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Finding A Cure for HIV-1 Infection

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Introduction

Remarkable advances have been made in the treatment of HIV-1 infection, but in the entire history of the epidemic, only one or two patients appear to have been cured. Here we review the fundamental mechanisms that render HIV-1 infection difficult to cure and then discuss recent clinical and experimental situations in which some form of cure has been achieved. Finally we consider approaches that are currently being taken to develop a general cure for HIV-1 infection.

The basic biology of retroviral persistence

The idea that HIV-1 infection might be curable is bold one. Fundamental aspects of the biology of HIV-1 make cure implausible. After HIV-1 enters a host cell, the viral genetic information is copied by the viral enzyme reverse transcriptase (RT) from RNA into a double stranded DNA molecule approximately 10,000 base pairs in length. This DNA copy is then inserted directly into the DNA of the host cell. The resulting proviral form of the viral genetic information persists in the host cell DNA, essentially functioning as a cellular gene, for as long as the cell survives. Although host cells have many interesting ways to defend themselves against retroviral infection,¹ they have no mechanism to eliminate the integrated viral genome (provirus). At the level of individual cells, infection is essentially permanent. The irreversible insertion of retroviral DNA into host cell chromosomes through the integration reaction confers upon the virus the stability of the host cells. Thus, a critical determinant of HIV-1 persistence is the lifespan of infected host cells. If the lifespan of an infected cell is short, and if *de novo* infection can be fully blocked, then cure is possible.

The lifespan of cells infected with HIV-1 was unknown until 1995 when two groups developed a way to analyze the turnover of free virus and infected cells *in vivo* in infected individuals.^{2,3} In untreated patients, HIV-1 replicates continuously, even during the asymptomatic phase between acute infection and the development of AIDS.⁴ Plasma virus levels are typically in the range of 10,000 – 100,000 copies of HIV-1 RNA per ml of plasma

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during this period. Over short times intervals (days to weeks), the levels of plasma virus remain roughly constant. George Shaw and David Ho realized that this is essentially a state of equilibrium in which the number of new cells becoming infected every day is roughly equal to the number of infected cells dying every day. If *de novo* infection is blocked with potent antiretroviral drugs, then the resulting decay in viremia should reflect the rate at which infected cells die. In landmark studies,^{2,3} these investigators measured changes in plasma virus levels shortly after patients were started on a potent antiretroviral drug such as a non-nucleoside RT inhibitor (NNRTI) or a protease inhibitor (PI). They made the surprising discovery that plasma virus levels fall rapidly and dramatically when *de novo* infection is blocked. A key feature of all currently approved antiretroviral drugs is that they block *de novo* infection of susceptible cells. They do not affect virus production by cells that already have an integrated provirus. This is true even of the PIs, which do not block the release of virus particles but instead prevent the maturation of the released particles into an infectious form.⁵ The rapid decay in viremia observed when *de novo* infection is blocked indicates that the lifespan of the infected cells that produce most of the plasmas virus is very short. Currently, the half-life is estimated to be less than one day.^{2,3,6}

It is unclear why most infected cells have a short half-life *in vivo*. It has been presumed that viral cytopathic effects lead to the death of cells that are expressing high levels of viral proteins, but the mechanisms remain unclear.⁷ Interesting recent work suggests that the CD4⁺ T cell depletion that is characteristic of untreated HIV-1 infection may result from an inflammatory form of cells death termed pyroptosis that is triggered by abortive infection of resting CD4⁺T cells.⁷ However, this finding does not explain the rapid death of productively infected cells. Infected cells can also be lysed by virus-specific cytolytic T lymphocytes (CTL) if viral peptides are presented on the cell surface in association with major histocompatibility complex (MHC) molecules.⁸ Some retroviruses like HIV-1 rapidly evolve escape mutations in epitopes recognized by CTL, thus thwarting this mechanism of elimination.⁹ Studies in the SIV model of HIV-1 infection suggest that CTL do not actually shorten the half-life of infected cells *in vivo*, although they clearly have a role in controlling viral replication.^{10,11} Although our understanding of the fate of infected cells *in vivo* is incomplete, the rapid decay of viremia in treated individuals makes it abundantly clear that most infected cells survive only a short period of time in the productively infected state.

