most extensive study aimed to determine whether the addition of Remune would confer added clinical efficacy to that achievable by cART. This was a multicenter, double-blind, placebo-controlled, randomized trial conducted at 77 centers in the United States, and involved 2527 cART-treated, HIV-infected patients with a CD4 T-cell level between 300 /μL and 549 /μL. There were no differences in HIV progression-free survival, changes in HIV RNA or CD4 cell counts when comparing Remune and placebo recipients.94

Subunit vaccines. Multiple strategies have been used to design therapeutic HIV vaccines based on peptides or recombinant proteins, including vaccination with a recombinant envelope protein,95,96 HIV p24-like peptides (Vacc-4x),97,98 a Tat protein99-102 and a combination of Nef-Tat fusion protein and envelope glycoprotein gp120 formulated with ASO2A adjuvant.103 The basis for the use of these candidates is that a therapeutic HIV vaccine that induces HIV-1-specific CD4+ T-cell responses could improve antiviral immunity, resulting in delayed disease progression. A number of clinical trials using recombinant gp120 or gp160 as immunogens have been performed both in combination and without antiretrovirals.104-109 Overall, these trials have not demonstrated clinical benefits, although all improvement in specific lymphocyte proliferation is observed in some of them.

Vacc-4x consists of four synthetic peptides corresponding to conserved domains of the HIV-1 protein p24 that preferentially include HLA-A2 restricted elements. To ensure optimal exposure of the immunogen to antigen-presenting cells (APCs) the vaccine was given intradermally together with granulocyte-macrophage colony-stimulating factor (GM-CSF).101 Forty HIV-infected patients on cART were vaccinated with either low-dose or high-dose Vacc-4x over 26 weeks. HIV-associated specific responses were safely induced in 90% of patients in a dose-dependent manner and were influenced by the HLA haplotype.102 Thereafter, cART was discontinued in patients for 14 weeks. Patients with the strongest delayed-type hypersensitivity (DTH) responses before treatment interruption tended to achieve lower actual HIV RNA levels.103 Finally, long-term HIV-specific immune responses and clinical outcomes were evaluated. Vacc-4x-induced cellular immune responses were unchanged 1.5 y after completing immunization, and 62% of patients were still off cART.104

Based on efficacy studies in animal models105 and those suggesting that the presence of an anti-Tat immune response correlates with a slower progression to disease,106 some authors have proposed that a preventive and therapeutic Tat protein-based vaccine deserves to be investigated. The first randomized, double-blind, placebo-controlled phase I vaccine trial based on the native Tat protein was conducted in HIV-infected asymptomatic individuals.107 The vaccine was well tolerated and induced and/or maintained Tat-specific T helper cell responses in all subjects, with a wide spectrum of functional anti-Tat antibodies. In addition,
Viral vector vaccines. Given the poor results obtained with Remune, subunit vaccines and vaccines using DNA as a vector, it has been proposed that outcomes could be improved by using viral vectors as immunogens. Live recombinant vaccines are made of a live viral or bacterial vector that is engineered to carry the genes that encode HIV antigens. The antigens are presented on the surface of the cell as externalized peptides in the context of MHC class I molecules, thus priming for CD8+ T-cell responses. The best studied vaccine vectors in humans are the pox viruses.112 Vaccinia virus engineered with HIV-1 genes has been shown to induce virus-specific cellular and humoral immune responses in immunodeficient individuals, as well as protection against simian immunodeficiency virus (SIV) infection when immunization with such constructs has been followed with boosting by recombinant proteins. However, due to the development of life-threatening disseminated vaccinia infections in immunocompromised hosts, the use of vaccinia vectors has been abandoned. The use of pox viruses as vaccines has been limited by obviating safety concerns and the availability of more immunogenic. In addition, there was a consistent decrease in the percentage of CD8 T cells and NK lymphocytes. The authors concluded that Tat immunization in patients on cART could help to restore immune homeostasis. The improvement in immune system function (as opposed to functional cure) is a benefit that some other investigators have claimed as an alternative primary end-point for therapeutic vaccines. However, the clinical relevance of the recovery of these parameters is not clear. Until this issue is clarified, functional cure remains the best objective for a therapeutic vaccine.

