HIV reservoirs and the possibility of a cure for HIV infection

S. Palmer1,2, L. Josefsson1,2 & J. M. Coffin3

From the 1Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet; 2Department of Virology, Swedish Institute for Infectious Disease Control, Solna, Sweden; and 3Department of Molecular Biology and Microbiology, Tufts University, Boston, MA, USA


Recent studies demonstrate that suppressive therapy can drive HIV-1 RNA levels to less than 50 copies mL⁻¹ in patient plasma. Yet, ultrasensitive assays show that most patients continue to harbour low-level persistent viremia. Treatment intensification studies indicate that low-level viremia could arise from several different sources. These sources include: (i) long-lived HIV-infected cells that replicate and produce virus; (ii) ongoing replication cycles in cells located in sanctuary sites where drug levels are suboptimal; and/or (iii) proliferation of latently infected cells with regeneration of a stable reservoir of slowly dividing infected cells. A well-defined latent reservoir of HIV is memory CD4+ T-cells where latency is established when an activated CD4+ T-cell becomes infected by HIV, but transitions to a terminally differentiated memory cell before it is eliminated. This review examines the dynamics and possible reservoirs of persistent HIV in patients on suppressive therapy, the mechanisms promoting viral latency and strategies to purge latent viral reservoirs. The promising research described here takes a number of steps forward to seriously address HIV remission and/or eradication.

Keywords: HIV eradication, HIV latency, HIV persistence, HIV reservoirs.

Introduction

The development of combination antiretroviral therapy remains one of the great triumphs of modern medicine. Despite its unquestioned success, current therapy for the treatment of human immunodeficiency virus (HIV) has a number of limitations. For example, although effective and life saving, it is not curative and does not eradicate infection. During combination antiretroviral therapy, reduction of HIV RNA levels to less than 50 copies mL⁻¹ is frequently achieved; however, residual low-level viremia can be detected in most patients using ultrasensitive assays [1–4]. Furthermore, interruption of treatment results in a rapid viral rebound arising from the persistent, residual viremia. Notably, this persistent viremia is evident even after seven years of therapy [5]. Therefore, effective therapy requires life-long adherence, which many patients find difficult to achieve.

The eradication of human immunodeficiency virus (HIV) from infected individuals is one of the major medical challenges of our time. For the past 25 years, this effort has attracted researchers in the fields of infectious disease, virology, molecular biology, drug development and many other related fields. In this article we review research into the source and dynamics of persistent viremia. We will focus on studies describing the levels and genetic make-up of persistent viremia during suppressive therapy and what this research can tell us about viral reservoirs and mechanisms of HIV latency. In the final section we will discuss current strategies for reactivation of the latent HIV reservoir, a step likely to be crucial for its eradication.

Persistent HIV viremia

Highly active antiretroviral therapy (HAART) effectively suppresses HIV RNA levels from an average of about 30 000 to below 50 copies mL⁻¹ (the lower detection limit of most commercial assays used in clinical practice) in the plasma of infected patients. However, if treatment is interrupted, the virus will rebound and become detectable again, often within two weeks [6]. Therefore, HAART does not eliminate HIV infection and residual low-level viremia has been detected using ultrasensitive assays [2, 3, 7–9] in 80% of treated patients. Notably, this viremia can persist for at least 7 years of suppressive therapy [5].
Owing to the fact that HAART prevents the transmission of infection to new cells and the half-life of plasma virions is 6 h, the levels of HIV RNA in the plasma are strongly correlated to the half-life of the cells producing the virus [10, 11]. After introduction of HAART, viremia declines rapidly and mathematical modelling has revealed at least four phases of viral decay corresponding to the half-lives of different populations of HIV-infected cells (Fig. 1). As HAART prevents new cycles of HIV infection, but has no effect on the death rate or viral production of already infected cells, each phase of the decline in viremia reflects the death or elimination of a different population of virus-infected cells. During the fourth phase, there is little or no viral decay and the levels of plasma HIV RNA remain stable ranging from ≤1–5 copies mL\(^{-1}\) with an overall median viral load of 3 copies mL\(^{-1}\) [1–3, 5].

