Reservoirs of Human Immunodeficiency Virus Type 1: The Main Obstacles to Viral Eradication

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Highly active antiretroviral therapy (HAART) has led to profound decreases in morbidity and mortality rates in human immunodeficiency virus type 1 (HIV-1)–infected persons, at least in the developed world. Many infected persons have plasma levels of HIV-1 RNA that are less than the limits of detection of most clinical assays as a result of combination antiretroviral therapy. Nonetheless, HIV-1 has not been eradicated by HAART. This has been shown to be because of latent HIV-1 replication-competent provirus in resting CD4+ T lymphocytes, cryptic viral replication below the limits of detection of most clinical assays, and, possibly, the presence of viral sanctuary sites. An understanding of these reservoirs for HIV-1 in the setting of virally suppressive HAART will be critical for the development of new approaches to induce HIV-1 remissions and for the exploration of the possibility of viral eradication in the future.

HIV type 1 (HIV-1), the causative agent of AIDS, is a lentivirus, a family within the broad group of viruses known as Retroviridae. Although HIV-1 inserts its genome into the host cell’s DNA (i.e., integration), this retrovirus also replicates efficiently and continuously in an infected host [1]. Thus, HIV-1 replicates in most untreated, infected persons at high levels throughout the infection, including the clinical quiescent phase. Levels of this active viral replication directly correlate with disease progression and patient survival [2, 3]. Combination therapeutics for HIV-1, or highly active antiretroviral therapy (HAART), have led to dramatic decreases in viral replication in vivo to less than the clinical limits of detection (i.e., usually plasma HIV-1 RNA levels of <50–400 copies/mL, depending on the clinical assay system used) and reductions in the rates of morbidity and mortality [4, 5].

Developments in the era of HAART have allowed clearer investigations of classic questions in human retrovirology, but new clinical problems have also been generated. Mechanisms of viral latency and hidden or “cryptic” viral replication can also now be addressed without the “noise” of active, virus-producing cells and high levels of cell-free virions as virally suppressive HAART “unveils” viral persistence. Furthermore, approaches toward possible viral eradication, or at least long-term remission, can be rationally studied. Nonetheless, it is critical to recognize that true viral eradication may be extremely difficult for many reasons. Of note, cells other than those in the immune system (e.g., in the kidney or heart) also appear to be infected at low levels with HIV-1 in vivo [6, 7]. I review the cellular and molecular reservoirs used by HIV-1 to persistently infect a host in the setting of clinically effective HAART.

HIV-1 PERSISTENCE

Latency, a general property of all retroviruses, is one of the diverse virus–host cell interactions that allows persistence of HIV-1 in the setting of effective, or virally suppressive, HAART [1]. “Retroviral latency” is defined as integrated provirus with no active transcription. Low-level chronic—yet productive—viral expression best characterizes persistent or cryptic replication. In the case of standard HAART, virus replication from previously infected cells is not fully ablated. Viral persistence takes place after initially successful virally suppressive therapy or effective antiviral immune responses in the infected host.
Such cryptic viral replication may also continue in immunologically privileged sites, such as the brain and testes. In addition, transcriptionally active but nonproductive viral infections (i.e., multiply spliced viral mRNA are expressed, but intact virions are not) may also occur (figure 1).

The HIV-1 replicative cycle contains multiple possible stages for latency and persistence via both pre- and postintegration into the human cell genome [1]. Initially, HIV-1 binds to cellular receptors and coreceptors (i.e., CD4 receptor and the chemokine coreceptors CCR5 and CXCR4), with subsequent viral core internalization and reverse-transcription of the viral RNA template into a double-stranded DNA intermediate. The viral DNA is then integrated into the host cell genome as proviral DNA. Transcriptional activation of the integrated provirus is controlled by a complex series of interactions between HIV-1 regulatory proteins (e.g., Tat and Rev) and cellular transcription factors, controlled by the state of activation of the host cell. The activated provirus produces various viral messenger RNA species and, in some cases, new virions [1].

Many studies have demonstrated that clinical HIV-1 latency, when defined as no virus expression in an entire untreated, infected person, does not exist at the host level during any stage of the disease [3]. Nevertheless, data indicate that, in the HIV-1–infected person, some cells contain proviral DNA, but they express little or no viral RNA and produce few or no virions [8]. In addition to postintegration latency, various states of preintegration HIV-1 latency have been described both in vitro and in vivo [9, 10]. As such, latency at a cellular level exists in vivo, and the number of latently infected cells may vary according to the stage of disease. As HIV-1 infects, in vivo, both CD4+ T lymphocytes and monocyte or macrophages, as well as other nonimmune-based cells [6, 7], the virus may maintain cellular latency by means of different molecular mechanisms in different cell types.