A clinical trial of the gp120/NeF/Tat/AS02A therapeutic vaccine in chronically HIV-1-infected volunteers on suppressive ART found the vaccine to be safe, well tolerated and able to induce strong gp120-specific CD4+ T-cell responses, and a higher number of vaccinees developed both HIV-1-specific CD4+ T-cell responses and CD8+ T-cell proliferation.107 Therapy interruption to determine whether these immunological responses might lead to improved control of HIV-1 viral load was not performed.

Vaccines using DNA as a vector. DNA vaccines are based on bacterial plasmids that express the desired antigen in situ, which leads to induction of an immune response of the Th1 type. The first trial, reported in 1998, included nine asymptomatic HIV-1-infected patients who were immunized with DNA constructs encoding the nef, rev or tat regulatory genes of HIV-1.113 The vaccination induced HIV-1-specific cellular responses, but was not able to control viral replication or change CD4+ T-cell counts. These results were confirmed with the same candidate in patients on cART.114 A further two candidates have been tested in HIV-infected patients on cART, the first encoding env/rev and gag/pol genes115 and the second encoding HIVA, an immunogen comprising HIV-1 clade A p24/p17 fused to a string of cytotoxic T-cell epitopes.116 These candidates were safe but their capacity to boost virus-specific CD4+ and CD8+ T-cell responses was poor. Given these results, novel means of vaccine delivery have been proposed for further improvement of DNA vaccine potency. DermaVir, a single plasmid DNA (pDNA) immunogen expressing 15 HIV antigens, has been formulated in a nanoparticle with polyethyleneimine-mannose and glucose, and has been developed for topical application to deliver DNA-encoded antigens into Langerhans cells.117 This candidate has recently been tested in the context of structured therapy interruption in a pilot clinical trial.118 Although HIV DNA immunization induces broader and higher magnitudes of HIV-specific immune responses compared with structured therapy interruptions alone, this candidate did not have any effect on the dynamics of viral rebound, set-point viral load or CD4+ T-cell counts.
without STIs. ALVAC-HIV vCP1452 did not affect viral load measures.124 A separate placebo-controlled study conducted in chronically-infected patients suggested that the immunogenicity conferred by the ALVAC vCP1452 candidate vaccine alone could be deleterious, since it was associated with higher virus production following treatment interruption.125 Further research suggested that ALVAC vCP1452 was only weakly immunogenic, it failing to elicit significant HIV-specific CD8 T-cell responses and inducing activated CD4 T-cell responses that could favor greater viral rebound during treatment interruption.126 These results appear to conflict with those of a clinical trial reported recently by Angel et al.127 This was a randomized, placebo-controlled, double-blind study in which effectively-treated HIV-infected individuals with high CD4 T-cell counts were randomized to receive ALVAC vCP1452 with Remune, ALVAC vCP1452 alone or placebo over 20 weeks. At week 24, participants interrupted cART. ALVAC with or without Remune did not lower the viral load set-point, although it tended to delay viral load rebound and was associated with a greater time to meet pre-set criteria to restart ART. In contrast to the findings reported by Autran et al.128 and Papagno et al.,129 the induction of helper CD4 T-cell responses was not associated with a worse virological outcome at all. At all events, further research is required to determine whether the best strategy to induce viral control in chronic HIV-infected patients should be based on inducing robust and broad CD8 T-cell responses. After cART interruption, 24% of the patients showed evidence of viral rebound, with a mean viral load of 0.8 log10 less than the other two groups, although the differences between groups were not significant. The absence of immune responses in the two vaccinated groups is disappointing and merits further investigation.