There are two different views regarding the source and/or dynamics of persistent viremia in patients on long-term suppressive therapy. One view holds that persistent viremia is the result of ongoing cycles of viral replication [2, 12, 13]. The other view maintains that this residual viremia is from long-lived cells that are infected prior to treatment initiation [3]. To settle this debate, the source of persistent viremia was recently characterized by quantifying the levels of viremia in patients on suppressive regimens (viral load < 50 copies mL\(^{-1}\)) who ‘intensify’ their standard regimen of three drugs by adding a fourth drug such as raltegravir. If persistent viremia decreases during this treatment intensification, it would suggest ongoing viral replication is contributing to persistent viremia in patients on effective HAART regimens. At least four studies, using a number of different treatment protocols, have shown no perceptible change in persistent viremia in patients receiving intensified treatment or statistical difference between the intensified and placebo arms of the study, implicating long-lived cells as the probable source [14–17]. This conclusion is supported by studies that demonstrate the lack of further evolution in the persistent virus population. Indeed, the emergence of genetically homogeneous subpopulations can often be observed in patients under long-term treatment, as well as in the viral population that rebounds during treatment interruption [18, 19]. The genetic homogeneity of these viral populations suggests that persistent viremia arises from long-lived latently infected cells rather than ongoing cycles of replicating virus. Further support for this comes from earlier studies which revealed that the level of persistent viremia is not related to treatment regimen but to pretreatment viral RNA levels, suggesting that the pretreatment viral set-point is correlated to the number of long-lived HIV-infected cells [3].

However, additional studies of treatment intensification with raltegravir, which blocks integration of HIV DNA into chromosomal DNA, have produced apparent conflicting results. In a minority (30%) of patients, an increase in episomal (unintegrated) HIV DNA was observed leading to the conclusion that active replication persists in some infected individuals on suppressive regimens.

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**Fig. 1** The four phases of viral decay after the initiation of highly active antiretroviral therapy and the virion producing cell populations related to each phase. The yellow cells represent activated CD4+ T-cells, the green cells represent partially activated CD4+ T-cells or other cell types, the blue cells represent resting memory CD4+ T-cell subsets or other cells and the red cells represent long-lived HIV-infected cells which could be resting memory CD4+ T-cells, or subsets of these cells.
In addition, a recent raltegravir intensification study with anatomic analysis of HIV infection in the gastrointestinal tract revealed no decreases in peripheral viremia but decreases were measured in unspliced HIV RNA in CD4+ T-cells obtained from the terminal ileum [21]. These results indicate that low-level viremia might arise from several different sources. These sources may include: (i) long-lived HIV-infected cells that produce virus; (ii) ongoing replication cycles in cells located in sanctuary sites where drug levels are suboptimal such as tissue-based foci of viral replication within CD4+ T-cells and/or myeloid cells; and/or (iii) proliferation of latently infected cells with regeneration of a stable reservoir of slowly dividing infected cells [1, 4, 9–11].

**Viral reservoirs and sanctuary sites**

During untreated infection, HIV predominantly replicates in activated CD4+ T-cells which die quickly either from viral cytopathic effect or from immune response [9]. After suppressive HAART is initiated, infection of new cells is prevented. Cells that are already infected produce virus and die as they would have without therapy. Measurement of decay of virus in plasma as a function of time since initiation of therapy thus reveals the kinetics of death of infected cells. The bulk of the plasma virus – 90% or so – decays in the first phase with a half-life of 1–2 days, and almost certainly reflects infection and killing of activated CD4+ T-cells (Fig. 1)[22, 23].

The kinetics of the second phase of decay is slower with a half-life of 12–14 days. The elimination of several different types of HIV-infected cells may contribute to this viral decay including macrophages, partially activated CD4+ T-cells and different types of dendritic cells. Macrophages are less susceptible to the viral cytopathic effects than activated CD4+ T-cells and the half-life of tissue macrophages can be several weeks [24, 25]. Partially activated HIV-infected CD4+ T-cells have been reported to have a longer turnover time than fully activated CD4+ T-cells [9]. Although it is unknown how long dendritic cells (DCs) can survive after HIV infection, in vitro studies show that infected myeloid dendritic cells can survive for more than 45 days [26]. Langerhans cells (dendritic cells in the skin) have been shown to have a half-life of about 15 days when infected [27, 28], whilst DCs in the mucosa have a shorter half-life of only 2–3 days [29]. However, HIV has not been found in DCs from the peripheral blood following 6 weeks of HAART which suggests that these cells do not contribute to long-term HIV persistence [30]. Follicular dendritic cells trap virions on their surface and these virions can remain infectious for at least 9 months [31] but it is unclear whether their slow release contributes to the different phases of decay. It has also been observed that in HIV-infected patients treated with a raltegravir-containing regimen, the proportion of virus attributable to the second phase is greatly reduced, suggesting that integration is delayed in the cells that make-up this phase [32, 33].

