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Review

HIV reservoirs, latency, and reactivation: Prospects for eradication

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ABSTRACT

Current antiretroviral therapy effectively suppresses but does not eradicate HIV-1 infection. During therapy patients maintain a persistent low-level viremia requiring lifelong adherence to antiretroviral therapies. This viremia may arise from latently infected reservoirs such as resting memory CD4+ T-cells or sanctuary sites where drug penetration is suboptimal. Understanding the mechanisms of HIV latency will help efforts to eradicate the infection. This review examines the dynamics of persistent viremia, viral reservoirs, the mechanisms behind viral latency, and methods to purge the viral reservoirs. This article forms part of a special issue of *Antiviral Research* marking the 25th anniversary of antiretroviral drug discovery and development, vol. 85, issue 1, 2010.

Contents

1. Introduction

The treatment and eradication of human immunodeficiency virus (HIV) are two of the major medical challenges of our time. For the past 25 years these challenges have attracted the best minds in the fields of infectious disease, virology, molecular biology, drug development, and many other related fields. Although current antiretroviral therapy is effective and life-prolonging it is not curative and does not eradicate HIV-1 infection. During combination antiretroviral therapy, reduction of HIV-1 RNA levels to less than 50 copies/ml is frequently achieved; however, residual low-level viremia has been detected using ultrasensitive assays. Interruption of treatment results in a rapid viral rebound arising from the persistent, residual viremia. Notably, this persistent viremia is present even after 7 years of therapy.

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In this article we review the characteristics of persistent HIV, the mechanisms that promote HIV latency, and potential approaches for therapeutically counteracting these mechanisms for the elimination of latent HIV reservoirs. We first discuss which cells and sanctuary sites are suspected of harboring HIV during effective antiretroviral therapy. We then review research into the dynamics and possible sources of persistent viremia. The source of persistent viremia is currently unknown: it could arise from ongoing cycles of replication in a sanctuary site where the drug levels are suboptimal, from long-lived productively infected cells, and/or from activation of viral expression from latently infected cell reservoirs. We then examine the molecular mechanisms of HIV latency. HIV latency was first discovered in cultured T-cells from HIV-infected patients. A subset of these T-cells only produced virus after stimulation with strong T-cell activators indicating they contained a replication competent transcriptionally silent provirus. Since this discovery, it has become evident that HIV latency is promoted by several different cellular and viral mechanisms. In the final section we review proposed methods for purging the latent HIV reservoirs. Stimulating the production of HIV from these reservoirs is crucial for the eradication of HIV.

2. Dynamics of viral decay

HAART can effectively suppress HIV RNA levels to below 50 copies/ml which is the lower detection limit of most commercial assays approved for clinical use. However, if treatment is stopped HIV becomes detectable again, often within 2 weeks (Davey et al., 1999). This is true even if HAART was initiated during acute infection (Markowitz et al., 2002). Soon after the introduction of HAART it became evident that it did not completely eliminate HIV from the plasma and that low-level persistent viremia could be detected in the individuals on suppressive therapy (Dornadula et al., 1999). Recently it has been established that persistent viremia remains detectable in plasma even after 7 years of HAART (Palmer et al., 2008).

Plasma virions have a half life of 6 h (Perelson et al., 1997). The level of HIV RNA in plasma is therefore strongly correlated to the half lives of the infected cells that produce HIV. HAART blocks new rounds of HIV replication and prevents new infections of cells. By measuring HIV RNA levels in plasma and using mathematical modelling it becomes evident that after the initiation of HAART plasma viremia goes through four phases of decay corresponding to the half lives of different populations of HIV-infected cells (Fig. 1). The first phase of decay corresponds to a population of cells with a half life of approximately 1–2 days, and the second phase 1–4 weeks (reviewed in Rong and Perelson, 2009). Two additional phases of decay has recently been identified during suppressive therapy in individuals with <50 copies/ml: a third phase of decay corresponding to cells with a half life of approximately 39 weeks and a fourth phase with no appreciable decay (Palmer et al., 2008). During the fourth phase the levels of HIV normally ranges from 1 to 5 copies/ml with an overall median viral load of 3.1 copies/ml (Maldarelli et al., 2007).

