

Novel immunological strategies for HIV-1 eradication

B Jülg^{1,2} and DH Barouch^{1,2*}

¹ Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University, Cambridge, Massachusetts, USA

² Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA

Abstract

Despite the significant advances in antiretroviral therapy (ART), HIV-1 is able to persist in cellular reservoirs. Preclinical studies suggest that the latent reservoir is established within days of virus exposure, even before virus can be detected in peripheral blood. Latently infected cells remain undetectable by the immune system and can persist for years without losing their ability to produce infectious virus when ART is discontinued. Novel concepts for viral eradication strategies combine pharmacological induction of latently infected cells to produce virus together with immune-enhancing interventions to enable the host to clear these cells. In this review, we describe the early establishment of HIV-1 latency and discuss current strategies to disrupt latency and potentially enable clearance of these persistently infected cells.

Keywords: HIV-1 reservoir, HIV-1 latency, HIV-1 cure, latency disruption, therapeutic vaccines

Introduction

Only one person, the ‘Berlin patient’, appears to have been cured from HIV-1. The mechanism behind this case of possible viral eradication included allogeneic haematopoietic stem cell transplants from a donor who carried a homozygous deletion in CCR5 [1]. For the vast majority of HIV-1-infected individuals, however, this is not a feasible therapeutic approach and at present no other cure strategies exist. While antiretroviral therapy (ART) has enabled pharmacological suppression of HIV-1 replication, it has failed to eradicate the virus. HIV-1 infection remains incurable as it establishes a pool of long-lived populations of memory T-cells in which replication-competent virus persists as integrated proviral DNA, despite ART. These cells are essentially invisible to the immune system as they lack active viral replication but are capable of reigniting new rounds of infection once suppressive ART is stopped. These latently infected cells present the biggest hurdle for cure approaches.

While complete viral eradication is the ultimate goal, the concept of a ‘functional cure’ has been introduced, which includes strategies that enable host control of the virus without the need for treatment. Several approaches have been proposed ranging from gene therapy to increased resistance of target cells to HIV infection [2] strategies that stabilise the latent reservoir by interfering with HIV production as it emerges from latently infected cells [3]. Another concept known as ‘shock and kill’ or ‘kick and kill’ has been proposed. This concept is to flush out HIV from the latent reservoir by activating proviral DNA in resting cells, hoping that these cells will start producing new virus, which may facilitate recognition and elimination by the immune system. This two-step approach, however, requires a latency-reversing strategy and an antiviral immune response in order to clear infected cells. The latter aspect has proven challenging due to the profound dysregulation of the host’s immune system caused by HIV-1. In this review, we discuss current immunological approaches for the shock and kill concept by focusing on strategies to disrupt latency and potentially enable clearance of persistently infected cells.

How early after exposure is a persistent infection established?

The recently published World Health Organization (WHO) guidelines for HIV-1 post-exposure prophylaxis (PEP) recommend prompt initiation of ART in less than 72 hours postexposure [4].

The timing recommendations are primarily based on early studies in non-human primates which demonstrated that animals that initiated ART within 24 hours post intravenous simian immunodeficiency virus (SIV) inoculation showed no evidence of viral replication following discontinuation of ART. However, extending the time to initiation of treatment from 24 to 48 or 72 hours post inoculation reduced effectiveness in preventing infection [5–7]. These studies provided the first evidence that HIV-1 establishes persistent infection early in its course.

The existence of a latent reservoir was first described nearly two decades ago [8–10] and it was assumed that the initial seeding of the latent reservoir occurs during acute infection when HIV-1-RNA levels reach their peak. This led to the hypothesis that early suppression of viral replication during primary infection might prevent the reservoir from becoming established. Chun *et al.*, however, demonstrated that ART initiated within 10 days of primary infection did not prevent the generation of latently infected CD4+ T cells; again indicating that the latent reservoir is established early in infection [11]. This was further highlighted by the case of the ‘Mississippi baby’, a child born to an HIV-1-infected viraemic mother who had detectable HIV-1-RNA levels and was started on ART 30 hours after delivery and treated for 18 months [12]. Although the virus was undetectable by 29 days, and remained so for 27 months after treatment was stopped, viraemia ultimately rebounded [13].

