Prospects for HIV-1 therapeutic immunisation and vaccination: the potential contribution of peptide immunogens

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Background: Human immunodeficiency virus (HIV-1) infection continues to challenge the development of antigen-specific immune-based strategies for the management (therapeutic immunisation) and prevention (vaccination) of HIV-1 infection. Objective: This review aims to assess current prospects for HIV-1 therapeutic immunisation with particular emphasis on the contribution of peptide-based immunogens. Methods: The potential for therapeutic immunisation to provide immunological support that can allow for prolonged safe ART-free periods is discussed in light of the Strategies for Management of Antiretroviral Therapy (SMART) study. Different approaches to peptide design are considered including the quality of T-cell responses desired. Results/conclusion: Synthetic peptide immunogens are amenable to modification to improve immunogenicity and reactivity to multiple virus subtypes. Ideally peptide immunogens should incorporate combinations that target restricted, relevant polyfunctional epitopes to regions of HIV-1 associated with control of infection. Peptides showing a beneficial effect following therapeutic immunisation may provide the basis for a future preventative vaccine.

Keywords: HIV-1, immunotherapy, peptide, preventative vaccine, therapeutic immunisation, therapeutic vaccine

1. Introduction

In the last few years two large clinical trials that enrolled several thousand patients have significantly influenced the prevention, care and treatment of HIV-1-infected individuals. The Strategies for Management of Antiretroviral Therapy (SMART) study had as its objective to determine the feasibility and safety of intermittent antiretroviral therapy (ART). This trial was halted early when patients in the drug-conservation group (on intermittent ART) were found to have more adverse events than patients on continuous treatment [1]. More recently, the STEP study (also known as HVTN 502 or Merck V520-023) analysing Merck’s promising recombinant Ad5 vaccine candidate was discontinued after the results of an interim report showed that the vaccine was ineffective in preventing new infections [2].

These results have prompted new considerations when designing interventions for the management (therapeutic immunisation) and prevention (vaccination) of HIV-1 infection. To date, clinical trials within these areas have been dominated by DNA prime–boost strategies using recombinant adeno- or poxviruses. This review will discuss current prospects for therapeutic and prophylactic approaches for HIV-1 infection in light of
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2. Current HIV-1 management

2.1 Antiretroviral therapy (ART)

The latest figures from UNAIDS show that in the course of 2007 there were approximately 33.2 million people living with HIV-1 [3]. Although this figure is reduced from previous years due to more accurate testing particularly in India, the number of infected individuals is still unacceptably high and continues to grow.

At present, the management of HIV-1 infection relies primarily on combination antiretroviral therapy (ART) (previously highly active antiretroviral therapy [HAART]) which normally consists of at least three different antiretroviral drugs that directly target steps in the virus life cycle to inhibit replication/production. ART suppresses HIV-1 to below the level of detection, defined as < 50 – 75 copies/µl blood; however reservoirs of infected cells persist in sanctuary sites such as the gut and brain that are poorly accessed by- or susceptible to ART. As a result, ART at this time cannot eradicate infection and must therefore constitute a life-long daily medication.

2.2 ART: benefits, limitations and challenges

ART has revolutionised the care of HIV-1-infected individuals, where it has been available, by converting a terminal disease to a chronic manageable infection. The major targets for antiretroviral therapy were initially the reverse transcriptase and the protease enzymes. Fuzeon (T20), the first fusion inhibitor, is generally used for treatment-experienced patients since this is injected subcutaneously. Two new ‘first-in-class’ antiretroviral medications were launched in 2007. These target virus entry (a CCR5 antagonist) and integration into host chromosomal DNA respectively. At present, these new treatments are more expensive and will therefore be used preferentially for treatment-experienced patients harbouiring viruses resistant to other antiretroviral medications.

Although ART has had a significant effect on the level of AIDS-defining illness in Europe and North America [4], the treatment remains costly; this also has an effect on its global availability. ART is also associated with side effects [5,6], which usually become more prominent after 3 years [7] and may also be influenced by host genetic factors [8]. For example, Efavirenz has been associated with teratogenic effects which are especially relevant as more women reach childbearing age [9].

(Multi)drug-resistant viruses continually emerge [10] and will eventually develop against the new viral medications as well. Drug-resistant viruses are transmissible, albeit usually with poor replicative capacity [11], restricting treatment options for newly infected individuals. Drug resistance monitoring is therefore of increasing importance as ART becomes more widely used, a feature that only adds to the financial burden of HIV-1 management, particularly in resource-poor settings.

The success of ART, however, poses new challenges. Although the mortality of HIV-1-infected individuals on ART is no longer always attributable to AIDS-related illness, mortality may now be linked to the cumulative irreversible effects of long-term ART use and/or the affects of persistent low level viral replication [12]. New medications are released due to their improved potency, short-term toxicity profile and convenience; however, the potential long-term effects are unknown. Furthermore, as patients live longer, the effect of continuous treatment on age-related conditions such as osteoporosis, cardiovascular disease, cancer and renal disease has become apparent.