The cellular sources of the third and fourth phases of decay have not been fully identified but these phases most likely represent additional classes of long-lived virus-producing cells. One well-accepted latent
reservoir of HIV is the stable infection of resting long-lived memory CD4+ T-cells. These cells may become infected directly or, more likely, when an activated CD4+ T-cell becomes infected and then subsequently transitions to a resting memory phenotype before HIV infection eliminates the cell (Fig. 2a). Transition to a memory cell is associated with a reduction in transcription factors required for HIV replication. Thus, switching to a memory cell allows this infected host cell to persist for years until it receives a stimulatory signal that activates the cell and concomitantly induces viral production. Resting memory CD4+ T-cells isolated from infected individuals on suppressive therapy can be activated by agents like anti-CD3 or by the combination of cytokines TNF-α, IL-2 and IL-6 to produce infectious virus [34, 35]. During antiretroviral therapy, these inducible cells decay very slowly with an average half-life of 44 months, indicating that under current treatment it will take over 60 years to deplete this reservoir [36]. The frequency of infected resting memory CD4+ T-cells is low, about one infected cell per 10^8 resting memory CD4+ T-cells (about 100-fold less than total HIV DNA–positive cells), and this reservoir is established during primary HIV infection [37, 38].

Recently, Chomont et al. have shown that during HAART, integrated HIV DNA can be found in two different subsets of CD4+ T-cells: central memory T-cells (Tcm) and transitional memory T-cells (Ttm). They found that Tcm cells are the major viral reservoir for patients with relatively high CD4+ T-cell counts, and the low proliferation rate of these cells allows them to persist at adequate levels for years. In patients with low CD4+ counts, the major reservoir is Ttm cells and these cells persist by homoeostatic proliferation making them a very stable viral reservoir. Furthermore, they observed that in individuals who have a lower CD4+ count the stability of the Ttm reservoir is mediated through IL-7 induced proliferation and that in these individuals plasma levels of IL-7 correlated inversely to the rate of decrease of the reservoir. This study suggests that there are at least two mechanisms by which the reservoir of infected resting memory CD4+ T-cells is maintained [39]. Recent studies indicate that the persistent viremia in 50% of patients is derived from memory CD4+ T-cells, which suggests that other virus-producing cellular sources may be responsible for the persistent viremia during the fourth phase of decay during HAART [18, 40]. An important caveat in the interpretation of these studies is that they assume that the properties of the bulk proviral DNA are representative of the small fraction (perhaps 1% or so) of the total HIV DNA–positive cells in patients on long-term suppressive therapy that is capable of being induced to make infectious virus in vitro. The large majority of proviruses are apparently incapable of activation, owing to accumulation of inactivating mutations, unfavourable integration sites or some other cellular feature. At the moment, there is no way to physically distinguish amongst these populations.

Although the bulk of evidence favours the conclusion that persistent viremia is owing to virus production from cells infected prior to initiation of therapy, it remains possible that low-level viral replication may occur in anatomical compartments or tissues where drug levels are suboptimal (Fig. 2b) [21]. Only about 2% of lymphocytes are in the circulation, the remainder are spread throughout the body, especially in lymphoid organs such as the spleen, lymph nodes and gut-associated lymphoid tissue (GALT) where the majority of viral replication takes place during untreated infection [41–44]. In fact, large numbers of lymphocytes are sequestered in the gastrointestinal (GI) tract. During the acute phase of HIV infection, CD4+ lymphocytes located in the GI tract are depleted and remain so throughout the course of the disease [13–15]. During suppressive therapy, the CD4+ T-cells in the GI tract are slowly but incompletely restored [45]. Although antiretroviral therapy greatly reduces HIV replication and immune activation, it is unclear whether low-level ongoing HIV replication is continuing in the gut of treated patients.

Also of note, patients infected with HIV-1 present hematopoietic abnormalities which are caused by HIV infection of the bone marrow [16]. A recent study has shown that HIV infects multipotent hematopoietic progenitor cells (HPCs) and that latent HIV infection was established in some of these HPCs [17]. The authors note in this study that further research is now needed to test whether persistent circulating virus in patients on suppressive therapy is partially derived from HPCs as was shown for resting memory T-cells.