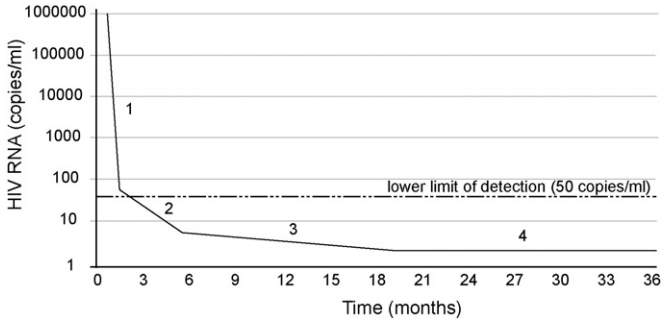

Fig. 1. After the initiation of HAART HIV there are four phases of viral decay which have been identified. *Phase 1* corresponds to a virion producing cell population with a half life of 1–2 days, presumably infected active CD4+ T-cells. *Phase 2* corresponds to one or several cell-populations with a half life/lives of 1–4 weeks, such as infected macrophages, dendritic cells or partially activated CD4+ T-cells. *Phase 3* corresponds to a population of cells with a half life of 39 weeks. *Phase 4*: a constant phase with no appreciable decline, caused at least partially by the activation of resting memory CD4+ T-cells that start to produce virions. Infected hematopoetic progenitor cells of the monocyte/macrophage cell line could also contribute to this phase.

3. Dynamics of persistent viremia

The dynamics of persistent viremia during HAART are debated. One view holds that there is ongoing viral replication during HAART, either in the presence of antiretroviral drugs (ARVs) or in "sanctuary sites" into which ARVs penetrate poorly. The other view is that persistent viremia arises from long-lived productively infected cells which were infected prior to therapy initiation.

Several studies have investigated whether there is viral evolution during HAART which would indicate ongoing viral replication. These studies have yielded conflicting results where some studies have found signs of viral evolution and some have not (Bailey et al., 2006; Kieffer et al., 2004; Nottet et al., 2009; Tobin et al., 2005). A major obstacle to these types of studies is that extensive analyses of viral sequences need to be done to exclude the possibility that a viral sequence that is found in a follow up sample was actually not present earlier and missed because of inadequate sampling.

There are several studies indicating that HAART completely inhibits viral replication. If ongoing replication continues drug resistance would evolve during therapy. However, studies have shown that in treatment naive individuals who start suppressive therapy and maintain their viral RNA levels below 50 copies/ml, no drug resistant mutations emerge (Hermankova et al., 2001; Persaud et al., 2004). During strategic treatment interruption the virus that emerges when treatment is stopped shows no evidence of evolution when compared to pre-treatment samples also indicating the lack of viral replication during therapy (Joos et al., 2008). If viral replication continues during suppressive therapy, low-level viremia would be reduced by the addition of another ARV. However, a recent study has shown that intensification of HAART with either efavirenz, lopinavir/ritonavir, or atazanavir/ritonavir in nine patients did not affect the levels of persistent viremia (Dinoso et al., 2009a). Moreover, it has been shown that the level of persistent viremia is not related to treatment regimen but to pre-treatment viral RNA levels suggesting that all treatment regimens completely inhibit viral replication and that the pre-treatment viral set-point is correlated to the number of long-lived HIV-infected cells (Maldarelli et al., 2007).

4. Viral reservoirs

A viral reservoir can be defined as a cell type or anatomical site where a replication competent form of the virus persists for a longer time than in the main pool of actively replicating virus (Blankson et al., 2002). During untreated infection HIV predominantly replicates in activated CD4+ T-cells (Zhang et al., 1999). They die quickly after infection, either from viral cytopathic effects or from immune response directed against these infected cells. HIV-infected activated CD4+ T-cells are probably responsible for the first phase of decay after initiating HAART as they have a half life of 1–2 days (Ho et al., 1995; Wei et al., 1995).

Several cell types could alone, or in combination with others, be linked to the second phase of decay. One is macrophages, they can become infected with HIV and are, at least in vitro, less susceptible

to the cytopathic effects of HIV than activated CD4+ T-cells (Ho et al., 1986; Nicholson et al., 1986). The half life of tissue macrophages depends on the type of tissue but can be several weeks. In addition, partially activated HIV-infected CD4+ T-cells may also contribute to the second phase of viral decay as they have been reported to have a longer turnover time than fully activated CD4+ T-cells (Zhang et al., 1999). Dendritic cells (DCs) can also contribute to this phase. It is not entirely clear how long an infected dendritic cell can survive and continue to produce virus. In vitro studies show that infected myeloid dendritic cells (MDC) can survive for more than 45 days (Popov et al., 2005). Langerhans cells (dendritic cells in the skin) have a half life of about 15 days when infected (Kalter et al., 1991) while DCs in the mucosa have a half life of just 2–3 days (Pugh et al., 1983). In individuals that have been on HAART for more than 6 weeks HIV DNA cannot be found in DCs in peripheral blood suggesting that they do not have a role in long-term HIV persistence (Otero et al., 2003). Follicular dendritic cells (FDCs) cannot be infected with HIV but they can trap virions on their surface where they can remain infectious for at least 9 months (Smith et al., 2001). It is unclear if release of such virus could contribute to the different phases of decay.