Additional studies by Perelson and colleagues¹² of patients starting combination antiretroviral therapy (ART) detected a second phase in the viral decay curve that was due to a second population of infected cells with a somewhat longer half-life (about 2 weeks). The nature of this cell population was unknown at the time and remains unclear.¹³ However the finding that most of the plasma virus is produced by cells with a relatively short lifespan (days to weeks) raised the possibility that HIV-1 infection might be cured simply by blocking new infection events and waiting for previously infected cells to decay. In fact, analysis by Perelson and colleagues suggested that cure could be achieved with 2-3 years of ART.¹² These findings were the basis of much of the optimism about cure that accompanied the initial finding the ART could reduce viremia to below the limit of detection of clinical assays. Unfortunately, the hope that ART alone could produce a cure was not realized. At about the time that effective ART regimens were being introduced, a

third population of infected cells with an extremely long half-life was identified^{14,15} and shown to allow viral persistence in the face of suppressive ART¹⁶⁻²¹. These are resting memory CD4⁺ T cells that harbor a latent form of the virus and represent the primary barrier to a cure.

HIV-1 latency

Viral latency is a reversibly non-productive state of infection of individual cells. For some viruses, such as those of the Herpes virus family, it is an important mechanism of persistence, allowing the virus to avoid immunologic clearance mechanisms between periods of active viral replication. Because HIV-1 replicates continuously in untreated individuals,⁴ HIV-1 persistence does not appear to require latency. Rather, the virus evades host immune responses through the rapid evolution of variants that are not recognized by antibodies or CTL.^{9,22,23} Thus it was not necessarily expected that HIV-1 could establish a state of latent infection *in vivo*. HIV-1 latency appears to be a somewhat accidental consequence of its tropism for activated CD4⁺ T cells. The virus readily infects and replicates in CD4⁺ T cells that have become activated in response to some antigenic challenge. HIV-1 gene expression is heavily dependent upon host transcription factors that are present in the nucleus of activated T cells, notably NFκB and NFAT.^{24,25} Following antigen-drive activation, some CD4⁺ T cells transition back to a resting state as long lived memory T cells capable of responding to the same antigen in the future. The resting state is largely non-permissive for HIV-1 gene expression, in part because key host transcription factors are excluded from the nucleus in resting CD4⁺ T cells. The end result is a stably integrated but transcriptionally silent form of the virus in a long-lived memory T cell. This allows HIV-1 to persist simply as genetic information, 10⁵ bases of DNA integrated somewhere into the 6 × 10⁹ base pair diploid human genome. In this form, the virus can persist unaffected by immune responses or antiretroviral drugs. However, if the relevant memory T cell becomes activated again by a future encounter with antigen, it can resume virus production. *In vivo* evidence for this form of latent infection was first obtained in the mid 1990's with the demonstration that populations of resting CD4⁺ T cells from infected individuals contained cells with integrated HIV-1 DNA but did not produce virus unless the cells were activated.^{14,15} A viral outgrowth assay in which resting CD4⁺ T cells are activated and cultured with CD4⁺ T cells from uninfected donors to allow outgrowth of virus was used to measure the frequency of latently infected cells.^{16,26} The frequencies are extremely low, on the order of 1 per million resting CD4⁺ T cells. However, the frequencies do not decline even after prolonged period of treatment with ART,¹⁶⁻²¹ and thus this latent reservoir is considered a major barrier to curing the infection.^{20,27} These points are summarized in Box 1. Intensive efforts, discussed below, are being directed at eliminating this reservoir to achieve a cure.²⁸

Defining a cure

Despite the renewed interest in HIV-1 eradication, it has become important to refine the definition of cure. Two types of cures are widely discussed²⁹. In a sterilizing cure, there is complete eradication of the virus. In light of the high fraction of defective viral genomes (discussed below), this definition could be further refined as follows: A sterilizing cure eliminates all replication-competent forms of the virus so that no viral reservoirs remain. By

this definition, a patient who retains some defective viral sequences would still be considered cured. Another form of cure is a functional cure in which the virus is not completely eradicated but rather is held in check by the host immune system. A functional cure could be an intervention that renders patients with progressive disease able to permanently control viral replication to below the limit of detection, thereby preventing clinical immunodeficiency and transmission. Examples of both types of cure are discussed below.