2.3 Other applications of ART

The concept of pre-exposure prophylaxis (PrEP) is also under investigation. PrEP is usually the administration of a single or preferably dual antiretroviral therapy to individuals at risk of infection [13,14]. Although this concept sparked some controversy initially particularly with respect to practicability, efforts to determine the potential efficacy of this approach have continued [15]. Preliminary mathematical modelling suggests that PrEP will be beneficial provided the medication(s) used are effective, their implementation is sustainable and adherence strong [16]. The mathematical modelling however is limited in that the potential effects of drug resistance, side effects and concomitant infections that influence susceptibility to HIV-1, have not been taken into consideration as yet. The true potential for PrEP will have to await the results of long-term clinical evaluation [17].

Postexposure prophylaxis (PEP) is usually combination ART administered shortly after occupational exposure and is continued for 4 weeks to inhibit the establishment of infection [18]. This short-term exposure to ART has been associated with side effects that are relieved on treatment discontinuation [19], however, potential long-term effects remain to be determined.

It is conceivable that, over time, the number and diversity of drug-resistant viruses may eventually affect the efficacy of post and pre-exposure prophylaxis. The development of a preventative vaccine that reduces the number of infections and lowers viral load, should infection occur, may later replace the need for PrEP.

3. Therapeutic immunisation

3.1 Background

The lack of immune correlates of protection for HIV-1 disease [20] hampers efforts to develop effective vaccines. However, results from therapeutic immunisation studies may be able to provide some insight by determining the overall effect of immunisation on CD4 count and viral load.
Multiply exposed seronegative individuals provide evidence that immune control is possible [21]. Interestingly, a pause in multiple exposures for a proportion of such individuals can result in late seroconversion despite earlier cell-mediated immunity (CMI) [22]. This suggests the CMI responses may not have induced sufficient memory or that mainly transient innate immunity was induced, such that boosting through multiple exposures was necessary to maintain immunity.

One other line of evidence that containment of infection is possible is the small percentage of infected individuals that can control infection in the absence of antiretroviral therapy for at least 10 years (long-term non-progressors (LTNPs)) [23]. However, signs of progression may eventually emerge after 10 years [24]. Elite controllers, in contrast, are defined as individuals that maintained viral RNA levels below the level of detection for one year. HIV-1 controllers appear to be infected with fully functional virus in the vast majority of cases, suggesting that host factors most frequently account for this virus control. Viraemic controllers maintain viral levels between 50 and 2000 copies/ml. Elite and viraemic controllers appear to have narrow CMI responses preferentially targeting Gag [25,26]. This is in agreement with increasing observations that Gag responsiveness is more often associated with virus control, whereas in one study immune responsiveness to Env was predominantly associated with higher viral set points [27]. The precise mechanism of action behind these observations, however, is presently unknown and cause and effect relationships should not be immediately assumed, as they may be related to levels of virus exposure and/or ongoing replication.

3.2 Aims of therapeutic immunisation

Antiretroviral therapies will continue to evolve in an effort to minimise side effects, circumvent existing drug resistance and potentially cure the infection. Single tablet combinations will improve adherence although drug resistance may eventually undermine their long-term efficacy.

The concept of therapeutic immunisation was first raised in 1987 for HIV-1 [28], although it may similarly be applied to other infectious agents inducing chronic infection. Therapeutic immunisation is often inaccurately referred to as therapeutic vaccination; however, the term vaccination can be misleading and strictly, should be reserved for prophylactic or preventative interventions [29]. Immunotherapy is usually associated with modulating the immune system through the use of cytokines rather than antigen-specific stimulation.

The main aim of therapeutic immunisation is to immunise individuals already infected with HIV-1 to induce antigen-specific immune responses (essentially T cell immunity) that can sustain immunological competence (control). Immunological competence during HIV-1 infection is clinically related to the level and stability of CD4+ T cells [30-32]. ART reduces virus levels to below the level of detection or, < 50 – 75 copies/µl by conventional clinical assays but does not eliminate non-circulating infected cells residing in tissues. Therapeutic immunisation therefore has a number of potential applications that could complement ART:

- To sustain immunological fitness or viral control during safe prolonged ART-free periods (i.e., allow ART to become a safe intermittent therapy).
- To sustain immunological fitness or viral control early in the disease course such that the initiation of continuous ART can safely be deferred.
- To maintain immunological fitness or viral control in individuals on continuous ART (particularly for patients who fail to regain or maintain CD4+ T cell counts).

Immunisation while patients are on ART has a number of advantages. ART allows for some immune reconstitution [33] where naive CD4+ T cells are generated that can be stimulated by therapeutic immunisation. Suppression of HIV-1 allows the immune system to focus on the administered immunogen in the absence of competing circulating virus. ART will also suppress any virus burst arising from HIV-1 infected CD4+ T cells activated as a consequence of immunisation [34]. The lack of success of earlier studies on HIV therapeutic immunisation may in part be because they were in the era prior to combination ART and the advantages of ART were unavailable at the time.