The sources of the third and fourth phases of decay have not been fully characterized but these phases most likely represent at least two additional classes of long-lived virus-producing cells. Resting memory CD4+ T-cells are a well-defined latent reservoir of HIV that most probably contribute to the fourth phase of viral decay. HIV latency is established in these cells when an activated CD4+ T-cell becomes infected by HIV but transitions to a terminally differentiated memory cell before HIV infection eliminates the cell. Transition to a memory cell can through a complex interaction between the cell and the virus result in a latent infection (this will be further explained in Section 6). Switching to a memory cell allows this infected host cell to persist for decades until it receives a stimulatory signal that activates the cell, concomitantly inducing viral production. During antiretroviral therapy these 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 (Siliciano et al., 2003). The frequency of infected cells among resting memory CD4+ T-cells is low, about 1 infected cell per 10^6 resting memory CD4+ T-cells with an overall size estimated to be around 10^6 cells (Chun et al., 1997). The reservoir of resting memory CD4+ T-cells is established during primary infection (Chun et al., 1998).

In a recent interesting study Chomont et al. showed that, in individuals on HAART, integrated HIV DNA can be found in different subsets of CD4+ T-cells, mostly in central memory T-cells (T_{CM}) and in transitional memory T-cells (T_{TM}) which express the immune activation markers and proliferation markers, PD-1 and Ki76, respectively. They found the major reservoir for immune responders is T_{CM} cells and the low proliferation of these cells allows them to persist for years. Alternatively, the major reservoir in patients with low CD4+ counts are T_{TM} cells and these cells persist by homeostatic proliferation making them a very stable viral reservoir. Furthermore they observed that in individuals who have a lower CD4+ count the stability of the T_{TM} 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: the long-term survival of infected T_{CM} cells and the homeostatic proliferation of infected T_{TM} cells (Chomont et al., 2009). However, other cells may be responsible for the persistent viremia during the fourth phase of decay during HAART. The results of two interesting studies have found that in about half of the individuals on HAART the persistent viremia is genetically homogeneous (referred to as predominant plasma clone or PPC). However, the sequences of these persistent viral populations were rarely found in the reservoir of circulating resting memory CD4+ T-cells. It has been proposed that the mechanism behind this homogenous population could be a rare infection event that establishes an integrated viral genome in a cell that has proliferative capacity, for example, a stem cell in the monocyte–macrophage lineage. This infected cell proliferates, copying the viral genome without introducing errors and generating an expanded set of progeny cells that release virus (Bailey et al., 2006; Brennan et al., 2009).

5. Sanctuary sites

It has been suggested that there might be anatomical compartments where HIV replication can take place in the presence of HAART due to poor penetration of ARVs or due to special biological properties of the compartments such as being an immuno privileged site. 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 (Gunthard et al., 2001; Pantaleo et al., 1993; Veazey et al., 1998). These tissues could also be the source of persistent viremia or be a reservoir for HIV during HAART. Chun et al. have found higher frequencies of infected cells in GALT than in peripheral blood mononuclear cells (PBMCs). They also found cross-infection between these compartments during HAART suggesting ongoing viral replication in the GALT which indicates that the GALT is a reservoir for HIV during HAART (Chun et al., 2008). HIV can penetrate into the central nervous system (CNS), presumably by the migration of infected monocytes/macrophages. In the CNS HIV can infect four types of macrophages: perivascular macrophages, meningeal macrophages, macrophages of the choroid-plexus, and microglia (Williams and Hickey, 2002). Also astrocytes can be infected even though it remains unclear if a productive infection can be established *in vivo* in this cell type (Brack-Werner, 1999). In untreated patients there are signs of "compartmentalization" in CSF and plasma, suggesting independently replicating viral populations (Harrington et al., 2009). Under HAART however, patients with <50 copies/ml in plasma have <50 copies/ml in CSF (Gunthard et al., 2001). It remains unclear if cells from CNS can serve as reservoir or maintain viral infection during HAART. The genitourinary tract has also been suggested to serve as a reservoir for HIV. In untreated patients there is viral compartmentalization between genital secretions and plasma (Kiessling et al., 1998; Vernazza et al., 1994). During HAART, HIV can be found in the semen, both as free virus and integrated in latently infected cells (Zhang et al., 1998). Interestingly, HIV has also been found in renal epithelium (Bruggeman et al., 2000). 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.