HIV-1 replicates extraordinarily rapidly in infected persons, with a virion half-life in plasma of minutes [11]. Most of this viral replication (considerably more than 99%) occurs in activated and productively infected CD4+ T lymphocytes in the peripheral blood and lymphoid tissue. Analysis of plasma viral decay in patients initially treated with potent combinations of antiretroviral drugs demonstrates a rapid first-phase decay of productively infected cells, a second-phase decay of long-lived cells (possibly from long-lived cells, such as tissue-bound macrophages), and then a third-phase decay of persistently infected cells (e.g., resting CD4+ T lymphocytes) [11]. Effective HAART blocks the virus from infecting healthy CD4+ T lymphocytes,
and the initial decrease in HIV-1 levels in the peripheral blood reflects the lifespans of cells that were infected before treatment was initiated. On the basis of these complex viral and cellular kinetics with slow decay of virus-infected cells in several phases, most HIV-1–infected persons would require complete suppression of viral replication for decades if one is even to consider viral eradication. However, this goal is hindered by several factors, including less-than-optimal treatment, HIV-1 infection in immunologically privileged sites, and partial drug sanctuary compartments in vivo (see the Potential HIV-1 Sanctuary Sites section).

**HIV-1 LATENCY IN RESTING CD4+ T LYMPHOCYTES IN VIVO**

In the peripheral blood of HIV-1–infected persons who have undergone treatment, persistently infected, nonactivated CD4+ T lymphocytes have been demonstrated to effectively suppress most productive viral infection [12]. Replication-competent viruses can be recovered from these proviral-positive cells after CD8+ T lymphocyte depletion in vitro [13–15]. Most of these viruses are CCR5-tropic [16]. In addition, this cell reservoir is established soon after primary HIV-1 infection [17] and can be activated by proinflammatory cytokines in vitro and potentially in vivo [18]. Suppressive HAART initiated before primary HIV-1 seroconversion and during perinatal HIV-1 infection has been shown to be unable to halt the development of this replication-competent virus reservoir in CD4+ T lymphocytes [17, 19].

Latent replication-competent HIV-1 provirus has been demonstrated in mainly resting memory (CD45RO) CD4+ T lymphocytes, but low levels of virus have also been found in resting naive (CD45RA) CD4+ T lymphocytes in patients receiving virally suppressive HAART. It was suggested that naive CD4+ T lymphocytes are rarely directly infected, but that the provirally harboring naive CD4+ T lymphocytes are generated by means of reversion from a memory phenotype [16]. In addition, direct HIV-1 infection of thymocytes in both children and adults may also lead to infection of naïve T lymphocytes [20].

Most viruses isolated from resting CD4+ T lymphocytes from the peripheral blood of patients with undetectable levels of viral RNA in plasma have few mutations that confer antiretroviral drug resistance [13]. Resistance mutations in the reverse-transcriptase (RT) and protease (PR) genes of HIV-1 are correlated with ongoing viral replication despite treatment, which suggests that these viral strains may represent “archival” species from a time before treatment, although this may change in the future if primary HIV-1 resistance to antiretrovirals continues to rise. It is of note that a study has demonstrated that, in some patients who are receiving virally suppressive HAART with transient spikes of virus of >50 copies/mL in plasma, resistance mutations may develop [21]. Data from the aforementioned studies demonstrated that, although defective proviruses accumulate in CD4+ T lymphocytes in vivo (i.e., “viral graveyard sequences”) [22], replication-competent provirus still exists in resting CD4+ T lymphocytes, which may hinder attempts at reducing the viral reservoir and may reseed the body with virus if HAART is discontinued. These possibilities, therefore, confound our attempts toward pharmacologically mediated virus eradication. In addition, a study has also demonstrated the presence of replication-competent HIV-1 in peripheral blood monocytes of patients who are receiving virally suppressive HAART [23].

Gut-associated lymphoid tissue also appears to be an important site for early HIV-1 replication in these mucosal lymphoid tissues [24]. Mucosal lymphoid tissue, with high baseline levels of T lymphocyte activation, may be a critical reservoir outside of the peripheral blood for HIV-1 residual disease in patients who are receiving suppressive HAART. Another potential reservoir for HIV-1 in certain patients who are receiving suppressive HAART appears to exist in the tubular epithelial cells of the kidney [7].

**POTENTIAL HIV-1 SANCTUARY SITES**

Tissues that maintain blood-tissue barriers, secondary to microvascular endothelial cell-fight junctions, may limit penetration of certain antiretroviral agents and act as partial drug sanctuaries. These compartments would potentially include the CNS, the retina, and the testes [1, 25, 26]. Although brain and retinal tissues are difficult to obtain from healthy HIV-1–infected persons receiving virally suppressive HAART for analysis, our initial studies demonstrated replication-competent HIV-1 provirus in the seminal cells of certain men who are receiving virally suppressive HAART. This reveals that HIV-1 may occur behind critical blood-tissue barriers in vivo [26]. An article suggests that true, complete viral sanctuaries may not be found in most areas of the body, although many potential sites were not analyzed in this small study [27].