3.3 Prolonged ART-free periods

When combination ART was introduced in 1996 treatment initiation was recommended at diagnosis ‘hit early hit hard’ [35,36]. Indeed, structured treatment interruptions met with more success for acutely infected individuals than for chronically infected individuals [37,38]. This may be related to number and nature of CD4 counts in acutely (newly) infected individuals compared with those of chronically infected individuals [39]. The limitations of ART following long-term use resulted in the recommendations for ART initiation being amended and based mainly on the level of CD4+ T cells in peripheral blood [32]. Instead of recommending ART at HIV-1 diagnosis, ART was indicated when CD4+ T cell counts fell below 200 cells/µl, which usually occurs later in the disease course. In practice it appeared that many initiated treatment at well below 200 cells/µl [40]. The results of the SMART study, however, have led to the suggestion that ART should be initiated when CD4 counts fall below 350 cells/µl rather than 200 cells/µl [41]. The most recent guidelines from the Department of Health and Human Services in the United States have taken this into consideration. ART is now indicated in patients with CD4 counts < 350 cells/µl or with a history of AIDS-defining illness [42].

The SMART study was large multi-centre trial that enrolled 5472 patients in order to determine the safety of ART-free periods where ART indication was based primarily...
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on CD4+ T cell counts [1]. This study was halted early in January 2006 due to adverse events. Patients had been divided into two arms, a continuous ART arm and an intermittent ART arm. In the intermittent ART arm, ART was discontinued when the volunteer had a single reading above 350 CD4+ T cells/µL. ART was resumed as soon as a single CD4 measurement below 250 CD4+ T cells/µL was observed. This resulted in patients being taken on and off ART at varying intervals without necessarily an opportunity for immune reconstitution while on ART. The results of the study suggested that treatment interruption was unsafe. The TRIVACAN study with the same CD4 start and stop regimen showed the same results as the SMART study [43]. Other smaller treatment interruption studies, for example the STACCATO and BASTA studies, found that prolonged treatment interruption was generally safe [44,45]. These were smaller studies where it was suggested that the rate of CD4 decline was related to the CD4 nadir.

Patients with higher preART CD4+ T cell counts have the potential for longer treatment interruptions compared with patients with lower preART CD4+ T cell levels [46]. Other studies have linked the duration of ART-free periods to the CD4 nadir where patients with nadir values of 350 or above were able to achieve ART-free periods of median 22 (6 – 46) months [47], 24 (16 – 28) months [48] and 15 months (mean) [49]. Furthermore, that patients having a nadir < 350 have been shown to achieve prolonged ART-free periods if they had gained > 150 CD4 cells while on ART [46]. None of these studies included therapeutic immunisation. However, a successful therapeutic vaccine could allow patients with lower preART CD4 levels to achieve similar treatment interruption stability provided they have experienced CD4 gain while on ART.

Treatment interruption is still a valid concept but it is important to identify a safe and effective means by which to stop all antiretroviral therapy [50], as well as to identify the optimal duration for both treatment interruptions and periods on ART. It is important that for planned treatment interruption, discontinuation of antiretroviral therapies should be according to the half-life of the treatments used, as delineated in current guidelines [42], to avoid functional monotherapy by a single drug with a long half-life such as efavirenz [51] which could then select for resistance mutations. Furthermore, to provide a beneficial effect, treatment interruptions supported by therapeutic immunisation would need to be of sufficient duration to alleviate ART side effects without compromising general immunological fitness and patient safety. Similarly, periods on ART would need to be of sufficient duration to recover stable CD4+ T cell counts to their original levels on ART.

The duration of ART-free periods supported by therapeutic immunisation should mainly be determined by immunological fitness (CD4 counts) in line with current treatment guidelines rather than by viral load. The precise effect on immunological fitness could be assessed by measuring the rate of CD4 decline in patients receiving therapeutic immunisation compared with a placebo group. CD4 counts during treatment interruption in the absence of therapeutic immunisation have been shown to reach preART levels within 3 months. Similarly, viral load tends to settle at preART values within 3 months of stopping ART [52]. The effect on viral load following therapeutic immunisation remains to be determined. The current focus of therapeutic immunisation is to reduce the viral set point, however, this may be a formidable challenge bearing in mind that viral reservoirs have never been successfully eliminated. However, viral rebound may be delayed. Whether the delay in rebound might be extended with each successive treatment interruption remains to be determined.

In an attempt to remove viral reservoirs, particularly those containing multi-resistant viruses, periodic ex vivo purging of infected monocytes from peripheral blood during treatment interruption has been investigated [53]. This approach has met with success in a small number of individuals. This form of intervention would probably only be practicable for highly treatment-experienced patients since purging of infected monocytes was done on a weekly basis.

Safe intermittent ART supported by therapeutic immunisation would carry the benefit of alleviating the financial burden on healthcare services as well as moderating side effects by reducing ART exposure. Alleviating side effects may also improve compliance, which, in turn, would positively affect drug resistance. Prolonged drug-free periods would also affect the development of drug-resistant strains because drug-resistant viruses have, in general, decreased replicative capacity and will therefore be competed out of the rebounding viral population during the ART-free periods resulting in the production of predominantly wild type viruses that are sensitive to ART [54]. However, it is unlikely that prolonged drug-free periods supported by therapeutic immunisation would eradicate resistant viruses since these may persist in latently infected cells even under effective ART [55].