In addition to the lymphoid system, the central nervous system (CNS) is a target for HIV-1 infection; and during primary infection, viral RNA has been detected in the cerebrospinal fluid (CSF) of patients [46, 47]. This infection causes a local inflammatory response which is detectable in the CSF of some HIV-infected individuals, although, in many, HIV-1 infection of the CNS is not clinically apparent [48]. In a subset of patients during the late stages of disease, HIV-1 infec-
tion of the CNS evolves to encephalitis and dementia. In addition, opportunistic infections of the CNS increase during late stage HIV infection. Recent studies suggest the CNS is a viral sanctuary site, as the HIV genetic populations in plasma and CSF are nearly identical during primary infection but as the disease progresses, these populations diverge [49, 50]. Moreover, patients with HIV-associated dementia have the most divergent HIV populations between plasma and CSF. The advent of HAART has reduced the levels of HIV RNA in the CSF and the incidence of dementia in HIV-infected patients has also greatly decreased [51, 52]. However, many drugs have suboptimal penetration of the CNS owing to restrictions of these drugs in crossing the blood-brain and blood-CSF barriers [53]. The limited penetration of the CNS by anti-HIV drugs allows the continual viral replication during HAART not only resulting in HIV drug resistance but is also the development of HIV-associated cognitive impairments. It is unknown whether this persistent virus in the CNS is contributing to the persistent viremia found in plasma of patients on suppressive therapy, although the genetic similarity of both the persistent virus population and the virus that appears following discontinuation of therapy to the pretherapy plasma virus argues against this possibility. In addition, a recent treatment intensification study revealed no decreases in plasma or CSF HIV RNA levels or changes in soluble inflammatory markers such as neopterin [54]. These results suggest that persistent viremia may arise from sources other than the CNS compartment.

In untreated patients, there is compartmentalization of virus between genital secretions and plasma [55, 56]. During HAART, HIV can be found in the semen, both as free virus and integrated DNA [57]. Interestingly, HIV has also been found in renal epithelium [58]. It remains to be determined to what extent GALT, CNS and the genitourinary tract contributes to the production of virions and the persistence of HIV during HAART.

Mechanism of HIV latency

The best-defined HIV reservoir is a small pool of latently infected resting memory CD4+ T-cells carrying an integrated form of the viral genome [59]. The precise molecular mechanisms contributing to the generation and maintenance of this reservoir, however, remain to be elucidated. As noted before, the scarcity of such cells, even amongst those carrying integrated HIV DNA, makes it virtually impossible to identify, isolate and study them directly. Therefore, studies to delve into mechanisms of latency have, for the most part, been confined to cells infected and manipulated ex vivo.

As the chromatin environment influences the expression of HIV, mechanisms which promote HIV latency in infected CD4+ T-cells include histone and DNA modification in the viral long terminal repeat (LTR). The recruitment of cellular histone deacetylases (HDACs) and CpG (CG-rich DNA) methylation, which closes the LTR chromatin structure, prevent HIV transcription and expression [60, 61]. The extent of DNA methylation has been studied in infected individuals and more hypermethylation was found in patients with <50 copies mL\(^{-1}\) than in patients with viral levels of >50 copies mL\(^{-1}\), and a recent study demonstrated that CpG methylation is greater in latently infected than in productively infected primary human T-cells [62]. Although CpG methylation once established is perhaps the major factor suppressing transcription of much of the genome, its establishment may depend on other factors which block gene expression in the first place. Thus, in the case of HIV, this modification is secondary to some other suppressive mechanism, such as lack of an appropriate activator. Furthermore, it is unclear whether reactivation of hypermethylated proviruses is a feasible mechanism to explain persistent viremia.

Several host transcription factors are maintained at low levels in resting CD4+ T-cells including nuclear factor kappa B (NF-kB), nuclear factor of activated T-cells (NFAT) and positive elongation factor b (P-TEFb). The up-regulation of these host factors may activate latently HIV-infected cells and increase HIV expression. In addition, low concentrations or impaired activity of the viral activator protein Tat and low levels of the cellular protein PTB, which causes a post-transcriptional block and degradation or translational suppression of viral mRNA, have been shown to be important mechanisms of HIV latency. (Reviewed in [63]).