A plasma membrane localized drug transporter, P-glycoprotein, has been shown to decrease the penetration of protease inhibitors across certain blood-tissue barriers. As such, substances that block this drug transporter will increase protease inhibitor concentrations across the blood-tissue barriers. This finding may represent a new pharmacological approach to target relative viral sanctuary sites with higher levels of antiretroviral agents [28]. It is of note, however, that most analyses do show parallel decay of HIV-1 in tissues and CSF, as compared with peripheral blood, in patients who receive virally suppressive HAART [29]. In addition, free-drug levels, rather than protein-bound drug concentrations, have rarely been analyzed in body sites outside of the peripheral blood.

Some studies show relatively poor penetration of certain an-
tiretroviral agents into the male genital tract [30]. Nevertheless, most, but not all [31], patients who receive virally suppressive HAART also experience a decrease in the corresponding seminal fluid HIV-1 RNA level, which can decrease to clinically undetectable levels [32]. This pattern has also been demonstrated in the cervicovaginal fluids of HIV-1–infected women who are receiving this type of antiretroviral therapy [33].

**CRYPTIC VIRAL REPLICATION**

Persistently infected CD4+ T lymphocytes that contain non-defective but quiescent HIV-1 proviral DNA and low levels of viral replication could account for the replication-competent virus within the peripheral blood CD4+ T lymphocytes and seminal cells of infected persons receiving HAART [26]. Viral replication could occur at such low levels that the virus would not be detectable in peripheral body fluid samples with standard clinical assays. Low-level productively infected cells might infect small numbers of target cells in the surrounding cellular microenvironment. One study demonstrated that, in a minority of patients who were receiving suppressive HAART, modest evolution of viral envelope sequences occurred over time [34]. Other studies have demonstrated that, during what appears to be full suppression of HIV-1 in the plasma, as determined by clinical RNA assays, there is ongoing viral replication in a majority of patients in some cohorts, as shown by evolution of viral sequences in cellular reservoirs [35]. It is of note that these archival viruses did not demonstrate antiretroviral resistance mutations, even in those strains that could undergo low-level replication.

In several studies [36, 37], sensitive measures of the “footprints” of persistent viral replication were used to evaluate HIV-1 mRNAs species and HIV-1 long-terminal repeat (LTR) DNA circles, which are formed by self-ligation of proviral DNA by cellular nuclear ligases after transport of the viral preintegration complex to the nucleus. LTR-DNA circles were demonstrated in the CD4+ T lymphocytes of most patients receiving suppressive HAART. The LTR circles, along with quasi–steady-state levels of HIV-1 mRNA, suggest the existence of low-level viral replication at some time in the past. A short in vivo half-life for HIV-1 2-LTR DNA circles has been suggested [37], but this is now controversial and requires further in vivo study. Our laboratories have demonstrated that levels of in vivo cryptic HIV-1 replication differ in specific patients who receive virally suppressive HAART and can be used to stratify these groups of patients with residual retroviral disease [38].

In one study [36], an additional complexity with regard to characterization of HIV-1 persistence during HAART was also demonstrated, because certain patients had cells with multiply spliced RNA out of proportion to unspliced viral RNA, a finding in agreement with in vitro models [39]. As multiply spliced viral RNA encodes regulatory proteins (and unspliced RNA encodes structural proteins), this pattern suggests transcriptionally active but nonproductive infection. These patterns of viral RNA species expression may be based on the state of host cell activation. Thus, several forms of cryptic replication might reset the virological clock by infecting previously uninfected cells in localized microenvironments.

Data have demonstrated that certain unstimulated, human leukocyte antigen–DR–negative CD4+ T lymphocytes in lymphoid tissue sampled from HIV-1–infected persons treated with virally suppressive HAART were positive for low levels of viral RNA [40]. HIV-1 replication was demonstrated previously to occur in activated memory CD4+ T lymphocytes in samples of both peripheral blood and lymphoid tissues [1]. These new data suggest that there may actually be a spectrum of cell types in a relatively inactive state that can still express low levels of viral RNA.

Most patients rebound rapidly (mean time, 16–17 days) with high levels of plasma viral RNA when standard suppressive HAART is discontinued, even when <50 copies/mL of plasma viral RNA has been demonstrated for significant periods of time [41]. This observation suggests not only the presence of persistently infected cells but also ongoing viral replication. One study showed that rebounding virus in certain patients after discontinuation of HAART does not appear to be from an outgrowth of latent provirus in resting peripheral blood CD4+ T lymphocytes, but the cellular reservoir that produces the rebound virus remains unclear [42]. Nevertheless, another study demonstrated that rebound virus in some cases can arise from the latent virus in resting CD4+ T lymphocytes [43].