6. Mechanisms of HIV latency

Many hypotheses have been proposed to explain the molecular mechanism of HIV latency (Fig. 2). As mentioned earlier the main source of latent virus in HIV-infected individuals treated with HAART is thought to be a small pool of latently infected resting CD4+ T-lymphocytes (Chun et al., 1995), thus these cells have been the main focus for investigation. However, due to the small size of this latent pool, the difficulties to enrich for these cells, and the fact that only a small portion of these latently infected cells harbor replicative competent virus (Hermankova et al., 2003; Finzi et al., 1997, 1999), many of the studies investigating the mechanism of HIV latency have been carried out in vitro using cell culture-based latency models. Nevertheless these studies have together with ex

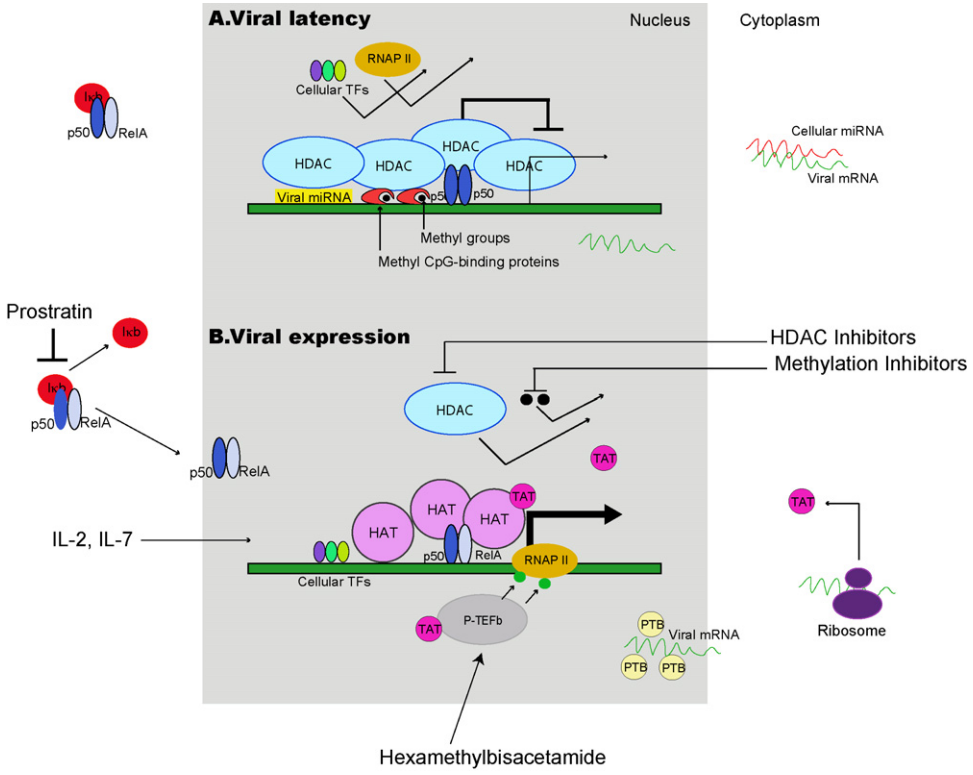

Fig. 2. Post-integration latency is a result of many different restrictions on HIV expression. (A) In the latent state, HIV expression is thought to be suppressed by HDACs recruited by several different factors, NF-κB p50:p50 homodimers, methyl-CpG-binding proteins, viral miRNA, which prevent binding of the transcription machinery to the viral LTR. Viral latency has also been proposed to result from the action of cellular miRNA on viral mRNA and inefficient transportation of viral RNA from the nucleus to the plasma which blocks translation of viral proteins. In addition active NF-κB is sequestered in the cytoplasm by the inhibitor of nuclear factor κB (IκB) in latent infection which prevents its positive regulation on viral transcription. (B) Viral expression occurs as a consequence of efficient binding of the transcription machinery to the viral LTR region. In the presence of active NF-κB p50:RelA heterodimers, HATs are recruited to the viral LTR where acetylation relaxes the chromatin structure allowing binding of the transcription machinery. A high level of nuclear PTB facilitates exportation of viral mRNA to the cellular cytoplasm where viral RNA is translated. Production of the viral protein Tat leads to a positive feedback loop when Tat recruits both HATs and P-TEFb (also recruited by NF-κB heterodimer) to the viral genome, thus promoting productive viral expression. Viral latency can be revoked by several different approaches: HDAC inhibitors which block the repressive action of HDACs, DNA methylation inhibitors which prevents the binding of methyl-CpG-binding proteins to the viral LTR, prostratin which activates NF-κB, hexamethylbisacetamide which promotes viral expression by activating P-TEFb and by the action of immune activators IL-2 and IL-7.

vivo analysis revealed many important aspects of the molecular mechanism of HIV latency.

Two different forms of latency are observed in resting CD4+ T-lymphocytes: pre-integration and post-integration latency. Pre-integration latency is a consequence of HIV fusion with non-dividing resting lymphocytes where the viral replication cycle is blocked prior to HIV DNA integration which leaves HIV DNA unintegrated in the cytoplasm outside the nucleus (Zack et al., 1990). The half life of this unintegrated viral DNA is short (1 day) (Pierson et al., 2002) thus this form of viral latency does not appear to contribute to the long-term viral persistence seen after prolonged periods of HAART. The block in DNA integration can however be revoked if activation of infected cells takes place with viral integration and productive infection as a possible consequence (Zack et al., 1990).