The first patient in whom a sterilizing cure appears to have been achieved is an HIV-1-infected adult male who was doing well on ART when he developed acute myelogenous leukemia³⁰. This patient is widely known as the “Berlin patient,” named after the site of his treatment. As part of the treatment for his leukemia, the Berlin patient received a bone marrow transplant from a carefully selected donor who was homozygous for a 32-base pair deletion in the HIV-1 coreceptor CCR5. ART was stopped at the time of the initial transplant, and despite a complex post-transplant course, the patient has remained aviremic off ART for over 5 years. Careful analysis of multiple samples from the patient with a variety of extremely sensitive techniques has failed to convincingly demonstrate persistence of HIV-1 in this patient³¹, and he is therefore considered to be the first patient to have been cured of an established HIV-1 infection. Interestingly, very different results were observed in two additional patients with HIV-1 infection who received bone marrow transplants for other indications³². In these patients (known as the Boston patients), the donors were wild-type for CCR5 and infection of susceptible donor cells was prevented by continuing ART throughout the transplant period. ART was stopped several years after transplantation, and the rebound in viremia which normally occurs about 2 weeks after interruption of ART was not observed. Instead, the patients maintained plasma virus levels below the limit of detection for many weeks, but both ultimately experienced a sudden and dramatic rebound of viremia to very high levels, 3 months and 8 months after ART interruption. In all three of these cases, the combination of the preparative regimens used and graft vs host disease eliminated most hematopoietic cells of host origin. Thus the latent reservoir was reduced in a non-specific fashion by targeting all host lymphoid cells. In the case of the Berlin patient, rebound did not occur because virus released from any residual latently infected cells would not be able to replicate in the HIV-1-resistant donor-derived cells that now comprise the patient's immune system. The viral rebounds observed in the Boston patients after prolonged periods of undetectable viremia nicely illustrate the concept of latent infection and suggest that only a very small number of residual latently infected cells may be sufficient to rekindle the infection in the setting of a susceptible immune system.

The other widely discussed case of a sterilizing cure is that of the “Mississippi baby”, an infant born to an infected mother who had no prenatal care.³³ At the time of birth, the baby's plasma HIV-1 RNA level was above 20,000 copies/ml, presumably reflecting *in utero* infection. An aggressive ART regimen was started within 31 hours of delivery and continued for 18 months. Treatment was then stopped against medical advice, but no rebound occurred for more than a year after interruption of ART. However, the baby was noted to have rebound viremia after an additional year of follow up, similar to the Boston patients. In this situation, it is the potential cure was postulated because treatment was

initiated before the latent reservoir in resting CD4⁺ T cells was established. There are few memory T cells at birth, and the memory cell compartment is largely generated in response to encounter with antigen in post-natal life.³⁴ Yet, in the end, sterilizing cure was not achieved.

As noted above, most productively infected cells have a very short half-life. The only HIV-1 reservoir that has been demonstrated to persist on a time scale of years in the setting of effective ART is the latent reservoir in resting CD4⁺ T cells. In the absence of this reservoir, the infection is curable if new infection of susceptible cells can be blocked. The Mississippi baby case is a testament to the remarkable efficacy of antiretroviral drugs.³⁵ Recent *in vitro* studies have provided evidence that the ability of ART regimens to block HIV-1 replication is actually much greater than previously thought.³⁵ Interestingly, Hepatitis C virus does not establish a stable latent reservoir and is proving to be readily curable with direct acting antiviral drugs.^{36,37} Together these findings emphasize the fact that the real barrier to HIV-1 cure is the latent reservoir. The Berlin patient appears to be the only example to date of a patient with an established latent reservoir who has been cured. Approaches to eliminate the latent reservoir are discussed at the end of this article.