The modified vaccinia virus Ankara (MVA) was developed toward the end of the smallpox eradication campaign in the 1970s, the aim being to obtain a vaccine with a better safety profile than the ones in use at that time. MVA was derived from vaccinia by over 570 passages in chicken embryo fibroblast cells (CEF).130 The complete genomic sequence has been sequenced and has a length of 178 kb. It consists of 193 open reading frames (ORFs), corresponding to 177 genes of which 25 are split and/or have suffered mutations resulting in truncated polypeptides. Three mutations affect antigenicity and immune responses, with some structural proteins, explaining the attenuated phenotype of MVA.131,132 There is clinical experience with MVA as a vaccine against smallpox in over 120,000 people.133,134 During extensive field studies, including with high-risk patients, no side effects were associated with the use of the MVA vaccine.135-137 The combination of a very good safety profile and the ability to elicit strong cellular immune responses, along with a highly immunogenic way makes MVA suitable as a vaccine vector. It can stimulate antibody and T-cell responses even in the presence of pre-existing antibodies,138,139 and it has been shown to be effective in primates and humans for several viruses, including SIV and SHIV.140-143 Harrer et al.144 investigated the safety and immunogenicity of an HIV-1 nef-expressing MVA in 14 cART-treated HIV-infected patients. Vaccination with MVA-nef was safe, was associated with recognition of new CTL epitopes, and after interruption of cART viremia remained below the pre-cART viral load in 9/14 patients. In another pilot study, vaccination of 16 HIV-1-infected cART-treated individuals with MVA expressing HIV-1 gag p24, p17 and a CD8 T-cell epitope string was not only safe but also significantly increased the frequencies of CD8 and CD4 T-cells secreting IFN-gamma and enhanced proliferative responses to gag peptides.145,146

Finally, in ACTG A5197, HIV-1-infected individuals who received a recombinant adenosine virus type 5 HIV-1 gag therapeutic vaccine had a 0.5 log10 lower PVL after a 16-week ATI, compared with those who received placebo.147,148

In summary, although most viral vector vaccines have been able to induce HIV-specific immune responses in clinical trials they have shown very limited efficacy in terms of controlling viral replication. New therapeutic strategies are therefore warranted.

Dendritic cell-based vaccines. Dendritic cells (DC) are the most potent professional antigen-presenting and presenting cells (APC), with the unique ability to induce both primary and secondary immune responses of CD4+ and CD8+ T lymphocytes.149-155 A wide variety of data in experimental in vivo and in vivo...
vitro systems have shown that DC are able to engulf exogenous soluble proteins, tumour cell lysates, and inactivated virus and virus-infected apoptotic cells, as well as presenting antigens not only in the MHC-class II pathway to helper CD4+ T cells (Th)146 but also in the MHC-class I pathway to cytotoxic CD8+ T lymphocytes (CTL). A phenomenon also known as "cross-presentation" or "cross-priming"156-158.

In vitro culture of human blood monocytes (MO) with GM-CSF and IL-4 gives rise to myeloid DC.159,160 Some data suggest that these human myeloid DC not only acquire naive CD4+ T cells but also promote their differentiation into Th1 cells, preventing the differentiation into Th2.170 In addition, antigen-pulsed DC secrete antigen-presenting particles (referred to as exosomes) which express functional MHC-class I and II and T-cell co-stimulatory molecules, and they are able to prime CTL in vivo and eradicate or suppress the growth of established murine tumors.171 Autologous DC pulsed in vitro with a variety of inactivated pathogens have also been shown to induce a potent protective immunity in murine models of human infections.155,156 All these data indicate that in vitro-generated DC may be the most potent cell-based adjuvant for a vaccine preparation designed to induce effective Th1 and CTL responses to tumor antigens and intracellular pathogens.