Therapeutic immunisation strategies that can complement ART and sustain CD4+ T cell levels in the absence of ART would make an important contribution to the overall management of HIV-1 infection.

3.4 Delaying ART

This concept is based on the potential for therapeutic immunisation to sustain CD4 counts in the absence of ART. The objective would be to slow CD4 decline, thereby delaying the need for continuous ART in newly infected individuals. However, such an approach would rely on accurate early diagnosis of infection.

Even prior to the advent of combination ART, sustained immune responses to the Gag protein, particularly p24Gag were found to be inversely correlated with disease progression [56-58]. These early observations, originally measuring antibody responses, have since been
corroborated and extended to include cell-mediated immune responses [27,59-60]. Hence, one therapeutic immunisation approach may be to sustain Gag-related immune responses. This in turn may serve to slow CD4 decline (thereby disease progression) and delay the need for ART. It may also allow for a slower build-up of viral reservoirs and result in a lower viral set point.

3.5 Continuous ART
ART suppresses virus replication but does not eliminate sequestered resting infected cells. In this way, by combining ART with a therapeutic immunisation eliciting an effective immune response, most probably CMI, virus levels may be suppressed to below the level of detection whilst infected cells are eliminated over time (thereby reducing viral reservoirs). If therapeutic immunisation can serve to sustain CD4 levels, it may also be used as an adjunct to ART particularly for patients that do not regain CD4+ T cell counts despite complete virus suppression.

4. HIV-1 vaccination

To date, there is no prophylactic (preventative) vaccine for HIV-1 despite over 25 years of intensive research and activity in the field. It became clear early on that conventional approaches to vaccine design aimed at inducing sterilising immunity faced a daunting challenge due to the hyper-mutability of the virus, the diversity of HIV-1 subtypes and the masking of critical epitopes. Initially vaccine approaches focussing on inducing neutralising antibody responses to envelope glycoproteins were not successful [61,62]. This field is now experiencing a renaissance since antibody approaches have been successful in preventing infections in non-human primates and represent one of the priorities of the Collaboration for AIDS Vaccine Discovery (CAVD) supported by the Bill and Melinda Gates Foundation. Vaccine approaches presently undergoing clinical evaluation aim to induce T cell immunity mainly through the use of DNA prime-boost strategies involving recombinant viruses such as adenoviruses and poxviruses [63,64]. Furthermore, since HIV-1 generally infects through mucosal membranes, the induction of mucusal as well as systemic immunity is likely to be necessary [65].

The expectations for a future prophylactic/preventive HIV-1 vaccine have now been moderated. As it appears unrealistic to date for HIV-1 vaccination to confer complete protection, it should nevertheless induce responses to HIV-1 that can reduce the risk of transmission and prevent disease progression should a vaccinated individual become infected, for example, induce a LTNP/elite progressor state [66]. This rationale was also a feature of the recent STEP study of Merck's recombinant Ad5 vaccine candidate.

The interim results from the STEP study have, however, questioned this rationale and, importantly, raised new considerations regarding the use of recombinant replicating viruses or other microbes as vaccines for HIV-1. Although the viral load in the infected persons was as high in the vaccinated individuals as in those who received a saline placebo, the effect on disease progression could take years to become clear. Adenovirus 5 (Ad5) is a common infection and patients were stratified according to their prior immunity (antibody titre) to Ad5. The original concern was that such prior immunity might lower the efficiency of immunisation through rapid elimination of the inoculated vaccine strain. However, initial analysis of the data suggested that patients with prior immunity to Ad5 in the test group were more likely to become infected than patients with prior immunity in the placebo group [67,68]. One plausible explanation so far for this conundrum, is that immune responses to the Ad5 vector produced inflammation and/or immune activation at the site of inoculation, resulting in greater numbers of activated T cells susceptible to HIV-1 infection [69]. However, because an empty adenovirus vaccine control was not given, this will remain speculation. Furthermore, the infections occurring in placebo recipients with higher Ad5 antibody levels occurred at a lower rate than in the Ad5-antibody negative placebo recipients, suggesting that adenovirus immunity alone had an effect on the rate of HIV infection, making the overall interpretation of the study very difficult.

Although the results from DNA prime–boost strategies with recombinant poxvirus vectors indicated poor immunogenicity in humans [70] despite high immunogenicity in nonhuman primates [71], a large efficacy clinical trial of 16,000 volunteers continues based on interim Data Safety Monitoring Board recommendations. Efforts are underway to improve the efficacy of this approach and several clinical trials are in progress [63,64].

5. The case for peptide immunogens

Peptide immunogens that could constitute future therapeutic immunisation strategies or even vaccines, will need to take into consideration the complexity of HIV-1 pathogenesis both with respect to the virus itself (antigen diversity) and the human host (human leukocyte antigen (HLA) diversity).