By use of a laboratory-based RT-PCR assay, which can quantify ≤5 copies/mL of plasma viral RNA, low (but detectable) levels of plasma virions were demonstrated in all HIV-1–infected patients with clinically undetectable levels of plasma HIV-1 RNA (i.e., <50 copies/mL) in a study [44]. This ongoing viral replication may infect cells in local sites, as well as cells at a distance within the body. The quantities of defective virions in residual plasma HIV-1 is still unknown and remains important for understanding possible viral spread within an infected host.

The viral decay characteristics in cells are different than the viral decay characteristics in plasma. In peripheral blood mononuclear cells, the first phase represents decay of productively infected and infected long-lived cells, whereas the second phase is decay of cell-associated viral DNA and mRNA from latently infected and nonproductively infected cells. Viral reservoir decay characteristics in one analysis have suggested that it will be necessary for patients treated with suppressive HAART to continue therapy for 60 years for potential viral eradication [45].
Unfortunately, even this estimate may not represent a worst-case scenario for patients, because this analysis did not fully take into account low-level viral replication in CD4+ T lymphocytes and tissues other than lymphoid organs.

Some patients with blood plasma viral RNA levels of <50 copies/mL who are receiving HAART still have low-level bursts of viral replication leading to >50 copies/mL in transient spikes, which carry risks of the evolution of viral resistance. The mean decay half-life of latent replication-competent proviral reservoirs is longer in patients with transient plasma viral RNA spikes, as compared with those patients who consistently maintain plasma HIV-1 RNA levels of <50 copies/mL [46]. A small study has suggested that low-level spikes of plasma HIV-1 RNA in patients receiving previously suppressive HAART do not appear to be associated with higher rates of viral rebound, at least in the short term [47]. Other preliminary studies, however, have shown lower CD4+ T lymphocyte counts in patients with plasma HIV-1 RNA spikes [48, 49]. It will be important to determine whether these viral spikes are due to insufficient plasma drug levels, depressed drug penetration into unique sanctuary sites, or just continued HIV-1 replication in the lymphoid tissues, as well as in peripheral blood cells.

The findings that 60 years of suppressive HAART would be necessary to possibly allow latent reservoir eradication were suggested to be owing more to the slow decay of a truly latent reservoir in patients who have no spikes of plasma HIV-1 RNA in the past decade, there has been a remarkable increase in the understanding of the pathogenesis of HIV-1 disease and in the rational design of antiretroviral therapy. Therefore, additional basic science and translational research is likely to aid us in the development of effective approaches with which to target residual HIV-1 disease.

**OBSTACLES TO VIRAL ERADICATION**

The treatment options for attempting to rid the body of cells infected with HIV-1, which can produce replication-competent virus in patients who are receiving effective HAART, are now beginning to be explored. On the basis of our understandings of molecular lentiviral pathogenesis, approaches can be developed to activate persistently infected cells, which might lead to virus-induced cell death and to the purging of the viral reservoirs. In theory, activation approaches for eliminating persistently infected cells would not lead to increases in newly infected cells, because virus spread would be prevented by HAART. The targeting of specific immunotoxins to deplete the HIV-1 proviral reservoir (after stimulation of viral transcription from the integrated proviruses) and the addressing of potential ongoing viral replication should now also be explored [52].

Because low levels of ongoing cryptic viral replication occur in many HIV-1–infected persons who are receiving effective HAART, “intensification” therapeutics must be added to initial combination therapy to ablate this viral replication if eradication is a goal. Therapeutic approaches that purge only the latent viral reservoirs will probably not be successful. In addition, further studies on the penetration of different pharmacological agents across blood-tissue barriers (e.g., blood-brain and blood-testes) will be of importance in attacking potential sanctuary sites, which may contain both cryptically replicating as well as fully latently infected cells. Certainly, combinations of each of these approaches may also be required for elimination of the HIV-1 reservoirs in vivo. As such, one should think of the treatment of HIV-1 as an oncological paradigm. This would include effective HAART as induction therapy and then additional approaches against HIV-1 latency, cryptic replication, and sanctuary sites for the removal of residual disease.

The complexity of viral reservoirs in HIV-1 infection is significant, and HAART, in its present form, is unlikely to eradicate HIV-1 in infected persons. Nevertheless, during the past decade, there has been a remarkable increase in the understanding of the pathogenesis of HIV-1 disease and in the rational design of antiretroviral therapy. Therefore, additional basic science and translational research is likely to aid us in the development of effective approaches with which to target residual HIV-1 disease.

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