Immune control and functional cure of HIV-1 infection

Given the difficulty of eliminating the latent reservoir, there has been great interest in another form of cure, the functional cure, in which the virus is not completely eradicated but rather is held in check by the host immune system. Elite controllers (EC) are patients who represent a model for a functional cure of HIV-1 infection.^{38,39} These patients are seropositive for HIV-1, but maintain levels of HIV-1 RNA that are below the limit of detection of current clinical assays. They differ from long term non-progressors (LTNPs) who are defined as having stable CD4 counts and no clinical symptoms regardless of their HIV-1 RNA levels.⁴⁰ The mechanism of virologic control in EC has been the focus of intense research. While several case reports have suggested that some EC and LTNPs are infected with attenuated or defective virus,^{41,42} other studies have shown that replication-competent virus can be cultured from CD4⁺ T cells in many EC.⁴³⁻⁴⁵ Furthermore full genome sequence analysis of replication-competent virus has not revealed any large deletions or signature mutations in the majority of EC⁴³, and transmission pair studies have proven that some EC are infected with viruses that were sufficiently pathogenic to cause AIDS in the source patients.^{46,47} Viral evolution has also been documented in EC,⁴⁸⁻⁵⁰ and a recent study showed that isolates cultured from EC replicate vigorously in a humanized mouse model of HIV-1 infection, leading to profound depletion of human CD4⁺ T cells in these animals.⁵¹ Taken together, the data suggest that unique host factors rather than infection with attenuated virus explain the majority of cases of elite control. Identification of these factors could clearly promote the development of strategies for a functional cure.

Among the many hypotheses to explain elite control, superior HIV-1-specific CD8⁺ T cell activity is the best established correlate of immunity in these patients. Certain class I MHC alleles such as HLA-B*27 and HLA-B*57 are overrepresented in every cohort of ECs studied.⁵²⁻⁵⁷ Furthermore, these HLA alleles are the only protective factors that have been identified in large genome wide association studies.^{58,59} Class I HLA proteins present

antigens to CD8⁺ T cells, and thus the mechanism by which these class I molecules contribute to protection from progressive HIV-1 infection may be through the presentation of critical HIV-1 epitopes to CD8⁺ T cells. The finding that HLA-B*27 and HLA-B*57 proteins present conserved immunodominant HIV-1 Gag epitopes is consistent with this hypothesis. While the low fidelity of HIV-1 reverse transcriptase allows for the accumulation of mutations that can be quickly selected when pressure is applied, the fact that these targeted epitopes are in conserved regions of an essential viral protein means that the mutations will come at a significant fitness cost to the virus. In support of the T cell hypothesis is the fact that HIV-1-specific CD8⁺ T cells in EC are more effective at producing multiple cytokines and chemokines and at killing HIV-1-infected CD4⁺ T cells than are CD8⁺ T cells from patients who have progressive disease.^{60,61} Furthermore, in the non-human primate model of elite control, depletion of CD8⁺ T cells with antibody results in the transient loss of control of viral replication which is re-established when the effect of the antibody wears off and CD8⁺ T cell numbers rebound.⁶² However, not all patients who have these alleles become EC,⁵² and a substantial number of EC do not have protective HLA alleles or strong CTL responses.^{53,57} Thus, while protective class I HLA alleles and strong HIV-1-specific CTL responses clearly play a key role in many ECs, they cannot explain all aspects of elite control.

A functional cure of HIV-1 infection can sometimes be achieved in adults by the initiation of ART shortly after seroconversion. Early trials showed that after a year of treatment, some patients who began therapy during acute infection maintained low or undetectable plasma HIV-1 RNA levels when therapy was discontinued.⁶³ Unfortunately, this virologic control was short-lived in the majority of patients, and by three years most of the patients were back on ART.⁶⁴ However recent studies have documented the long term control of viral replication in some patients who were treated during primary infection. Salgado et al. described a patient who was treated during symptomatic primary infection and subsequently maintained plasma HIV-1 levels below the limit of detection for more than 9 years after stopping therapy.⁶⁵ Saez-Cirion et al. have described 14 patients who maintained some degree of control of viral replication after interrupting treatment.⁶⁶ It is impossible to determine whether these patients were destined to become EC before treatment was initiated, but some patients had extremely high viral loads and severe clinical symptoms which are not often seen in primary infection in ECs.⁶⁶ Saez-Cirion et al. estimate that 5-15% of patients treated during acute HIV-1 infection may become post treatment controllers. This is much higher percentage than the reported prevalence of EC (0.5 to 1 %) in many large cohorts.⁴⁰