5.1 Challenges

The efficacy of peptide-based interventions will be dependent upon peptide design, route of administration and choice of adjuvant. As synthetic entities, peptide immunogens are simple in that they can be administered directly without the need for a replicating vector. HIV-1 shows extensive genetic diversity and has the ability to escape immunological pressure through mutation of both potential neutralising domains for antibody responses as well as cytotoxic T lymphocyte (CTL) epitopes for cell-mediated immunity. Whilst escape from neutralising antibodies does not hinder viral replication by itself, mutations of certain epitopes for cell-mediated immunity usually come at a fitness cost. Another form of immunological
escape involves the viral regulatory proteins, such as Nef, that have the capacity to downregulate HLA classes I and II rendering infected cells undetectable to immune surveillance [72] or Tat inducing hyperactivation, anergy and apoptosis of bystander cells.

A significant challenge to developing immune-based interventions is that HIV-1 infects CD4+ T cells, macrophages and dendritic cells, the very cells that are pivotal for inducing an immune response. Whilst productively infecting activated HIV-1-specific CD4+ T cells, HIV-1 remains latent in resting cells. This feature may hamper the success of immunogens administered to chronically infected individuals in the absence of ART.

Taken together, these features of HIV-1 present a formidable challenge to the success of any immunological intervention targeting HIV-1 prevention (vaccination) or management (therapeutic immunisation).

5.2 Considerations for peptide design
The considerations for peptide design described here are also applicable to other epitope-based strategies used to design novel recombinant proteins or DNA constructs encoding novel proteins.

5.2.1 The diversity of HIV-1
The diversity of HIV-1 variants and novel circulating recombinants represents a significant challenge to peptide design. HIV-1 is divided into three groups M (Main), O (Outlier) and N (non-M non-O). The majority of virus variants belong to group M which includes subtypes or clades A-D, F, H and J-K which differ by ∼25 – 35% in their env and 15% in their gag gene sequences.

Peptides that are designed based on diversity within only one specific virus subtype would probably restrict their use to the geographical regions where these strains predominate. They would be less effective against strains arising from other subtypes or novel recombinants. Ideally, peptide immunogens should be broadly reactive against multiple virus clades. Efforts to generate such peptides include basing peptide design on consensus or ancestral sequences since these might have the potential for broader cross-clade responses [73,75]. Both consensus and ancestral sequences are derived from sequence information collated in databases. However, the level of sequence information available for each subtype in these databases will vary, resulting in an over representation of certain subtypes. This in turn may affect the fidelity of the derived consensus with respect to the other virus clades. Although both consensus and ancestral sequences are artificial, it is anticipated that they will share sufficient similarity to multiple variant circulating strains.

Peptide design may also focus on conserved domains, which are defined as regions with low mutation frequency and should provide broader crossreactivity to multiple virus strains. Such sequences are probably conserved because integrity of the region is necessary to maintain an essential function. Perturbation of such regions by mutation may therefore affect viral fitness [76]. Indeed, therapeutic immunisation with respect to peptides directed at conserved domains of the major capsid protein (p24) within Gag showed little mutation over time, indicating that immune escape from such regions may be restricted because it comes at a fitness cost [77].

The mutability of HIV-1 may lead to changes that affect epitope presentation at the cell surface. Mutation can result in poor binding to anchor domains on the HLA molecule [78], failure to interact correctly with proteins and enzymes involved with antigen processing, and/or altered structural interactions with the HLA bearing the epitope and the T cell receptor. Such mutations may therefore affect the magnitude of the T cell response or ultimately induce immune escape. Similarly, by designing peptides to regions harbouring drug-resistance mutations, it has been suggested that immune escape may confer drug susceptibility and thereby prolong the life expectancy of ART regimens [79].

The multitude of virus strains suggests that therapeutic immunogens must contain multiple epitopes to ensure sufficient potential to target a diversity of virus strains and human leukocyte antigens (HLA). One concern is that peptides, by being short molecules, will carry too few epitopes to be effective and at worst drive immune escape to those particular regions of the virus.

Efforts to maximize the number of epitopes available include artificially stringing together multiple potential CTL epitopes [80] and designing compound peptides [81]. Compound peptides consist of concatenating 9-mers where potential epitopes have been identified following analysis of proteasome cleavage, transporter associated with antigen presentation (TAP) transport and trimming by peptidases in the endoplasmic reticulum. However, these approaches may lead to the generation of junctional epitopes unrelated to HIV and it is unclear how these may affect immunogenicity. These approaches await verification in in vitro systems or animal models. Although concatenating epitopes seems logical, it is also interesting that epitopes in nature tend to be overlapping.

An alternative approach to polyvalent vaccines is to develop mosaic proteins derived from different HIV-1 subtypes or gene sequences optimised to carry the maximum number of common T cell epitopes [78]. As such these represent natural sequences from multiple different strains. This concept has so far only been assessed in mice as part of a DNA prime-boost strategy using a recombinant vaccinia virus vector [74]. Broad T cell responses were observed to peptide epitopes corresponding to subtype A, B and C viruses. However, since these studies involved mice that are resistant to HIV infection and their MHC proteins are different from HLA molecules, human clinical trials will be...
required to assess whether the immune responses induced will be effective.

