Efforts to eliminate the latent reservoir in resting CD4+ T cells: strategies for curing HIV-1 infection

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Abstract

Since the introduction of the first antiretroviral drug, zidovudine, in 1987, over 25 different antiretroviral agents from six different drug classes have been approved for the treatment of HIV-1 infection. Today, combination antiretroviral therapy (ART) is extremely effective in suppressing HIV-1 replication, providing durable control of the virus in adherent patients. However, despite the effectiveness of ART in blocking viral replication, HIV-1 infection cannot be cured by ART alone because HIV-1 establishes a state of latent infection in a small pool of resting, memory CD4+ T cells. Some new developments in the search for a cure are discussed.

Keywords: HIV-1, resting, memory CD4+ T cells, latent reservoir, cure

Introduction

Since the introduction of the first antiretroviral drug, zidovudine, in 1987, over 25 different antiretroviral agents from six different drug classes have been approved for the treatment of HIV-1 infection [1]. The modern era for treatment of HIV-1 infection began in the mid 1990s with three-drug combinations targeting the viral enzymes reverse transcriptase and protease. Later, new classes of antiretroviral drugs inhibiting viral entry and integration were introduced. Recently, treatment has been greatly simplified through the combination of multiple antiretroviral drugs into a single pill taken once daily. Combination antiretroviral therapy (ART) is extremely effective in suppressing HIV-1 replication, providing durable control of the virus in adherent patients.

However, despite the effectiveness of ART in blocking viral replication, HIV-1 infection cannot be cured by ART alone because HIV-1 establishes a state of latent infection in a small pool of resting, memory CD4+ T cells. When patients start ART, plasma virus levels undergo rapid, biphasic decay to clinically undetectable levels (20 copies of HIV-1 RNA/mL of plasma). All of the drugs used to treat HIV-1 infection act to stop new cells from becoming infected but none blocks virus production by cells that already have an integrated provirus. Therefore, this decay reflects the rapid turnover of the two populations of infected cells that produce most of the plasma virus; namely productively infected, activated CD4+ T cells, which have a half-life of <1 day, and a second population of infected cells that have a slower decay rate, possibly infected macrophages or infected CD4+ T cells that are in a lesser state of activation. Based on these decay kinetics, hope was raised in 1997 that ART might produce a cure in infected individuals within 2–3 years [2]. However, this did not occur because there is a third population of infected cells with a much slower decay rate. These are latently infected, resting memory CD4+ T cells [3,4]. They arise as a consequence of the normal physiology of CD4+ T cells. HIV-1 replicates mainly in activated CD4+ T cells and these infected cells die quickly, usually within a day. On rare occasions, activated CD4+ T cells become infected with HIV-1 as they are returning to a resting memory state at the conclusion of an immune response. This state is non-permissive for viral gene expression. The result is a stably integrated but transcriptionally silent form of the viral genome in a long-lived memory T cell. Latently infected cells are unaffected by host immune responses or ART. Therefore, this cell population is the major barrier to curing HIV-1 infection. Thus, although ART suppresses viral replication, and adherent patients do extremely well, a therapeutic strategy that targets latently infected cells is required to cure HIV-1 infection. Moreover, due to the multifactorial nature of HIV-1 latency, combinations of different HIV-1 cure strategies may be required to reach a sterilising cure. Some new developments in the search for a cure are discussed in this issue.

Defining the challenges

There are many challenges in eliminating latently infected resting CD4+ T cells. Latency is established extremely early in infection. While early treatment may impact the size of the reservoir, a stable reservoir of latently infected cells with replication-competent virus is seeded within days of infection. This has been dramatically demonstrated in elegant experiments in the simian immunodeficiency virus (SIV) model, as described by Dan Barouch in this issue. Once established, the pool of latently infected cells is extremely stable. Recent studies described by Frank Maldarelli in this issue suggest that latently infected resting CD4+ T cells can proliferate [5–7], through the normal homeostatic proliferation process that maintains immunological memory, through clonal expansion upon antigen encounter [8], or through processes driven by viral integration into host genes involved in cell proliferation. The proliferation of latently infected cells presents another challenge for eradication. Several recent studies suggest that latency reversal may be stochastic, meaning that multiple rounds of activating stimuli may be required to mobilise the entire reservoir for elimination by the 'shock and kill strategy', a widely discussed approach for HIV-1 cure. This approach involves drugs that can reverse latency ('shock') without inducing global T cell activation followed by a mechanism to ensure that the infected cells die after latency reversal ('kill'). Several histone deacetylase (HDAC) inhibitors and protein kinase C (PKC) activators have been identified as potential latency-reversing agents (LRAs) in *ex vivo* assays using resting CD4+ T cells isolated from patients on ART [9]. Recent clinical trials have suggested that some of these agents have the ability to reverse latency *in vivo* as measured both by small transient increases in intracellular HIV-1 RNA and plasma virus in some patients [10]. However, a decrease in the latent reservoir has not yet been observed. This suggests that some degree of T cell activation is required for infected cells to die either by viral cytopathic effects or host immune responses after latency reversal.