The site of viral integration into the host genome can affect the transcription rate of HIV. The integration of HIV proviruses has been found to occur in both actively and actively transcribed genes [64, 65]. The reduction of viral transcription in actively transcribed gene regions of resting memory CD4+ T-cells may be owing to transcriptional interference [66]. Transcriptional interference takes place when cellular transcripts initiated outside the integration site of HIV read through the HIV genome and disrupt the transcription of HIV by displacing the viral transcription...
factors assembled at the HIV promoter causing a decrease in HIV expression.

Owing to difficulties in using primary human T-cells for investigation of HIV latency, most studies to date have utilized infected cell lines for modelling viral latency [65, 67, 68]. Studies based on these cell lines have revealed many important mechanisms of HIV latency but an infected cell line may not truly reflect the latent state in vivo. Therefore, new ex vivo models based on primary human CD4+ T-cells have been developed by several researchers and most of these promising ex vivo latency models use human primary CD4+ T-cells infected with HIV [62, 69–72]. These new models have allowed for detailed studies of the signalling pathways which can reactivate latent HIV. They underline the importance of NFAT, the tyrosine kinase Lck and cellular transcription factor binding sites such as Sp1 and kb/NFAT for optimal reactivation of latent HIV in memory CD4+ T-cells. In addition, these relatively new ex vivo models revealed that HIV was not reactivated after incubation with compounds such as the HDAC inhibitor valproic acid; the NF-kB activating agent prostratin; PMA; and TNF-α. These compounds had previously been shown to reactivate HIV in cell line-based models [70]. These discrepancies further stress the importance of using ex vivo models instead of models based on infected cell lines. Moreover, these ex vivo models did demonstrate the importance of heterochromatic structures owing to histone deacetylation and DNA methylation for promoting HIV latency.

A recent study revealed that HIV integration can happen in both active and resting primary human T-cells and that in both cell types, integration was favoured in active transcription units [73]. A modest difference between resting and active cells was found and integration into actively transcribing genes was reduced in resting cells. The finding that latency can be established in resting T-cells conflicts with commonly held beliefs that HIV latency is established only in activated T-cells. Additional studies are needed to investigate the contribution of resting T-cells to persistent viremia.

In addition, the role of myeloid cells in maintaining the latent reservoir should be investigated as it is generally assumed that HIV persists in both lymphoid and myeloid cells [74, 75]. For example, activated macrophages residing within tissues interact with CD4+ T-cells via cell-to-cell contact, and via release of inflammatory and regulatory cytokines. These interactions may contribute to HIV persistence.

**Strategies for purging the latent HIV reservoir**

The mechanisms behind HIV latency are extremely complex; and to efficiently eliminate the viral reservoir, many different strategies have to be explored. Reactivation of these latently infected cells which would eliminate the HIV-infected cell either by viral cytopathic effect or natural immune response in combination with antiretroviral therapy to prevent new infections is currently considered the best strategy, but not the only, for eradication of HIV.

Earlier studies have shown a nonspecific activation of resting memory T-cells and an increase in HIV replication by CD3 monoclonal antibodies or by combination of cytokines TNF-α, IL-2 and IL-6 [34, 35]. In addition, the cytokine IL-7 has been shown to reactivate HIV primary human T-cells and thymocytes in vitro and is well tolerated in HIV-infected patients [76–79]. Patients infected with HIV are currently receiving IL-7 as part of an ongoing clinical trial to determine if IL-7 reduces their latent HIV reservoir (ERAMUNE, http://www.clinicaltrials.gov). The outcomes of this trial could help in the development of new and important therapies for HIV eradication. Upregulating cellular transcription to induce HIV gene expression has been proposed as another strategy for reducing latent HIV reservoirs. This includes inhibiting class I HDACs because these HDACs promote latency by regulating genome structure and transcriptional activity. A recent report has shown that synthetic class I HDAC inhibitors can reactivate latent HIV in a Jurkat cell model of latency and in resting CD4+ T-cells isolated from patients better than class II HDAC inhibitors by inducing LTR expression [67]. However, the coadministration of the HDAC inhibitor, valproic acid and HAART gave mixed results [80, 81].