The mechanisms through which post-treatment controllers suppress viral replication are not known. It is unlikely that infection with attenuated virus can explain post-treatment control since many of these patients had high levels of viremia in primary infection. The frequency of HIV-1-infected cells in cohort studied by Saez-Cirion et al. was much lower than the frequency normally seen in patients on HAART and appeared to be decreasing over time in some patients.⁶⁶ However, in the case reported by Salgado et al., the frequency of HIV-1-infected cells was comparable to the frequency seen in patients on suppressive ART.⁶⁵ Furthermore in another trial in which patients were treated during acute HIV-1 infection, these patients were not able to control viral replication once treatment was discontinued

despite having very low frequencies of latently infected cells.⁶⁷ Thus it appears that having a small HIV-1 reservoir is neither necessary nor sufficient for post-treatment control. While early treatment leads to the maintenance of HIV-1-specific CD4⁺ T cells that are normally depleted during chronic HIV-1 infection, loss of control was shown to occur in the presence of these CD4⁺ T cell responses, suggesting that they are not protective by themselves.⁶⁴ The post-treatment controllers studied by Saez-Cirion et al. did not have a high incidence of the protective class I HLA alleles.⁶⁶ Interestingly, HLA-B*35, an allele that is normally associated with rapid disease progression, was actually over-represented in this cohort. Robust CD8⁺ T cell responses that are often seen in EC were not found in these post-treatment controllers,^{65,66} and in one case, high titers of neutralizing antibodies to autologous virus excluded.⁶⁵ Thus, the mechanism of post-treatment control of HIV-1 replication remains elusive.

Elite control and post-treatment control are examples of a functional cure of HIV-1 infection, and understanding the mechanisms responsible for both phenomena may lead to the development of a therapeutic vaccine that controls HIV-1 replication without eradication (See Chapter XX). This may be analogous to the licensed vaccines for Herpes zoster which significantly reduce the frequency of episodes of shingles but do not produce sterilizing immunity in older patients with chronic infection. It should be noted that in some patients, elite control comes at a price. There is a higher incidence of immune activation^{68,69} and higher levels of some inflammatory biomarkers in EC than patients on suppressive ART regimens.^{70,71} Thus the goal should be to develop a vaccine that prevents both viral replication and immune activation and inflammation.

Targeting the latent reservoir

Although ART effectively suppresses viral replication, the basic biology of the latent reservoir necessitates lifelong treatment to avoid viral rebound.²⁰ In most patients, rebound occurs within about two weeks of treatment interruption.⁷² There is genetic evidence that rebound originates from the latent reservoir,⁷³ although other stable reservoirs could certainly contribute. In any event, unless a method for inducing effective immune control of HIV-1 replication can be developed, elimination of the latent reservoir will be required before patients can be safely taken off ART. As discussed above, allogeneic bone marrow transplantation with cells from a donor homozygous for a CCR5 deletion has generated the only cure of a patient with an established latent reservoir. However, the difficulties and dangers associated with bone marrow transplantation will likely restrict the use of this approach to patients who require a transplant for other conditions, and the current emphasis is on ways to directly specifically target the latent reservoir.

The most widely discussed general approach for eliminating the reservoir, termed “shock and kill”, involves the use of latency reversing agents to increase HIV-1 gene expression in latently infected cells. This would be done while the patients remained on ART so that the released virus would not infect new cells. It is hoped that the cells would then die from viral cytopathic effects or be lysed by virus-specific CTL. Recent studies of HIV-1-induced cell death focused on innate immune mechanisms that detect the presence of viral infection and initiate signaling cascades leading to cell death by pyroptosis.⁷ However, innate immune