The temporal dynamics of the seeding of the viral reservoir has also been studied in animal models. Hu *et al.* showed that virus crosses the mucosal barrier within hours of vaginal exposure to high doses of SIV [14], followed by establishment of a founder population of infected cells [15]. These *in vivo* tissue studies suggested that spreading of the infection into the draining lymph nodes, and later into secondary lymphoid organs, occurs rapidly after inoculation and requires the local expansion of the founder population of infected cells [16,17]. Nishimura *et al.* reported large numbers of resting CD4+ T cells carrying integrated SIV and simian-human immunodeficiency virus (SHIV) DNA as early as 7 days post infection in rhesus macaques [18]. More recent data proposed an even earlier dissemination of SIV infection and creation of a latent reservoir. Whitney *et al.* initiated ART in rhesus macaques on days 3, 7, 10 or 14 after infecting them with SIV. While ART significantly reduced plasma virus levels in all animals, it completely blocked primary viraemia in the animals that initiated treatment on day 3. Moreover, these animals had no detectable proviral DNA in their peripheral blood mononuclear cells, although proviral DNA was readily detectable in lymph nodes and gastrointestinal mucosa. Once ART was stopped, all animals demonstrated viral rebound, confirming early seeding of the viral reservoir within the first 3 days and prior

*Corresponding author: Dan H Barouch, Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center E/CLS-1045, 330 Brookline Avenue Boston, MA 02215, USA
Email: dbarouch@bidmc.harvard.edu

to measurable viraemia [19]. While studies in non-human primates enable us to precisely measure the timing and location of the seeding of the reservoir, these animal models necessitate using an SIV challenge dose that is higher than the typical amount of HIV-1 to which humans are exposed during sexual transmission.

Clinical implications of early HIV-1 reservoir seeding

While early initiation of ART does not prevent establishment of the latent reservoir [11], several lines of evidence show that initiating ART during the acute/early phase of the infection correlates with a lower pool of HIV-1 reservoir cells [20–22]. Ananworanich *et al.* reported undetectable total HIV-1-DNA levels in a small group of acutely infected subjects undergoing an extended antiretroviral regimen [20]. In another study, significantly lower HIV-1-DNA levels could be measured in subjects who initiated antiretroviral therapy up to 4 months into acute infection [21]. Early ART was also shown to promote long-term viral control in some infected individuals after treatment had ended [22,23]. Despite these promising results, the recent data suggesting that the viral reservoir may be established before viraemia [19] make it likely to be impossible to treat HIV-1 early enough to avoid reservoir seeding and thus additional strategies are likely to be required for virus eradication. Nevertheless, early treatment could be beneficial by reducing the barrier to cure [24].

Strategies to disrupt latent infection

The absence of viral gene expression enables evasion of latently infected CD4+ T cells from immune surveillance [25]. Activation of these cells may reverse the latent state, produce replication-competent virus and render these cells susceptible to cytolytic T lymphocytes (CTLs) or viral cytopathic effects [9,26]. Attempts to activate bulk T cells globally *in vivo* have resulted in effective latency reversal; the concomitant cytokine release, however, caused significant toxicity and prohibits this strategy for clinical use [27]. Thus, several groups of latency-reversing agents (LRAs) have been identified with the goal to induce viral replication while avoiding global immune activation.

Multiple compounds have been proposed including: histone deacetylase inhibitors (HDACi); DNA methyltransferase inhibitors (DNMTi); histone methyltransferase inhibitors (HMTi); protein kinase C (PKC) activators; Toll-like receptor (TLR) agonists; phosphatase and tensin homologue (PTEN) inhibitors like disulfiram; and others. All of these agents have demonstrated latency-reversing activity *in vitro* but only a few LRAs have undergone clinical evaluation in HIV-1-infected humans [28]. HDACis are currently the most advanced compounds for clinical evaluation as LRAs, as these molecules have been investigated intensively as anti-cancer drugs, and several agents are FDA approved for treatment of malignancies. The HDACis vorinostat, romidepsin and panobinostat have been evaluated in ART-suppressed individuals [29–31], but results so far have been unimpressive. The best studied HDACi, vorinostat (SAHA), induced a significant increase in cell-associated unspliced HIV-RNA in 90% of patients but had no effect on plasma HIV-RNA levels, concentration of integrated DNA or inducible virus in CD4+ T cells [30]. A second study to assess the effects of vorinostat on HIV-RNA expression in resting CD4+ T cells of patients on stable ART is currently enrolling. Similarly, panobinostat increased cell-associated RNA without impacting integrated HIV-1-DNA levels [31]. Romidepsin has been the only HDACi so far that has been shown to elicit detectable increases in plasma HIV-1-RNA in a small group of aviraemic patients using quantitative clinical assays [32]. A larger trial is currently enrolling to confirm these results. Administration of the PTEN inhibitor disulfiram resulted in a transient increase in single-copy assay viraemia but failed overall to reduce the size of the latent reservoir [33].