It has been reported that an inactivated SIV-pulsed therapeutic dendritic-cell vaccine was able to elicit effective and lasting SIV-specific cellular and humoral immunity. This study found that after three immunizations administered at two-week intervals the SIV-specific cellular DNA and the SIV plasma RNA levels decreased by 50-fold and 1,000-fold, respectively. This decrease in viral load was negatively correlated with SIV-specific T-cell responses. Neutralizing antibody responses also increased significantly and remained increased throughout the follow-up period.195 Finally, two studies in a hu-PBL-SCID mouse model suggest that mice immunized with inactivated virus-pulsed dendritic cells elicit a potent immune response against HIV, which protects the animals from virus challenge.148-150.

There are at least ten published clinical trials of DC immunotherapy for HIV infection in humans.147,148-158 Most of them found that DC immunotherapy elicited immunological responses, even though the design of the trials and the HIV antigens used to pulse DC were very different. It should be noted, however, that virological responses, even though the design of the trials and the HIV antigens used to pulse DC were very different. It should be noted, however, that virological responses were only observed in four of these trials.148,149,154,155

Two of these four trials were performed in untreated patients. The first was a preliminary, non-controlled, non-randomized study that showed striking results for a MD-DC-based vaccine loaded with high doses of chemically-inactivated (with aldrithiol-2) autologous virus in untreated HIV patients.163 Plasma viral load (PVL) decreased by 90% for at least one year in 18 of 18 patients. This decrease in PVL was associated with strong and sustained HIV-1-specific cellular responses. The second trial was a double-blind, placebo-controlled study using the same vaccine and in patients of the same type of patients (HIV-1-infected patients off cART).170 Only a modest virological response, which was maintained for at least 48 weeks in the vaccinated group, was observed.170 This change in VL in the immunized patients was small (ranging from 0.30 to 0.60 log10) but clinically significant when compared with the placebo group, and it was maintained up to week 48. However, this small decrease in VL in immunized patients was inversely correlated with a weak increase in HIV-1-specific CD8+ T-cell responses.

The other two trials involved antiretroviral-treated patients. The first was an open, randomized (2:1) clinical trial using heat-inactivated autologous virus in cART-treated HIV-1-infected patients, and reported partial and transient control of viral replication after interruption of antiretroviral therapy.164 In this study, autologous DC vaccine pulsed ex vivo with heat-inactivated autologous HIV-1 elicited only weak Th1- and HIV-1-specific CD8+ T-cell responses. Recently, R Enterprise et al.,172 in an uncontrolled study, reported the final results of an immunotherapy regimen consisting of MD-DC electroporated with mRNA encoding autologous HIV-1 antigens (Gag, Nef, Rev, Vpr); this was administered monthly in 33 subjects in four intradermal doses in combination with cART, followed by two more doses during a 12-week cART interruption (STI). They found that eight out of 24 subjects (35%) met the criteria for the primary endpoint of three instances of viral load <1,000 copies/mL, while 11 out of 24 subjects (46%) met the criteria for the secondary endpoint of three instances of viral load <10,000 copies/mL. At week 12 of the STI 16/24 subjects responded with a mean reduction in viral load of ≥1.2 log compared with their pre-cART viral load value. These data are impressive and are similar to those reported by Lu et al.,163 with HIV-1-infected patients (who were vaccinated when treated with cART). These data need to be confirmed in a randomized, placebo-controlled study. A review of DC-based vaccine clinical trials has recently been published.173

Conclusions

Our knowledge of the immunological control of HIV viral replication and the causes of cellular and humoral immune deterioration is limited, and we lack immunological methods able to demonstrate an efficacious immune control of HIV in vivo. The efficacy of immune therapy and therapeutic vaccines has been modest in the best of cases, although some preliminary results with dendritic cell-based vaccines seem promising. Efforts must now be redoubled in order to improve our understanding of the mechanisms of protection, virological control and immune deterioration, as without this knowledge an efficacious therapeutic vaccine remains a long way off. Given the toxicity and long-term efficacy problems associated with current drugs the search for therapeutic vaccines must be seen as a priority line of investigation.

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