Post-integration latency is suggested to result when infected active CD4+ T-lymphocytes revert to a resting memory state. As described earlier, this latent state is extremely stable and is necessary for the persistence of HIV during HAART. In patients receiving HAART with <50 copies/ml this type of latently infected cells does not produce detectible levels of virions in the absence of activating stimuli (Chun et al., 2003) and are not recognized by the immune system or antiretroviral compounds. Many different mechanisms have been proposed to establish and maintain this post-integration latency in HIV-1 infection. Some of the more important ones are thought to be: viral integration sites, chromatin environment, lack of key transcription factors, impaired viral activator Tat and RNA interference all which will be reviewed hereafter.

7. Site of viral integration into the host genome

The position of genes within chromosomes has been shown to affect their rate of transcription. This has also been proven to be true for HIV as several studies have revealed evidence that the site of viral integration into the host genome plays a central role in the establishment of post-integration latency. There are conflicting reports as to where the integration occurs. Initial studies from in vitro infection of a T-lymphocyte cell line indicated that latent provirus integrate in, or close to, repetitive DNA elements in heterochromatin (Jordan et al., 2003). On the other hand Han et al. showed that HIV-1 genomes in resting CD4+ T-cells preferentially reside within actively transcribed genes (Han et al., 2004). Additional in vitro studies also demonstrated HIV-1 integration into actively transcribing genes (Klase et al., 2007; Lewinski et al., 2006; Schroder et al., 2002). In 2005, Lewinski et al. found that low levels of HIV-1 expression in a Jurkat T-cell latency model is associated with HIV integration into either centromeric heterochromatin, large tracts of the human genome lacking protein-coding genes known as gene deserts, or very highly expressed cellular genes (Bisgrove et al., 2005). The finding that latent HIV proviruses can exist in transcriptionally active genes can be explained by the phenomenon of transcriptional interference, a mechanism that arises when two gene promoters are in close proximity (Adhya and Gottesman, 1982). If HIV integrates with the same polarity as the host gene ongoing cellular transcription from an upstream promoter can interfere or suppress the downstream promoter. This

occurs when RNA polymerase II "reads through" the downstream gene and disrupts the binding of certain transcription factors bound to the downstream viral promoter (Greger et al., 1998). Alternately, if HIV is integrated with opposite polarity, the host gene transcriptional interference has been proposed to occur due to collision of polymerases from the cellular and viral promoters (Bisgrove et al., 2005).

8. Chromatin environment—*Histone deacetylation*

In addition to the site of integration the chromatin structure at the viral LTR region has been shown to play a critical role in viral suppression. In transcriptionally silent state the LTR region is packed into a heterochromatic structure where two nucleosomes nuc-(0) and nuc-(+1) covers recognition elements for the sequence-specific transcription factors (Verdin et al., 1993). Several studies have shown that modification of these nucleosomes play a crucial role in the regulation of viral transcription. Histones are subject to several post-translational modifications, where acetylation has been associated with nucleosome remodeling, accessibility of DNA for transcription factors and transcriptional activity (Lee et al., 1993). The level of acetylation is a result of histone acetylase and deacetylase activities and in cellular models of HIV latency the histones composing nuc-(0) and nuc-(+1) are deacetylated, suggesting a critical role of histone deacetylases (HDACs) in post-integration latency. By using chemical HDAC inhibitors, with histone acetylation as a consequence several studies have proven the role of HDAC in HIV latency (see Section 11) (Van Lint et al., 1996; Laughlin et al., 1993; Ylisastigui et al., 2004).

9. Chromatin environment—*DNA methylation*

Methylation of DNA is another mechanism by which level of transcription can be modulated. High levels of CpG (CG-rich DNA) methylation are known to prevent the binding of basal transcriptional machinery. In addition methylated CpG recruits methyl-CpG-binding proteins which form complexes with histone deacetylases (reviewed in Bird and Wolffe, 1999). The first indication of methylation as a possible factor in HIV latency was illustrated by Bednarik et al. when they obtained reactivation of a stable integrated HIV1-LTR-CAT plasmid by the use of the methyl inhibitor 5-aza-cytidine (Bednarik et al., 1987). Methylation of CpG sequences in the HIV LTR region has further been shown to strongly suppress expression of HIV pointing out its possible role in HIV latency (Bednarik et al., 1990; Schulze-Forster et al., 1990). However, this mechanism of latency has been challenged (Pion et al., 2003) and the contribution of CpG methylation *in vivo* is still not known.