sensors generally recognized viral DNA intermediates generated early in the virus life cycle, during the reverse transcription and integration reactions.^{7,74} It is less clear that the innate immune system can sense the reactivation of a latent provirus, which essentially functions as a cellular gene. Thus it is not yet clear that infected cells will die following the reversal of latency, particularly when latency is reversed using strategies that do not induce T cell activation and high level virus gene expression. It may therefore be necessary to rely on other immune effector mechanisms, especially virus-specific CTL, to eliminate infected cells after reversal of latency. However, CTL responses wane in patients on ART, presumably due the lack of antigen stimulation, and a recent *in vitro* study finds poor killing of infected cells following reversal of latency.⁷⁵ Thus it may be necessary to combine some form of therapeutic vaccination with latency reversing strategies. Alternatively, novel immunotoxin-based strategies may promote the elimination of these cells.⁷⁶ In any event, the first step is to reverse latency and up-regulate HIV-1 gene expression.

Early approaches to reversing latency relied on global T cell activation.⁷⁷⁻⁷⁹ However, there was often unacceptable toxicity, likely due to the release of large amounts of proinflammatory cytokines by the activated T cells. These results focused discovery efforts on the identification of agents that reverse latency without global T cell activation. The identification of latency reversing agents has been aided by a variety of *in vitro* models of HIV-1 latency, including transformed cell lines and primary CD4⁺ T cells that have been infected with HIV-1 constructs carrying reporter genes and then manipulated in various ways to obtain cell populations in which viral gene expression has been turned off.⁸⁰⁻⁸³ Using these *in vitro* models of latency, several different classes of latency reversing agents have been identified (reviewed in⁸⁴).

Most prominent among the classes of latency reversing agents identified to date are the histone deacetylase (HDAC) inhibitors. In *in vitro* models of HIV-1 latency, histones positioned at specific locations in the HIV-1 long terminal repeat (LTR) can inhibit HIV-1 gene expression,⁸⁵ and therefore HDAC inhibitors, which have the effect of decreasing interactions between histones and DNA, might be expected to increase HIV-1 gene expression in the same way that they affect expression of a large number of host genes. Several HDAC inhibitors have been developed for the treatment of various cancers, and some of these drugs have been shown to reverse HIV-1 latency in model systems.⁸⁵⁻⁸⁸ In a pioneering study, Archin et al. showed that a single dose of the HDAC inhibitor vorinostat caused a modest increase in intracellular levels of transcripts containing HIV-1 *gag* sequences.⁸⁹ However, a follow-up multiple dose study failed to show a sustained increase in HIV-1 gene expression.⁹⁰ Other studies with vorinostat have failed to detect induction of HIV-1 gene expression or virus production by cells from infected individuals.^{91,92} In addition, there is some evidence that the effect of this drug on latent HIV-1 operates through a different mechanism.⁹³

Another important class of latency reversing agents are the protein kinase C (PKC) agonists including prostratin and bryostatin.⁹⁴⁻⁹⁸ The activation of PKC is a downstream event in the activation of T lymphocytes through the antigen receptor, and it is therefore not surprising that PKC agonists induce expression of latent HIV-1 in several

model systems. However, it is not yet clear whether these agents can be used safely in patients.

Several other classes of latency reversing agents have been identified, but in most cases the mechanisms involved have not yet been elucidated (reviewed in reference ⁸⁴). A major problem is that the *in vitro* models used to study latency and to identify potential latency reversing agents may not accurately mimic the state of latently infected cells *in vivo*. A recent study ⁹² has shown that with the exception of bryostatin, the leading candidate latency reversing agents fail to upregulate HIV-1 gene expression in resting CD4⁺ T cells from patients on ART. It is possible that no single agent may effectively convert the transcription environment in a resting T lymphocyte into one that is permissive for HIV-1 gene expression. Therefore effective reversal of latency is likely to require carefully chosen combinations of agents.