Peptides of 15 amino acids in length have usually been used in in vitro assays to delineate regions of a protein inducing a proliferative response or resulting in cytokine release. This has led to the hypothesis that groups of overlapping synthetic peptides (OSP) may be used as candidate immunogens [82]. Cocktails of 15-mers would therefore result in multiple epitopes to multiple HLA. However, being such short sequences, it is conceivable that they may interact with HLA class II only at the cell surface. They may also be trimmed by cell surface proteases so that they generate smaller peptides that bind to HLA class I. It is not clear how this will affect the immune responses generated or whether intracellular processing will be a more effective means of generating long-term CTL responses.

One potential problem that will be encountered by any polyvalent vaccine is the potential for certain epitopes to exert dominant responses that overshadow those of other epitopes in the cocktail [74]. This is also an important consideration when using viral vectors such as poxviruses as they may also express dominant epitopes. Dominant epitopes, however, are not necessarily the ones that elicit protective responses. Epitope hierarchy is not necessarily due to tight binding to HLA but rather linked to the magnitude and kinetics of peptide processing. Epitope dominance has been shown to be influenced by the flanking sequences. Interestingly, transposition of flanking sequences for one immunodominant epitope to one that was subdominant enhanced the production and antigenicity of the subdominant epitope [83].

Although multi-epitope approaches aim to ensure reactivity to diverse viruses in populations with diverse HLA, it is interesting that in individuals that control infection, the CTL response is not broad but restricted to the Gag protein.

5.2.2 HLA diversity
In addition to HIV-1 diversity, there is also great diversity in the human population determined by human leukocyte antigen (HLA). HLA class I antigens are associated with presenting antigen to CD8+ T cells whereas HLA class 2 present to CD4+ T cells. Interestingly, certain HLA types have been associated with improved control of infection particularly the class I antigens B57 and B27 [84]. A higher proportion of LTNP's and elite controllers possess these antigens [27,28]. In contrast, one other HLA marker, HLA-B*35 has been associated with an accelerated onset of AIDS [85]. The precise mechanism for these effects remains elusive and has been the subject of intense study. Possible explanations include restriction of viral escape and that B57 responses in particular, were associated with a narrow range of Gag-specific epitopes. This is based on the remarkable observation that closely related HLA molecules (e.g., HLA-B*5703 and HLA-B*5701 which differ in only two amino acids) may bind the same epitope but stimulate T cell repertoires with different properties. Individuals expressing HLA-B*5701 tend to express T cells recognising dominant Gag epitopes as well as epitope variants whereas individuals with HLA-B*5703 recognise a more restricted T cell repertoire [86].

Finally, the apparent HLA restriction of a peptide or group of peptides may be overcome by peptide dose. In a dose-finding study of a peptide-based therapeutic immunisation candidate carrying HLA-A2 epitopes, HLA-A2-positive individuals responded to both low and high peptide doses whereas HLA-A2-negative individuals mainly responded if they received the higher dose [87]. This suggests that peptides, on processing, generate multiple epitopes to diverse HLA. By increasing the ‘concentration’ of diverse epitopes generated, the potential for broader recognition with alternate HLA antigens available in the host is enhanced.

5.2.3 The quality of CD4 and CD8 responses
In order to provide cell-mediated immunity, peptides of interest should carry epitopes that can stimulate the induction of polyfunctional CD4+ and CD8+ T cell responses that are associated with long-term memory. This comes from observations that progressors tend to have mono-functional cell responses (CD4+ and CD8+ T cells releasing only IL-2 and IFN-γ) whereas LTNP's have polyfunctional CD4+ T cells producing both IL-2 and IFN-γ reflecting memory T cells at different stages of differentiation. IFN-γ is an early effector response that will decline with viral clearance whilst IL-2 secretion is typical of long-term memory (predominantly CD4+ T cell responses). It is therefore important to assay both parameters when assaying the potential of peptide antigens.

If therapeutic immunisation is to be used to support ART-free periods, it is important that CD4+ T cell numbers are sustained. The use of IL-2 alone to sustain CD4+ T cell numbers is possible but does not induce antigen-specific responses. This provides evidence that it is not only the quantity but the quality of CD4+ T cells that is important [20]. In this way, IL-2 may be combined with therapeutic immunisation to potentially improve CD4+ T cell numbers and the effect of therapeutic immunisation [89-92].

5.2.4 The route of administration
HIV usually infects through mucosal membranes and it is therefore important to induce not only systemic but also mucosal immunity. The majority of approaches used so far have targeted intramuscular immunisation (inducing mainly systemic immunity) and more recently approaches targeting dendritic cells. Nasal, vaginal or rectal administration represent approaches to induce mucosal immunity [93].