10. Accessibility of key transcription factors

Several host transcription factors (TFs), including nuclear factor kappa B (NF-κB), nuclear factor of activated T-cells (NFAT), stimulatory protein 1 (Sp1), lymphoid enhancer-binding factor (LEF-1), and T-cell specific factor-1α (TCF-1α) (reviewed in Rohr et al., 2003), have been shown to be important for regulation of HIV transcription where NF-κB is one of the more important and of focus in this review. Levels of NF-κB vary depending on the state of cellular activation. In resting memory T-lymphocytes the active form of NF-κB (p50:RelA heterodimer) is sequestered in the cytoplasm by the inhibitor of nuclear factor κB (IκB), while NF-κB p50:p50 homodimers occupies the NF-κB binding sites at the viral LTR region. Together with other host regulatory proteins NF-κB homodimers have been shown to recruit HDACs to the viral promoter blocking the binding of proteins involved in the transcription machinery and repressing HIV LTR activity (Blankson et al., 2007). Stimulation of resting T-cells releases NF-κB heterodimers from its inhibitor and active NF-κB moves into the nucleus where it displaces p50 homodimers from the viral LTR. Once bound to the viral promoter these proteins recruit the cellular histone acetyl transferase (HAT) p300 for additional relaxation of the chromatin and other central proteins of the cellular transcription machinery, such as positive elongation factor b (P-TEFb), thereby promoting efficient transcriptional initiation and elongation of the viral genome (Barboric et al., 2001; Gerritsen et al., 1997). These events induced by active NF-κB appear to be important for the activation of HIV expression, as low levels of NF-κB p50:RelA has been shown to correlate with suppressed levels of HIV expression (Nabel and Baltimore, 1987). Inadequate nuclear concentrations of active NF-κB in resting T-lymphocytes have therefore been proposed to contribute to HIV latency (Bisgrove et al., 2005).

11. Viral activator protein Tat

Nuclear presence of active NF-κB and several cellular proteins leads to initiation of viral transcription and production of early short viral gene products. The late phase of transcription and efficient production of long viral RNA transcripts is however regulated by the viral activator protein Tat (Kao et al., 1987). Tat binds to the transactivation response element (TAR) in the viral LTR and plays a crucial role in efficient RNA polymerase II elongation (RNAP II) by recruiting P-TEFb in close proximity of the C-terminal domain (CTD) of RNAP II (Parada and Roeder, 1996). P-TEFb phosphorylates serine-2 residues in the CTD which is required for effective elongation by the polymerase complex. In addition Tat has been shown to associate with other important coactivators such as p300 that further opens the LTR chromatin structure through histone acetylation (Ott et al., 1999) Thus, cellular concentrations of NF-κB and other cellular transcription factors are not sufficient for productive viral expression, rather concentrations and functionality of Tat determines viral expression. Low concentrations or impaired activity of Tat have further been proven to maintain HIV-1 post-integration latency in a latently infected cell line (Emiliani et al., 1998) and latently infected CD4+ T-lymphocytes from infected individuals (Yukl et al., 2009). In addition to Tat, Rev, Vpr and Nef have also been proposed to pay roles in HIV-1 latency (Pomerantz et al., 1992; Levy et al., 1995; Niederman et al., 1989).

12. Post-transcriptional regulation

Viral latency has also been linked to a post-transcriptional block. It has been demonstrated that multiple spliced (MS) viral RNA in resting T-lymphocytes from patients on HAART are strictly localized to the nucleus, with no production of early viral proteins as a consequence and thus interrupting the positive regulation of Tat seen in activated cells. Over-expression of the host protein PTB, a protein involved in post-transcriptional regulation of gene expression, revoked this block by localizing viral MS RNA to the cytoplasm indicating that the low levels of PTB in resting T-cells may cause the HIV latency (Blankson et al., 2007). However an earlier study did not find evidence for MS transcription in latent infected resting T-lymphocytes indicating that HIV post-integration latency is regulated at the level of mRNA production rather than at the level of nuclear export of viral mRNAs (Hermankova et al., 2003).

13. RNA interference

Other possibilities for HIV latency have been evaluated since the identification of microRNA (miRNA) encoding sequences in the HIV-1 genome and the discovery of functional virus derived miRNAs, suggesting a role for RNA interference (RNAi) in the regulation of HIV-1 gene expression. RNAi functions either through

post-transcriptional gene silencing through homology-dependent degradation and/or translational suppression of mRNA or through modification of chromatin structures. HIV has been proposed to use these mechanisms in several ways to mediate viral latency (reviewed in Corbeau, 2008). Two studies by Omoto et al. have showed evidence of nef miRNA in HIV-1 infected cells and a nef miRNA dependent downregulation of HIV-1 transcription (Omoto and Fujii, 2005; Omoto et al., 2004). Furthermore another viral miRNA from the viral TAR element exists in cultured HIV-1 infected cell lines and in HIV-1 infected T-lymphocytes and has been shown to exert gene downregulatory effects through the recruitment of HDACs to the LTR region (Klase et al., 2007; Ouellet et al., 2008). In addition to viral miRNA targeting viral RNA several viral miRNA are thought to target host cellular mRNA and vice versa. Recently several host miRNA that target sequences in the 3′ end of HIV RNA, thus silencing almost all viral messenger RNA, have been shown to be over expressed in resting T-lymphocytes (Huang et al., 2007).