Despite these challenges, the cure of a single patient, the 'Berlin patient', has led to renewed optimism that HIV-1 may be a curable

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infection if all latently infected cells with replication-competent virus can be eliminated. This patient was doing well on ART when he developed acute myeloid leukaemia. After a conditioning regimen of extensive chemotherapy and total body irradiation, he was given an HLA- matched haematopoietic stem cell transplant (HSCT) from a donor who was homozygous for the 32-base pair deletion in the HIV-1 co-receptor CCR5 [11]. Essentially the patient's immune system was replaced with cells that were resistant to HIV-1 infection. Antiretroviral therapy was discontinued at the time of his initial transplant, approximately 8 years ago. To date, no residual virus has been detected. Recently, two additional cases (the 'Boston patients') were presented in which HSCTs were given to HIV-1-infected recipients who had malignancies [12]. However, unlike the case of the Berlin patient, the HSCT that these patients received were from donors who had only wild type CCR5 alleles. Therefore, the patients had to remain on ART during and after the transplant period to protect the new donor cells from becoming infected. Both patients experienced a prolonged period with no detectable viraemia after ART interruption before a delayed viral rebound occurred. Presumably, reactivation of a small number of latently infected cells that were still present led to the viral rebound. While HSCTs are not a generalisable approach to HIV-1 cure and will only be done in patients with malignancies, these cases have been very instructive and have stimulated HIV-1-cure research. As discussed in this issue, a variety of different approaches are being developed.

Combination therapy for a cure

There is increasing recognition that it may be necessary to combine multiple strategies to maximise the possibility of cure. These include early treatment, combinations of latency-reversing agents (LRAs) and multiple strategies for the killing of infected cells. Early treatment may reduce the initial duration of viral seeding, thus reducing the size of the latent reservoir [13]. In some patients, such as the Mississippi baby [14], this may allow drug-free, virus-free remission for years before eventual viral rebound. Rare individuals receiving early treatment, such as those in the VISCONTI cohort [15] and the French teen [16], may become long-term controllers after treatment interruption. The mechanisms involved remain unclear. However, as discussed by Jülg and Barouch, early treatment is unlikely to prevent establishment of the latent reservoir. With regard to the shock part of the shock-and-kill strategy, combinations of multiple LRAs may be required. Since HIV-1 latency involves multiple mechanisms, drugs targeting different mechanisms may be required. It has been shown in *ex vivo* studies that combination of different classes of LRAs, such as PKC activators and HDAC inhibitors, may provide synergistic latency reversal [17]. Combinations of different classes of LRAs may replicate the success of ART in targeting different steps of the life cycle. Finally, immunotherapies targeting HIV-1-infected cells may be required for effective killing following latency reversal. Such strategies include therapeutic vaccination, cell-based therapies, broadly neutralising/functional antibodies and CTL exhaustion reversal (as reviewed in this issue by Jülg and Barouch).

HIV-1 latent reservoir may be dynamic

The half-life of the latent reservoir is estimated to be 44 months [18,19]. Some HIV-1-infected cells may die from normal turnover or from viral cytopathic effects or immune killing after latency reversal [20]. However, the reservoir may be replenished through homeostatic proliferation [21]. Two independent studies identified a unique form of clonal expansion of HIV-1-infected cells in which proviruses had integrated into cancer-related genes [5–7]. It was proposed that HIV-1 might drive aberrant proliferation through integration into proliferation-related genes, as discussed by Maldarelli in this issue. Taking together the fact that a significant proportion of HIV-1-infected cells may undergo clonal expansion and the observation that the size of the latent reservoir declines minimally over time, it is reasonable to conclude that the size of the latent reservoir reflects a dynamic equilibrium between opposing factors including clonal expansion, homeostatic proliferation, immune clearance and the cellular dynamics of memory CD4+ T cell responses to antigen (such as the expansion of a tumour-specific, HIV-1-infected clone [8]). It remains unclear how HIV-1-cure strategies may alter the dynamics of the clonally expanded cells. First, while the majority of the clonally expanded HIV-1-infected cells harbour defective proviruses [5], reflecting the composition of the pool of defective proviruses in resting CD4+ T cells [22], replication-competent proviruses have been identified from clonally expanded cells in a single case [8]. Secondly, little is known about how latency reversal may affect the dynamics of clonal expansion. In the worst-case scenario, if an LRA stimulates HIV-1-promoter activity and drives aberrant proliferation, the number of HIV-1-infected cells may paradoxically increase. Whether clonally expanded HIV-1-infected cells harbour replication-competent HIV-1, whether such cells can be eliminated through immunotherapy, and whether HIV-1-cure strategies may paradoxically increase the number of these clonally expanded cells through preferential HIV-1 stimulation and aberrant proliferation, are all critical questions that remain to be answered.