Mode of delivery will influence which antigen-presenting cells may take up the peptides and their potential to present
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epitopes of interest on HLA classes I and/or II. It will also affect the type of adjuvant and the half-life of the peptides. Intramuscular injection will result mainly in exposure of the antigen to macrophages that take up exogenous antigens primarily through the HLA class II pathway leading to a Th2 response. In order to get antigen presented in the context of HLA class I and elicit a Th1 response, peptides need to be introduced into the cytoplasm. Lipopeptides for example, include a fatty acid addition to achieve this (89,90). Alternatively, peptides may be introduced into the skin directly via intradermal injection. Dendritic cells in the dermal layer are capable of cross presentation and can therefore transfer antigen into the cytoplasm, where peptides may be transported through the proteasome to the HLA class I presentation pathway. The ability of dendritic cells to migrate provides the theoretical possibility that dendritic cells may also be able to influence T cells of the gut mucosa. However, this possibility needs to be investigated further.

Dendritic cells can be of myeloid or lymphoid origin. In tissues such as the skin, myeloid dendritic cells include Langerhans cells and dermal dendritic cells. Dendritic cells can be isolated and exposed to peptides ex vivo or alternatively accessed through intradermal injection. Specialised plasters for accessing the dermal layers have been devised but have only been used so far for DNA vaccines incorporated into nanoparticles (94).

Lu et al. showed the potency of dendritic cells in therapeutic immunisation. Dendritic cells were bombarded ex vivo with the patient's own virus that had been chemically inactivated. The patients demonstrated a transient but nevertheless dramatic reduction in viral load (95). Similar results have also been reported for people on ART (96). However, ex vivo administration on a mass immunisation scale will be cumbersome and care must be taken to ensure sterility of the culture mixture can lead to tolerance (97). Such a strategy may not be affordable or practicable for the majority of patients and healthcare providers but could result in important insights for further studies.

Transcutaneous delivery of peptides following mechanical micro abrasion of the skin surface has also been evaluated in a mouse model. This approach enhances access and delivery to dendritic cells directly within the dermal layers of the skin. A fusion peptide was used as immunogen containing both CTL and T helper epitopes. Adjuvants included cholera toxin (CT), heat labile enterotoxin from Escherichia coli (LT) or CpG oligonucleotides. Interestingly in this mouse model, both systemic and mucosal immunity was triggered. However, should this method be applicable to humans, alternate CpG oligonucleotides will be needed in order to induce responses human dendritic cells (98).

Lipopeptides (HIV-1 peptides carrying a palmitoyl fatty acid chain and covalently linked to a T helper tetanus toxoid peptide) (89,90,99) represent an approach with several different candidates that are undergoing clinical evaluation. Lipopeptides have been used in conjunction with IL-2, which stimulates T cell proliferation and a recombinant poxvirus (90) prior to a 3-month treatment interruption. Among the immunised group 24% had a lower viral set point after 3 months compared with 5% of the controls. When the study ended, patients were followed until they resumed ART and the overall median treatment interruption extended to 35 weeks. It is unclear whether the observed effects were due to the lipopeptides or to IL-2. However, a clinical trial studying one lipopeptide cocktail was recently suspended due to adverse events where a patient experienced neurological symptoms (100). Further meta-analysis of all lipopeptide trials however, showed that lipopeptide vaccine safety was acceptable (101). More recently it was shown that lipopeptides administered intradermally induced equivalent immune responses as those induced via the intramuscular route when only 100 μg of peptide was used compared with 500 μg. Furthermore, that the route of administration affected the T cell responses induced; the intradermal route induced greater CD8 responses compared with the intramuscular route that induced greater CD4+ T helper responses (102).

Another peptide candidate comprising peptides modified by amino acid substitution to the HIV-1 p24 protein similarly showed a lower viral set point after 3 months of treatment interruption amongst high-responder patients compared with low-responders (103). Responders were identified by virtue of their delayed type hypersensitivity responses (DTH). Sixty two per cent of the patients were still off ART 1.5 years after completing immunisation. Eighty four per cent of the patients still had positive DTH responses and 78% had detectable peptide-specific T cell proliferative responses at this time suggesting that immunological memory to the peptides had been developed. DTH high-responder patients had a delayed return to ART compared with DTH low-responders (104) showing indirectly improved preservation of CD4+ T cell numbers in patients with renewed immune responses to p24Gag following peptide immunisation.

Despite the success of dendritic cell immunisation, it is important to keep in mind that HIV-1 can infect dendritic cells and induce tolerance. In addition, dendritic cells may potentially enhance infection by binding HIV-1 envelope glycoproteins by virtue of the DC-specific intercellular adhesion molecule grabbing nonintegrin (DC-SIGN). HIV-1 bound to the surface of dendritic cells can be transported to the lymph nodes and infect CD4+ T cells during antigen presentation. Dendritic cells tend have long contact with CD4+ and CD8+ T cells enhancing the potential virus transmission. Sustained dendritic cell numbers appear to be associated with long-term non-progression since patients infected for over more than 10 years (in the absence of ART) retain their dendritic cell numbers. This suggests that the number of circulating DCs may also play a role in the control of viraemia (105). Dendritic cells in chronically infected individuals are defective and it is unclear whether long-term ART can reverse this. In this way, whether maintenance of
CD4+ T cell numbers following therapeutic immunisation will similarly sustain dendritic cell numbers and thereby prevent disease progression remains to be determined.