14. Methods for purging the latent HIV reservoirs

Persistent viremia during suppressive antiretroviral therapy could arise from long-lived productively infected cells, and/or from activation of viral expression from latently infected cell reservoirs. Latently infected resting CD4+ T-cells are hard to target since they in all aspects apart from the integrated HIV DNA genome are similar to uninfected cells of the same type. Therefore, many strategies for HIV eradication involve activating latently infected cells to induce the expression of the integrated HIV genome making it vulnerable to immune-mediated killing and antiretroviral therapy.

A number of ways have been proposed for the reactivation of latently HIV-infected cells. First, latently infected cells have been exposed to cytokines such as interleukin-2 (IL-2) and interleukin-7 (IL-7) (Chun et al., 1999; Scripture-Adams et al., 2002). Earlier studies have shown that patients treated with IL-2 plus HAART have fewer resting memory CD4+ T-cells than patients treated with HAART alone (Chun et al., 1999). As previously mentioned, a recent study has shown that IL-7 can also promote HIV latency in patients with low CD4+ cell counts. Second, upregulating cellular transcription to induce HIV gene expression has been proposed. This includes inhibiting HDACs which promotes latency by regulating genome structure and transcriptional activity. A recent report has shown that synthetic HDAC inhibitors can reactivate latently HIV-infected cells in vitro (Archin et al., 2009). However, the coadministration of the HDAC inhibitor, valproic acid, and HAART gave mixed results (Blankson et al., 2007; Lehrman et al., 2005). In addition, DNA methylation also reduces intracellular transcriptional activity and elimination of HIV from a latent source may be enhanced by combining DNA methylation inhibitors with HAART (Kauder et al., 2009). Another positive regulator of HIV expression is active NF-κB (p50:RelA as described above). Using cell line cultures Williams et al. showed that prostratin, a phorbol ester which is nontumorgenic, activates the NF-κB pathways leading to an increased binding of active NF-κB to the HIV-1 LTR (Blankson et al., 2007; Williams et al., 2004). Moreover, a recent study has shown that prostratin up-regulates the HIV-1 expression in peripheral blood mononuclear cells from patients on HAART and down-regulates CD4 receptor expression (Kulkosky et al., 2001). Due to the fact that NF-κB promotes global T-cell activation, it may be of importance to identify other transcription factors which activate the viral LTR in a NF-κB-independent manner. A recent study by Yang et al. have identified that the transcription factor Est1 reactivates latent HIV in resting memory T-cells from patients on HAART without causing general T-cell activation (Yang et al., 2009). In addition, hexamethylbisacetamide a hybrid bipolar compound tested in oncology trials, recruits cellular kinases to the LTR which induces efficient Tat-independent HIV-1 expression. This compound also appears to down-regulate CD4 expression (Choudhary et al., 2008; Contreras et al., 2007; Klichko et al., 2006; Young et al., 1988). A third method increases HIV gene expression by altering the effects of non-coding cellular miRNAs. Cellular miRNAs have been reported to contribute to HIV-1 latency in memory CD4+ T-cells. Binding of cellular miRNAs to the 3′ end of HIV-1 messenger RNAs inhibits viral protein translation in cells and thus promotes latency. Moreover, these cellular miRNAs are at higher levels in memory CD4+ T-cells compared to activated CD4+ T-cells. When cellular miRNAs were inhibited protein translation in memory CD4+ T-cells increases indicating the manipulation of cellular miRNAs could be a unique avenue for purging the latent HIV reservoirs (Huang et al., 2007). Moreover, reactivation of latent viral reservoirs may require combinations of different HIV-1 activators. A recent study has shown that combining valproic acid and prostratin or suberoylanilide hydroxamic acid (SAHA) another HDAC inhibitor and prostratin more efficiently reactivated HIV-1 production in cell lines and cells isolated from patients receiving HAART than each compound alone (Reuse et al., 2009).

Several notes of caution are necessary. Owing to the virus–host relationship, seeking to purge the latent HIV reservoir runs the risk of fully activating the patient's immune system. In addition, any future approach to eradicating latent HIV reservoirs must avoid the generation of new HIV infection resulting from the activation of these latently infected cells. In looking ahead to clinical trials in humans, a consensus is building in the field to carry out these trials in the presence of a HAART "shield". However, early experimentation of new reactivation strategies need to be conducted in appropriate animal models.