Similar to multiple-drug HIV therapy which interferes with different stages in the viral life cycle, antilatency compounds have been combined to purge and reactivate latent HIV. A recent study revealed that in patients on suppressive therapy (HIV RNA < 50 copies mL⁻¹), a combination of prostratin with either valproic acid (long approved – at low doses – for the treatment of epilepsy) or suberoylanilide hydroxamic acid (SAHA) (a recently approved HDAC inhibitor for clinical use as cancer therapy) was more efficient than each compound alone in reactivating latent HIV in CD8 depleted PBMCs [82]. Synergistic reactivation of latent HIV was also achieved by combining HDAC inhibitors and NF-kB activating agents [83]. This study demonstrated that in PBMCs and Jurkat-cell-
based systems, a combination of SAHA and prostratin synergistically reactivates latent HIV. This SAHA and prostratin combination targeted a wide range of latent integration sites, acted effectively against viral variants that have acquired mutations in their promoter regions and functioned across five different HIV-1 subtypes. These results emphasize the possibilities of using combination antilatency therapy and may explain earlier disappointing results from a clinical trial using valproic acid alone to treat patients on suppressive HAART [80]. Moreover, SAHA by itself reactivated latent HIV in cell lines and CD4+ T-cells isolated from patients on suppressive HAART. SAHA is approved by the US Food and Drug Administration for clinical use, making it a promising candidate for future clinical trials [84, 85]. Currently there are large-scale screening efforts ongoing to find novel compounds to reactivate latent HIV using latently HIV-1-infected reporter cell lines that allow for high throughput drug screening [86]. In addition, resting memory CD4+ T-cells from patients on suppressive HIV therapy are being used to screen potential reactivating agents [67].

NF-kB inducing agents such as prostratin have shown promise in reactivating HIV from latently infected cells. However, NF-kB promotes global T-cell activation running the risk of systemic inflammatory response in patients. Recently, two transcription factors Ets-1 and VII-Ets-1 have been found which reactivate latent HIV in a NF-kb-independent manner [87]. In addition, a compound, 5-hydroxynaphthalene-1,4-dione (5HN), has been found which reactivates latent HIV by producing a strong but short burst of NF-κB activity. This NF-κB activity was sufficient to trigger initial HIV-1 Tat production, which then induced HIV-1 expression [88].

To further elucidate where HIV persists during therapy and to test compounds for the eradication of HIV, an appropriate animal model is necessary. Ideally, this model should show similar patterns of viral decay and CD4+ T-cell increase during therapy as HIV-infected humans starting HAART. The therapy used in the animal model should also simulate HAART. A recent study revealed that SIV-infected macaques treated with a combination of four antiretroviral drugs revealed a similar pattern of viral decay and reduced frequencies of circulating resting CD4+ T-cells similar to HIV-infected humans starting HAART making this an attractive macaque model for studying HIV latency [89, 90]. However, the macaque model has two major disadvantages: (i) rhesus macaques are expensive as they have to be maintained on therapy for a long time for studies of viral persistence, and (ii) there are important genetic differences between SIV and HIV. Therefore, a ‘humanized’ bone marrow/liver/thymus mouse model populated with both human B- and T-cells that can be infected with HIV is an interesting alternative animal model for researching viral reservoirs and compounds that purge these reservoirs [91].

Clinical approaches to HIV eradication

The observation that an increase in viral RNA was measured in HIV-infected individuals treated with intravenous immunoglobulin (IVIGs) for Guillain-Barrés syndrome resulted in a study of the possible use of IVIGs to eradicate HIV [92]. The administration of high dosage of IVIGs to patients on HAART decreased the latent reservoir, as measured by a decrease in quantifiable HIV in resting memory CD4+ T-cells, in about half of the patients revealing how bedside observations may point the way to new important anti-latency therapies. It is assumed the mechanism for how IVIGs decrease the latent reservoir in HIV patients is related to the effects of IVIGs have on the activation, differentiation and effector functions of dendritic cells, and T- and B-cells [93].

Also of note is the interesting case of a HIV-infected patient in Germany, apparently now cured of the disease following a stem cell transplant from a donor, homozygous for the CCR5 Δ32 mutation, which prevents expression of this HIV-1 coreceptor. Despite receiving no antiretroviral therapy in the 3.5 years since receiving the transplant, this patient has experienced no viral rebound [94]. Such procedures are not presently a viable solution for tens of millions of HIV-infected individuals worldwide. Nevertheless, it is an intriguing and promising result which informs efforts to develop a cure for HIV infection.