6. Concluding comments

Recent advances in the field of HIV research have provided important information for the design of therapeutic antigens. Ideally, therapeutic immunisation should stimulate polyfunctional CD4+ and CD8+ T cell responses that can decelerate CD4+ T cell decline, reduce or eliminate viral reservoirs, lower the viral set point, induce long-term memory and confer a LTNP status or ultimately an elite controller status on infected individuals. Although there has been much focus on generating multi-epitope antigens, it appears that control of infection is associated with restricted immune responses to particular regions of HIV-1 within the Gag proteins.

Peptides represent simple synthetic entities that can be specifically designed and screened to include epitopes of interest that stimulate polyfunctional CD4+ and CD8+ T cells. The number of epitopes used can be controlled in part by peptide length. In this way they may provide immune responses that reflect the restricted responses associated with virus control in nature. Epitope dominance can be assessed in the context of different peptide cocktails as well as by investigating flanking sequences. Such flexibility of design is not available for larger protein antigens (or DNA corresponding to such antigens) or recombinant virus vectors.

Peptide design and optimisation may allow for the development of promising candidates for therapeutic immunisation. A combination of such candidates showing beneficial effect may then form the foundation for a future prophylactic HIV-1 vaccine.

7. Expert opinion

The development of effective therapeutic immunisation interventions for the management of HIV-1 infection remains an important goal of HIV research. ART-free periods represent one means to alleviate the high cost of ART, combat the emergence of drug-resistant viruses and alleviate long-term side effects. The SMART study, which is the largest so far assessing treatment interruptions, however, concluded that treatment interruption was unsafe, particularly in the absence of immunological support. This will therefore have implications for including a placebo control group in clinical trials assessing therapeutic immunisation strategies since a placebo group will stop therapy in the absence of immunological support. However, inclusion of a placebo group should be possible if patients are returned to ART when CD4+ T cell levels fall below 350 cells/µl—since this recommendation arose from the SMART study. Other smaller studies using peptide-based interventions (some in conjunction with recombinant virus vectors and cytokines) have indicated that ART-free periods may be safe if carried out in conjunction with therapeutic immunisation. However, larger clinical trials will be needed to verify these observations.

Approaches currently under investigation that focus on eliciting cell-mediated immunity include viral vectors, DNA prime–boost strategies, and peptides. DNA vectors have been found to provide only weak priming and prior immunity to recombinant virus vectors may either impede effect or increase susceptibility to infection. Peptides represent much simpler synthetic entities and may function in the absence of a recombinant viral vector. Peptides can be based on conserved, consensus or ancestral sequences to acquire broad reactivity to multiple virus subtypes. Peptide design can identify epitopes following *in silico* assessment of their processing and presentation (i.e., using computational methods including bioinformatics tools) complemented with *in vitro* laboratory analyses. Furthermore, peptides can be of sufficient length to encompass a discrete and limited number of epitopes that induce relevant polyfunctional T cell responses (although the issue of epitope dominance will have to be addressed for peptide cocktails). It is likely that in the next few years there will be significant focus on HIV-1 Gag since immune responses to these proteins have been associated with control of infection and a lower viral set point. Since certain HLA types are associated with improved control of infection, efforts will be made to identify how this is achieved and whether similar properties can be conferred on other HLA types. This will require a detailed analysis of the epitopes of these HLA molecules and their flanking sequences that confer dominance.

For future therapeutic immunisation or vaccination to be effective it is paramount that the antigens are immunogenic and that they induce relevant immune responses with concomitant long-term memory. Future research is likely to focus on different routes of administration to try and achieve both mucosal and cell-mediated immunity. Although *ex-vivo* pulsing of dendritic cells has been shown to be effective, it is likely that administration *in situ*, for example through intradermal injection, will ultimately be simpler. Intradermal injection may also be as effective as systemic immunisation but at lower antigen concentrations which will also be cost effective. Identifying new routes of administration will also require identification of suitable adjuvants for optimal effect.

One other aspect that will become of increasing importance in the field is the issue of HIV-1-induced chronic immune stimulation. Disturbing evidence is emerging that HIV-1-induced immunological defects are still occurring even following sustained viral suppression in the presence of ART. Indeed, T cell activation levels in the presence of effective ART remain higher than those in uninfected individuals and may in part explain the loss and/or lack of optimal gain in CD4+ T cell counts despite effective viral
suppression below the level of detection. Further research will be required to understand the mechanism(s) involved in chronic immune stimulation. Interventions that can abrogate chronic immune stimulation would complement other therapeutic immunisation interventions and enhance the safety of ART-free periods.

Effective therapeutic immunisation may eventually be a combination of different interventions providing targeted immune responses to eliminate infected cells and alleviate chronic immune stimulation. Furthermore, such interventions could potentially confer a long-term nonprogressor/elite controller status and/or form the basis of a future preventative vaccine.

**Declaration of interest**

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