Preclinical data have also shown the potential of TLR7 agonists in SIV-infected rhesus macaques on ART. All animals developed transient increases in plasma viral load and decreases in cellular viral DNA levels, suggesting a latency-reversing and reservoir-reducing effect of this compound [34]. A clinical trial is now under way in ART-treated HIV-1-infected humans.

Concern has been raised that single agents might target only specific quasispecies of latent virus or have activity against specific cell types alone [28]. This suggests that a combination of several latency-reactivating agents targeting distinct pathways might be required to successfully mobilise the latent reservoir [35].

Strategies to enable clearance of persistently infected cells

Latency reversal alone is not likely to be sufficient to reduce the size of the reservoir. A second step will therefore probably be necessary to clear infected cells. Multiple potential strategies have been proposed to boost immune responses via immunisation or by immunomodulatory interventions. Other exogenous interventions like administration of broadly neutralising antibodies or adoptive transfer of modified antiviral T cells have been proposed as well.

Therapeutic vaccination

T cell responses have been implicated in suppressing HIV-1 replication in acute infection and have been associated with ongoing viral control in a subset of individuals who are able to control HIV-1 to low or undetectable RNA levels without ART [36,37]. These individuals maintain robust levels of highly functional CD8+ T cell responses that are able to control HIV-1 by selectively killing virus-producing cells [38]. Induction of potent antiviral T cell responses is therefore the goal of therapeutic vaccination strategies with the objective to improve host control of virus replication and/or reduce the size of the viral reservoir. So far, a number of therapeutic vaccine modalities have been tested in humans to boost pre-existing immune responses to HIV-1 [39–42]. While the majority of these vaccine concepts proved immunogenic, most studies failed to show significant virological effects and in particular did not enable sustained interruption of ART [42]. These previous therapeutic vaccine studies did not include LRAs, and studies combining LRAs with vaccines are currently ongoing.

The concept of enhancing a host's immune responses by therapeutic vaccination faces several key challenges. Recent data suggest that most of the harboured viruses in the latent reservoir contain CD8+ T cell escape mutations [43,44]. Successful vaccine strategies would therefore be required to elicit CD8+ T cell responses against previously untargeted epitopes as well as expand responses against subdominant, un-escaped epitopes. Furthermore, continuous antigenic stimulation during untreated HIV-1 infection results in chronic immune activation, immune exhaustion and loss of functional HIV-1-specific effector cells [45]. While antiretroviral therapy reverses some of the exhaustion by reducing pathogen burden, recent data suggest that HIV-1-specific CD8+ T cells from ART-suppressed individuals retain a transcriptional program poised for PD-1 expression, suggesting an imprinted exhaustion status of HIV-1-induced T cell responses [46]. An effective eradication strategy is therefore likely to require interventions to improve the quality of HIV-1-specific immune responses instead of expanding pre-existing immune responses that have already failed to control the infection.

Several novel vaccines have been developed that not only demonstrate improved immunogenicity compared to previous vaccine concepts, inducing broad and durable cellular immune responses, but also showed promising results in preclinical protection studies [47–53]. These vaccine concepts are likely to be evaluated as therapeutic vaccines in clinical trials over the next few years.

Viral vector-based vaccines

Cytomegalovirus (CMV) vector vaccines have been shown to induce broad cellular immune responses in rhesus macaques and have led to virological control and likely clearance in approximately half of the vaccinated animals against a stringent challenge with simian immunodeficiency virus (SIVmac251) [50–52]. CMV vaccines induce persistent and highly functional effector T cells, probably due to persistent viral replication. Moreover, they have been shown to induce unconventional class II-restricted CD8+ T cell responses. In particular, the latter provides a potentially important benefit for therapeutic vaccine indications, as CMV-elicited CTL responses will likely target novel epitopes that were not previously subject to immune selection pressure [54]. CMV vectors are expected to enter clinical trials in the next few years.

Adenovirus serotype 26 (Ad26) -based prime-boost vaccine regimens, such as priming with Ad26 and boosting with the poxvirus modified vaccinia Ankara (MVA) vector, have proven highly immunogenic and have afforded partial protection against acquisition of infection as well as reduced set-point viral loads following stringent SIVmac251 challenges in rhesus monkeys [47,48,55]. In contrast to Ad5-based vaccines, which failed as both a prophylactic and a therapeutic vaccine [42,56], Ad26 has several advantages that make it a promising alternative serotype adenovirus vector: (1) Ad26-based prime-boost regimens have demonstrated partial protective efficacy in the stringent rhesus monkey challenge viral models in which Ad5-based regimens have failed [47,48,53,55], suggesting their potential for clinical utility; and (2) Ad26 induces different innate immune profiles that may reduce undesirable inflammatory responses and result in more functional T cell phenotypes. The Ad26/MVA vaccine platform also can express bioinformatically optimised HIV-1 ‘mosaic’ Env/Gag/Pol antigens, which substantially augmented cellular immune breadth as compared with natural sequence or consensus antigens in rhesus monkeys [57], potentially resulting in responses against both the natural and escape sequences of individual epitopes. This may increase the likelihood that CTL responses against relevant sequences in the viral reservoir are induced. Ad26 vaccines have been assessed in several Phase 1 studies and are currently being evaluated in Phase 1/2a studies.