The CCR5Δ32 phenotype is being replicated in HIV-infected individuals through the use of gene modification. The expression of the CCR5 coreceptor is eliminated from CD4+ T-cells and hematopoietic stem or progenitor cells (HSC) by zinc-finger nucleases (ZFNs) which permanently disrupt the CCR5 open-reading frame [95, 96]. The use of adenovirus vectors to deliver these CCR5-targeted ZFNs to pri-
mary human CD4+ T-cells resulted in HIV-resistant primary CD4+ T-cells that expanded stably in culture [96]. The transplantation of these cells into immunodeficient mice and subsequent challenge with CCR5-tropic HIV-1 resulted in a statistically significant reduction in plasma viral RNA in the mice receiving the ZFN-modified T-cells compared to the mice which did not receive these cells. Additional studies of immunodeficient mice transplanted with ZFN-modified HSC revealed that 6 weeks after infection with a CCR5-tropic HIV-1, viral loads decreased and their transplanted human CD4+ T-cells increased to preinfection levels. In fact, HIV-1 could not be detected by PCR quantification 10–12 weeks postinfection at necropsy. In addition, the levels of CCR5-negative human cells had expanded indicating a selective advantage for these cells [95]. These CCR5-modified T-cells remain susceptible to CXCR4-tropic strains of HIV-1 and, therefore, phenocopy the HIV-1 resistance in patients with the naturally occurring CCR5Δ32 allele. Based on the positive results from these preclinical studies, the therapeutic value of CCR5-knockout T-cells is being evaluated in a phase 1 clinical trial. Three different patient cohorts are being enrolled in this trial: patients infected with multidrug resistant virus; patients with controlled viral infection who have normal CD4+ T-cell levels; and patients infected with drug-sensitive virus but who have low CD4+ T-cell levels (ClinicalTrials.govNCT00842634). CD4+ T-cells from these patients will be exposed to CCR5-targeted ZFNs ex vivo. The resulting CCR5-knockout cells will be expanded for 10 days and then transferred to the patient. The overall aim of this trial is to replicate the CCR5Δ32 phenotype in HIV-infected individuals and it will be completed in 2011.

Obviously, caution in approaching therapies that meddle with control of immune activation is warranted. The very nature of the virus-host relationship means that seeking to purge the latent HIV reservoir runs the risk of triggering a systemic patient immune reaction. In addition, any future clinical approaches to eradicating latent HIV reservoirs must avoid the generation of new HIV infection resulting from the activation of these latently infected cells. In carrying out initial exploration of these methods, researchers will need to integrate appropriate animal modelling first to avoid such risks arising in humans. Even then, experience with potent immune activators in other fields has shown that animal models may not successfully predict the human reaction to therapies that can affect the immune response at a basic level.

Conclusion

Under the most effective current antiretroviral therapy, HIV infection is well suppressed but not eradicated. During combination antiretroviral therapy, reduction of HIV RNA levels to less than 50 copies mL⁻¹ is frequently achieved; however, ultrasensitive assays detect the persistence of viremia in approximately 80% of patients on suppressive HAART for periods exceeding 7 years. The source and dynamics of this persistent viremia are currently under investigation. The important conclusion that emerged from this work is that antiviral strategies alone will never be adequate to eliminate HIV infection; to accomplish this goal, we must address HIV latency and its implications. A well-defined latent reservoir of HIV is resting memory CD4+ T-cells, which might be infected directly at low frequency, or arise when an activated CD4+ T-cell becomes infected by HIV, but transitions to a terminally differentiated memory T-cell before HIV infection eliminates the cell. However, other cell types such as hematopoietic stem cells or cells of the monocyte/macrophage lineage may also contribute to the latent reservoir. A number of mechanisms, such as the chromatin environment, the viral integration site, down-regulated transcription factors, impaired activity of Tat and suppression of viral mRNA leading to reduced viral protein translation, have been shown to play a role in maintaining HIV latency. Whilst further research is needed, recent studies have introduced new insights into the mechanisms of latency and persistent HIV reservoirs. Considerable effort is now underway to apply the findings of these mechanistic studies to the clinical problem. The problem of HIV remission and/or eradication from an infected patient is an extremely difficult one, but well worth the effort.

Conflict of interest statement

No conflict of interest was declared.

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Correspondence: Sarah Palmer, Department of Virology, Swedish Institute for Infectious Disease Control, Nobels väg 18, 171 82 Solna, Sweden.

(fax: 46-08-337272; e-mail: Sarah.Palmer@smi.se)