Alternative strategies

The “shock and kill” strategy is only one of many approaches currently being explored in the search for a cure (Table 1). As mentioned above, early initiation of ART can reduce the size of the latent reservoir, but generally does not prevent its establishment. Near complete elimination of the reservoir has been achieved by allogeneic bone marrow transplantation strategies, but this dangerous intervention has only succeeded in a single case where the donor's cells were resistant to HIV-1 infection as a result of a homozygous deletion in CCR5. Recent advances in gene editing technology ^{99,100} will allow facile modification of host genes like CCR5 in hematopoietic stem cells, and several groups are exploring the idea of rendering hematopoietic stem cells from patients genetically resistant to HIV-1 infection, for subsequent reinfusion into autologous hosts. The major problem with this strategy is how to eliminate all of the non-modified host cells. In allogeneic transplants, preparative regimens and graft vs. host disease produce a near complete elimination of host cells, but this does not happen in autologous transplants. Ultimately, the most feasible general cure strategy may be one that enhances antiviral immune responses to the state that is observed in EC. Exciting recent studies by Picker and colleagues have shown that a cytomegalovirus-based SIV vaccine given prior to exposure can clear an established SIV infection, ¹⁰¹ presumably by generating CTL that lyse infected cells before they have a chance to revert back to a latent state.

Clinical aspects of HIV-1 eradication strategies

Although the cure case described above has generated optimism regarding HIV-1 eradication, considerable challenges remain. One frequently voiced concern is that reversal of latency will lead to *de novo* infection of additional cells. Implicit in the “shock and kill” strategy is the possibility that virus production will transiently increase when latency reversing agents are administered. However, modern ART regimens have remarkable efficacy ³⁵, even in patients who begin therapy with very high levels of viremia. It is therefore likely that these regimens will prevent *de novo* infection by any viruses released following reversal of latency. As an additional safeguard, intensification of ART with additional antiretroviral drugs can be incorporated into latency reversing strategies.

Another important clinical question is how to measure the reservoir in clinical practice. At the present time, there is no clinical assay for the latent reservoir and no reason to measure it in the management of patients with HIV-1 infection. However, as clinical trials of eradication strategies proceed, it will become important to measure the reservoir. The reversal of latency in shock and kill trials may lead to transient increases in viremia detectable with standard clinical assays, which have a limit of detection of 20-50 copies of HIV-1 RNA/ml of plasma. To date, such increases have not been convincingly demonstrated, suggesting a lack of efficacy.^{89,90} Most patients on ART have trace levels of viremia that are below the limit of detection of standard clinical assays.¹⁰²⁻¹⁰⁵ This residual viremia can be detected with special research assays and is typically on the order of 1 copy/ml of plasma. These assays may prove useful in eradication studies, but it is likely that an effective strategy will produce increases in viremia that are readily measureable even with standard clinical assays.

Ultimately it will be necessary to demonstrate that the intervention has reduced or eliminated the latent reservoir. The reservoir was originally defined with a quantitative viral outgrowth assay (QVOA) in which the frequency of resting CD4⁺ T cells that produce replication-competent virus following cellular activation is determined^{15,16,26}. This assay provides a definitive minimal estimate of the frequency of latently infected cells, but it is expensive and time consuming. Currently the QVOA is performed only in a small number of research laboratories. Because of the low frequency of latently infected cells, this assay requires large volumes of blood (>100 ml) to detect latently infected cells. Any substantial (multi-log) reduction in the size of the reservoir would render the detection of latently infected cells unlikely without an even larger input of cells, for example through leukapheresis.

It is also possible to detect the integrated viral genome with PCR assays.^{14,15,106-108} Interestingly, most PCR-based assays give infected cells frequencies that are 2-3 logs higher than, and poorly correlated with, infected cell frequencies determined by viral outgrowth.¹⁰⁹ An explanation for this discrepancy has recently been provided by Ho et al.¹¹⁰ They demonstrated that most viral genomes detected by PCR have readily identifiable defects that would preclude replication. These include lethal G→A hypermutation introduced by the host restriction factor APOBEC3G^{1,111} and large internal deletions. However, this study also found that the number of intact genomes that were capable of giving rise to infectious virus significantly exceeded the number measured in the QVOA, suggesting that a single round of T cell activation used in this assay does not induce all of the functional latent virus present. Thus, the QVOA likely underestimates the size of the reservoir while PCR-based assays dramatically overestimate reservoir size.