DNA-based vaccines

Several plasmid DNA vaccines expressing HIV-1 genes have been evaluated although their initial immunogenicity was poor [54]. Since then, novel concepts of vaccine delivery have been developed to further improve DNA vaccine immunogenicity. In particular the delivery of DNA in association with electroporation and intradermal administration has been shown to increase gene expression and vaccine-induced responses [58,59]. In particular, DNA vaccines have been shown to prime immune responses but require other viral vectors or proteins for boosting. Several concepts are being tested currently for therapeutic interventions in early phase trials in ART-suppressed individuals, including a DNA prime, MVA boost regimen vaccine displaying Env, Gag and Pol proteins, and a plasmid DNA encoding Env/Gag/Pol/Nef/Tat and Vif combined with an interleukin-12 plasmid DNA followed by a boost with a recombinant vesicular stomatitis virus (rVSV) (clinicaltrials.gov). A DNA vaccine encoding 15 HIV-1 proteins administered by skin patches was safe in ART-naïve subjects but the impact on plasma RNA copies was only modest [60].

Dendritic cell-based vaccines

A recent study demonstrated that *ex vivo* generated dendritic cells (DCs) loaded with HIV-1 lipopeptides were immunogenic in patients on ART, increasing breadth, magnitude and functionality

of T cell responses and leading to a 10 times lower plasma viral load post treatment interruption [61]. Along the same lines, Garcia *et al.* showed that monocyte-derived dendritic cells (MD-DCs) pulsed with autologous inactivated whole HIV-1 elicited efficient HIV-1 specific immune response that inversely correlated with plasma viral load set-point post cessation of ART [62]. While this vaccine regimen did not impact cellular HIV-1 DNA levels, it may have delayed the expansion of integrated HIV-1 DNA after ART interruption [63]. However, other DC-based vaccines have proven minimally immunogenic [64]. Results from a Phase 2a trial, in which intradermally injected autologous DCs loaded *ex vivo* with RNA encoding the patient’s own HIV-1 antigens plus CD40L demonstrated a delay of ART resumption after treatment interruption, but no improvement of CD4+ T cell counts [65].

Cell-based therapies

Adoptive transfer

The adoptive transfer of T cells recognising multiple viruses (e.g. CMV, Epstein–Barr virus) has shown promise in reconstituting antiviral immunity in immunocompromised patients [66] and represents a potential strategy to enhance viral control and potentially clear persistently infected cells in HIV-1 infection. Adoptive transfer of HIV-1-specific T cells also may provide two advantages over vaccine-induced T cell responses, as the phenotype and specificity of T cells can be controlled and the limitations imposed by eliciting an immune response in an immunodeficient state may be circumvented. Infused HIV-1 specific CD8+ T cell clones persisted in individuals on ART, maintained proliferative capacity upon encountering cognate antigen and localised to mucosal tissues [67]. T cells expanded *ex vivo* against multiple HIV-1 peptides enabled increased clearance of reactivated latently infected cells *in vitro* [68]. This approach, if combined with latency-reversing agents, might facilitate the clearance of the latent reservoir.

T cell receptor (TCR) modifications and chimeric antigen receptors (CARs)

Another strategy to direct T cells towards viral antigens has been adapted by genetically modifying peripheral blood cells with a molecularly cloned TCR. The high-affinity TCR against the HLA-A2 Gag epitope SL9 was identified to enhance effector functions in transduced T cells and also to recognise common escape variants of SL9, suggesting the high potential of these cells to overcome immune escape [69]. A Phase 1 clinical study testing the *in vivo* efficacy of these high-affinity Gag-specific T cells in ART patients has been performed but no results have been reported so far. While TCR-modified T cells are directed against specific HIV-1 antigens, CAR-transduced T cells combine the specificity of an antibody with the signalling of a T cell receptor and are not limited by class I expression. While the first generation of CAR-transduced T cells, expressing a CD4 molecule on its surface that was fused with the CD3zeta signalling domain, showed excellent persistence as well as retention of receptor expression *in vivo*, antiviral effects in clinical trials however, were minimal [70]. Newer generation CAR-transduced T cells containing additional intracellular motifs from co-stimulatory molecules have shown promise in the cancer field [71] and could potentially enhance clinical efficacy against HIV-1-infected cells.