Measurement of the size of the latent reservoir: taking defective proviruses into consideration

As efforts to target the latent reservoir intensify, a clinical assay to measure reservoir reduction is urgently needed. An accurate and clinically feasible assay for the size of the latent reservoir is necessary to compare the effects of different strategies and to identify patients who may have reached a level of reservoir reduction that would allow a prolonged remission after interruption of ART [23]. Unreliable reservoir assessment may expose patients to unnecessary pharmacological toxicities and premature decisions regarding treatment interruptions. Currently, available assays include a culture-based quantitative viral outgrowth assay (QVOA), HIV-1 RNA-based assays, and HIV-1 proviral DNA-based measurements. While the QVOA remains the gold standard assay for the frequency of cells harbouring inducible, replication-competent proviruses, it is too complex for clinical use. Quantitative measurement of HIV-1 proviral DNA is technically more feasible; however, the vast majority of proviruses detected by these assays are defective. In addition, PCR-based assays show no correlation with the size of the latent reservoir measured by the QVOA [22,24]. RNA-based quantitative assays, which measure supernatant or cellular HIV-1 RNA copies upon stimulation of resting CD4+ T cells, allow for comparison of the effects of different LRAs [9,10,17]. However, except for assays performed in a limiting dilution format, they do not measure the number of latently infected cells, as numerous copies of HIV-1 RNA can arise from a single cell or numerous cells upon stimulation. In this issue, we report on the *2015 Towards an HIV Cure Symposium* at the International AIDS Society (IAS) Annual Meeting in Vancouver where several groups reported advances in assays to quantify the size of the latent reservoir. To simplify the limiting dilution step of QVOA, Lee *et al*. [25] used deep-sequencing of HIV-1 RNA from a bulk viral outgrowth culture to identify the number of different viral species in culture. They observed a twofold higher frequency of inducible viruses compared with standard QVOA. Massanella *et al*. [26] reported two assays to shorten the duration of the lengthy QVOA: a supernatant HIV-1 RNA assay termed 'inducible HIV reservoir-modified QVOA' (mQVOA) and a cellular HIV-1 RNA measurement

termed 'inducible cell-associated RNA expression in dilution' (iCARED). Compared with the standard QVOA, which measures induced replication-competent HIV-1 at a frequency of ~1 per million resting CD4+ cells, sizes of the latent reservoir measured by these RNA assays are slightly higher (~5–45 per million resting CD4+ T cells). Given that the standard QVOA can detect a single latently infected cell, it is not clear whether the new assays are actually more sensitive or detect viruses that cannot grow out in a QVOA. The discordance between the production of replication-competent virus and the measurement of supernatant HIV-1 RNA indicates the pitfalls of using supernatant HIV-1 RNA to represent the presence of replication-competent viruses. As described by Pollack *et al.* [27], reconstructed, patient-derived defective HIV-1 proviruses may be transcribed and translated, depending on the nature of the defect, which complicates the estimation of replication-competent viruses if RNA-based quantification is used as the sole measurement. Thus, while RNA-based HIV-1 quantification provides faster turnaround than the standard viral outgrowth assays, it may be more useful as a measure of HIV-1 RNA transcription than an absolute representation of replication-competent virus measurement.

Progress towards curing HIV-1 infection

There has been significant progress in therapeutic strategies to eliminate the latent reservoir in resting CD4+ T cells, the major barrier to curing the infection. Insight into mechanisms that establish and maintain latency have led to numerous ongoing clinical trials. Improved methods for measuring the reservoir will help to determine whether these interventions are decreasing the size of the reservoir and may stimulate further clinical trials in the same way that the introduction of an assay for plasma HIV-1 RNA stimulated the development of an extremely effective arsenal of antiretroviral drugs. As is illustrated by the HIV-1 eradication studies reviewed in this issue, we are hopefully at the beginning of an exciting era that will culminate in a scalable approach for cure.

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