Assuming that it will eventually be possible to develop strategies that produce a multi-log reduction in the latent reservoir, it is interesting to consider the clinical consequences for treated patients. With this degree of reduction, the latent reservoir may no longer be measurable with any assay. The only way to determine whether the strategy has been effective will be to interrupt ART and wait for viral rebound. Normally rebound occurs within a few weeks of interruption. This time interval likely reflects the fact that multiple latently infected cells are being activated per day. The time to rebound represents the time

needed for antiretroviral drugs levels to decline sufficiently plus the time needed for viruses released from these cells to grow out. However, if the latent reservoir is reduced to the point where the activation rate is less than one cell per day, then there will be an additional, highly variable delay in time to rebound reflecting the stochastic activation of surviving cells in the reservoir. Some patients may experience a very late rebound, many months after interruption of ART. The Boston patients and the Mississippi baby described above nicely illustrated this problem. It is therefore important to consider how patients receiving eradication therapy will be monitored. Failure to detect and rapidly treat rebound viremia will likely result in reseeding of the latent reservoir to pre-intervention levels, and improper treatment or poor adherence could lead to the evolution of drug resistant virus. Thus, curative strategies present many challenges beyond the initial step of finding ways to reverse HIV-1 latency.

Concluding thoughts

Given the remarkable success of ART and the likelihood that adherent patients on modern ART regimens will have a near normal lifespan^{112,113}, it is important to consider how finding a cure for HIV-1 infection should fit into research priorities. The success of ART certainly alters the criteria for an acceptable curative protocol. No intervention that poses the risk of significant morbidity or mortality would be acceptable given the alternative of lifelong treatment with relatively well tolerated ART regimens. Nevertheless, a strong justification for eradication research can be found in considering the global scale of the epidemic. A great deal of evidence suggests that all infected individuals should be treated with ART. Treatment outcomes are consistently better in patients who start treatment earlier¹¹⁴. The stability of the latent reservoir means that treatment should be continued for life.^{19,20} The number of infected individuals currently stands at approximately 35 million and continues to increase with time. Whether it will be possible to treat this large and growing population with ART for life is unclear. Currently, well less than half of infected individuals are on ART, and many of them are receiving suboptimal regimens. A simple and safe eradication strategy that allows patients to stop ART for a significant period of time might allow treatment to be more widely distributed and thereby help to curb the spread of the epidemic.

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Key Points

- HIV-1 can persist in a latent form that is not affected by immune responses or antiretroviral drugs.
- This latent reservoir is the major barrier to curing HIV-1 infection.
- Several recent developments have provided hope that a cure can be achieved, but many obstacles remain.

Box 1. Key points regarding basic HIV-1 biology and the cure of HIV-1 infection

- Irreversible integration of the viral genome into host cell DNA confers upon the virus the stability of the infected host cell
- Most productively infected host cells die rapidly.
- Antiretroviral drugs act by blocking *de novo* infection of host cells and can produce a complete or near complete inhibition of new infection events.
- Since productively infected cells die rapidly and ART blocks new infection, the infection would be curable with ART if there were no long-lived infected cell population.
- A long lived population of resting memory CD4+ T cells carrying latent, replication-competent HIV-1 genomes is present in all patients and represents a major barrier to curing the infection.

Table 1

Approaches to Curing HIV-1 Infection (see text for references)

Approach	Type of cure	Example	Latent Reservoir	Population	Current status
Shock and kill	Sterilizing	None yet	Must be reduced by >3 logs	Could be applied to all patients on ART	Effective latency reversing agents needed. Simultaneous boosting of CTL responses may be required. Long term monitoring for rebound required.
Immune control of HIV replication	Functional	Elite controllers (EC)	Present but smaller than typical patient	Goal is to convert all patients to EC	Unclear how to render most patients able to control HIV replication
Early treatment (adults)	Functional	Visconti cohort	Present	Limited to those treated very soon after exposure	Mechanism unclear, many studies in progress
Early treatment (infants)	Sterilizing	Mississippi baby	Early treatment prevents formation	Infants born to infected mothers	Additional studies in progress.
Hematopoietic stem cell transplantation	Goal is a sterilizing cure	Berlin patient, Boston patients	Very small residual reservoir can lead to rebound unless donor cells are resistant	Currently, possible only in patients requiring bone marrow transplantation for other indications	One cure to date with resistant donor cells