Monoclonal antibodies

Broadly neutralising antibodies (bNAbs)

Early trials in humanised mice, and later in infected individuals, using combinations of first generation bNAbs (2G12, 2F5, 4E10 etc.) failed to lead to virological control and resulted in the emergence of antibody-resistant variants [72,73]. Over the past few years, many new, more potent bNAbs have been identified

and have shown promise in preclinical protection studies and therapeutic trials. These bNAbs were not only capable of protecting from SHIV infection in macaques [74–76], but administration of bNAbs has also been shown to suppress viraemia in HIV-1-infected humanised mice [77]. More recent data from two non-human primate studies confirmed that bNAbs were able to reduce plasma viraemia in SHIV infected macaques [49,76]. Infusion of one particular mAb, PGT121, resulted in not only rapid and profound suppression of plasma viral RNA but also substantial reductions of proviral DNA in peripheral blood, lymph nodes and gastrointestinal mucosa [49]. In particular the latter observation has led to a resurgence of interest in evaluating bNAbs for therapeutic indications in humans. A first-in-human study demonstrated that a single administration of the bNAb 3BNC117 was able to reduce viral loads in HIV-1-infected individuals by 0.8–2.5 log₁₀ [78]. Other bNAbs (VRC01, PGT121, PGDM1400) and bNAb combinations are currently being evaluated or planned for clinical evaluation in HIV-1-infected individuals, and it remains to be determined what effect broadly neutralising antibodies may have on the viral reservoir. Another caveat regarding broadly neutralising mAbs is their limited accessibility to certain anatomical reservoir sites, such as the central nervous system.

Broadly functional antibodies

In addition to direct neutralising activity, certain antibodies are able to specifically recruit antibody fragment crystallisable (Fc)-dependent antiviral activities such as antibody-dependent cellular phagocytosis (ADCP) or antibody-dependent cellular cytotoxicity (ADCC). These functions might be particularly important for the eradication of infected cells. The viral envelope is presented in various forms on the surface of infected cells throughout the infectious life cycle, and might be a target for ADCC [79]. Interestingly, ADCC-inducing antibodies are enriched in spontaneous controllers of HIV-1 [80–83]. Monoclonal antibody therapy concepts should therefore include broadly functional antibodies able to broadly recognise and destroy infected cells through the recruitment of the antiviral activity of the innate immune system.

Reversing exhaustion

Exhaustion of pre-existing T cells is characterised by the loss of important effector functions and represents a challenge for therapeutic strategies that are geared towards utilising existing immunity to clear latently infected cells. During progressive HIV-1 infection with persistent antigen exposure, increased expression of inhibitory receptors like PD-1, CTLA-4, LAG-3, and TIM-3 on HIV-1-specific T cells is associated with greater immune dysfunction that is only partially restored with ART [84–86]. Novel immunotherapeutic strategies have been developed to reverse this state of exhaustion by inhibiting the PD-1 pathway and to restore the ability of T cells to inhibit HIV-1 replication in animal models [87,88]. Moreover, blocking PD-1 has shown efficacy in the cancer field [89] and in another chronic viral infection, where a single dose of an anti-PD-1 antibody appeared to contribute to hepatitis C virus therapy [90]. A Phase 1 trial of an anti-PD-L1 antibody in ART-suppressed HIV-1-infected individuals is currently evaluating the safety and efficacy of this approach.

Conclusions

Recent advances in our understanding of viral latency reversal have generated enthusiasm that an HIV cure may be possible. While clinical trials with LRAs have yet to show robust and reproducible induction of plasma viraemia, these concepts are promising and are actively being explored. However, improved LRAs are likely to be required. Another challenge for a successful ‘kick and kill’ strategy

is the need for a more effective ‘kill’. Inducing potent anti-HIV immune responses has proven difficult in HIV-1-infected individuals but several novel vaccine concepts are currently under clinical evaluation. The recent identification of potent and broadly neutralising antibodies and promising preclinical data suggest that administration of these antibodies could be another path to reduce virally infected cells following latency reversal. Overall, significant progress has been made over the last decade to develop strategies that may target the viral reservoir. Over the next few years clinical trials should help define which of these avenues may be promising cure strategies to pursue in HIV-1-infected individuals.

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