15. In vitro, ex vivo and animal models of HIV latency

In order to analyze the ability of new compounds to activate the production of HIV from latently infected cells, in vitro cell lines mimicking the HIV-1 latent state have been developed. These include chronically infected cell lines and clones such as ACH2 T-cell line, the U1 promonocytic cell line and the JΔK T-cell line which do not express HIV-1 genes unless they are treated with cytokines or mitogens (Antoni et al., 1994; Folks et al., 1987, 1989). Studies using these cell lines have indicated that post-transcriptional mechanisms are involved in latency as the HIV-1 RNA detected in unstimulated ACH2 and U1 cells were not full length but rather singly or multiply spliced RNA fragments (Pomerantz et al., 1990). However, these cells continue to proliferate and therefore do not truly represent a latent state. A latently infected Jurkat cell line encoding the enhanced green fluorescent protein (GFP) as a marker for Tat-driven HIV LTR expression has been used to study the inhibition of transcription initiation of the HIV-1 LTR (Jordan et al., 2003). More recently this cell line has been used to determine the ability of valproic acid, SAHA, and new class I and II HDAC inhibitors to induce HIV expression by measuring the LTR-driven expression of GFP using flow cytometry (Archin et al., 2009). The ideal cells for studying HIV reactivation are latently infected resting memory CD4+ T-cells directly isolated from patients. Typically lymphocytes are obtained by continuous flow leukophereis or from fresh whole blood which is treated to density centrifugation on a Ficoll–Hypaque gradient. To harvest HIV-infected resting CD4+ T-cells most researchers use a two step method which yields a population of 99% pure CD4+ resting T-cells. In the first step, unwanted cell-populations such as CD8+ T-cells, activated CD4+ T-cells, monocytes, B cells, and NK cells will be negatively selected by exposing them to monoclonal antibodies with cell specific surface markers and magnetic beads conjugated with sheep anti-mouse immunoglobulin. As a second purification step, fluorescence-activated cell sorting is used to positively select resting CD4+ T-cells based on size and phenotype (Archin et al., 2009;

Lehrman et al., 2005; Reuse et al., 2009; Siliciano and Siliciano, 2005). Typically a subset of these purified cells are then treated with different HDAC inhibitors and/or prostratin and several days later the supernatant is tested quantitatively for HIV RNA and the frequency of HIV outgrowth in cultures treated with these compounds is compared to the frequency of HIV outgrowth in a subset of resting CD4+ T-cells treated with a mitogen (Archin et al., 2009; Reuse et al., 2009). However, due to the limited numbers of circulating latently infected cells and the difficulty in identifying infected versus uninfected resting CD4+ T-cells it remains difficult to use primary cells for testing the ability of new compounds to activate viral expression from these latently infected cell reservoirs.

Appropriate animal models could also help in better characterizing viral reservoirs. Simian immunodeficiency virus (SIV)-infected nonhuman primates have served as important models for understanding HIV pathogenesis. Recently, SIV-infected macaques treated with a combination of four antiretroviral drugs have shown similar pattern of viral decay during therapy and reduced frequencies of circulating resting CD4+ T-cells harboring replication competent virus as HIV-infected humans starting HAART (Dinoso et al., 2009b). However, the SIV model has two major disadvantages. First, rhesus macaques are costly and in high demand, and must be housed in accredited primate facilities. Second, although SIV is quite similar to HIV, there are some important genetic differences between these two viruses. Therefore a "humanizied" BLT (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 in Melkus et al. (2006) and Sun et al. (2007).

16. Conclusion

Current antiretroviral therapy effectively suppresses but does not eradicate HIV-1 infection. During combination antiretroviral therapy, reduction of HIV-1 RNA levels to less than 50 copies/ml is frequently achieved; however, residual low-level viremia has been detected using ultrasensitive assays. The source and dynamics of this residual viremia are currently being investigated by many researchers. A well-defined latent reservoir of HIV is memory CD4+ T-cells where HIV latency is established 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 of the monocyte/macrophage linage may also serve as long-term reservoirs of HIV. Several mechanisms have been shown to play a role in maintaining HIV latency including viral integration sites, chromatin environment, down-regulated transcription factors, impaired activity of Tat, and cellular miRNA interference with viral protein translation. However, further studies are needed to fully understand the mechanisms that promote HIV latency *in vivo*. Recent studies have introduced new insights in persistent HIV reservoirs and the mechanisms of latency. These studies point to an important conclusion: any long-term strategy for eliminating HIV infection must address HIV latency and its implications. While many challenges remain for HIV eradication the promising approaches outlined here will illuminate our understanding of HIV latency and ultimately benefit persons infected with HIV.

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