Understanding HIV Latency: The Road to an HIV Cure

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Abstract

Treatment with antiretroviral therapy dramatically increases the survival of HIV-infected individuals. However, treatment has to be continued for life because it does not lead to the full eradication of infection. HIV persists in resting $CD4^+$ T cells, and possibly other cell types, and can reemerge from these cells when therapy is interrupted. Here, we review molecular mechanisms that have been proposed to contribute to HIV latency, as well as the relative roles of *cis*- and *trans*-acting mechanisms. We also discuss existing and future therapeutic opportunities regarding HIV latency that might lead to a future cure for HIV infection.

INTRODUCTION

HIV (type 1) is an incurable infection that causes AIDS. Although antiretroviral therapy (ART) suppresses HIV to undetectable levels, interrupting ART causes the virus to rapidly rebound to pretreatment levels. Thus, HIV-infected individuals must commit to lifelong ART to keep HIV replication suppressed. Critically however, ART does not fully prevent pathology or restore a normal lifespan in HIV-infected patients (1). These limitations are due to drug side effects (reviewed in 2) and/or incomplete viral suppression, particularly in viral anatomical sanctuaries (3). Moreover, with continuous and expanded usage of ART, HIV is becoming progressively more drug resistant. This erodes the efficacy of ART, especially in settings with limited resources or inadequate access to therapy. Thus, lifelong ART is not a sustainable solution to treating HIV/AIDS at either an individual or global scale, and curative therapies are urgently needed.

To develop curative treatment for HIV, we must understand the site of HIV persistence under ART. An important source of viral rebound is a reservoir of long-lived, quiescent, memory $CD4^+$ T cells that harbor integrated latent HIV proviruses. There, the HIV provirus lies transcriptionally silent and immunologically inert. Under circumstances that are not entirely clear, but that may include reactivation of the T cell by its cognate antigen, the T cell adopts a cellular state that promotes and sustains productive HIV transcription, and virus production resumes.

Several therapeutic approaches are being considered to control or eliminate the HIV latent reservoir. These involve either a complete elimination of all persistent HIV (sterilizing cure) or the immunological control of persistent HIV (functional cure). The "shock and kill" approach is the main focus of current research efforts for a sterilizing cure. In this approach, small molecules that activate HIV transcription would be used to force the reactivation of latent HIV in memory $CD4^+$ T cells under the cover of ART. Subsequently, reactivation of HIV expression would induce viral cytopathic effects, immune clearance, and cell death, thereby purging latently infected cells while uninfected cells are protected by ART (4). Challenges to this approach include the heterogeneity of latent HIV and the lack of evidence that lymphocytes, in which HIV is reactivated, are eliminated from the latent pool (reviewed in 5–7). Recent studies have also illustrated that different latent viruses might become differentially reactivated in response to different drugs (8–10), and that HIV-latency reactivation might behave in a stochastic rather than deterministic manner when challenged by small molecules (10). Additionally, many of the non-reactivated proviruses appear to be replication competent, indicating that the latent reservoir may be up to 60-fold larger than previously estimated (10).

The complex mechanisms of HIV transcriptional regulation, as well as HIV latency, have been recently summarized in a number of excellent reviews (5–7, 11). In this review, we highlight the core principles that regulate HIV transcription and then focus on two broad and highly variable mechanisms that control HIV latency: (*a*) *cis*-acting mechanisms, dependent on the site of integration of the virus into the genome and the local chromatin environment at that site, and (*b*) *trans*-acting mechanisms, including basal and activated transcription factors, their regulation by the state of activation of T cells, and the environmental cues that these cells receive.

HETEROGENEOUS CELLULAR AND VIRAL MECHANISMS REGULATE HIV-1 LATENCY

HIV transcription is regulated by a variable combination of cellular and viral processes that include different integration sites and different levels of critical transcription factors (TFs) present in different lymphocytes. This heterogeneity yields a number of possible distinct basal transcriptional states for the HIV promoter soon after infection. Importantly, if and when basal HIV transcription

reaches a certain threshold, the HIV-encoded Tat protein becomes synthesized and accumulates within the infected cell. Once Tat has accumulated to a critical level, it further activates HIV transcription by binding to an RNA stem loop structure called the *trans*-activation response (TAR) element present at the 5′ extremity of all viral transcripts and by recruiting the positive transcription elongation factor–b (pTEFb), which dramatically activates HIV transcription rate. This leads to further Tat expression and the creation of a feed-forward loop. This modular organization of HIV transcription is responsible for the ability of HIV to adopt two different transcriptional functional states, i.e., latent and productive, and implies that the factors that control basal HIV transcription and/or Tat stability are critical in determining the entry of HIV into a productive versus latent state.

In the following section, we focus on the *cis*-acting cellular and viral components that heterogeneously regulate HIV-1 transcription. These components include (*a*) viral integration site and proviral orientation, (*b*) genomic architecture, and (*c*) stochastic gene expression.

Viral Integration Site and Proviral Orientation

Because the eukaryotic chromatin environment is highly heterogeneous in terms of transcriptional activity and histone posttranscriptional modifications, it has long been suspected that integration of HIV into certain unique chromatin environments might lead to HIV transcriptional repression and latency, whereas integration into other sites might lead to active transcription and productive infection (12, 13). However, HIV integrates into the host cell genome in a nonrandom manner—the HIV integrase protein interacts with the cellular coactivator lens epithelium-derived growth factor (LEDGF/p75) (14) and, to a lesser extent, histidine-rich protein (HRP-2) (15). This interaction directs the HIV preintegration complex to the introns of actively transcribing genes. There, HIV preferentially integrates into the outward-facing major groove of nucleosomal (AT-rich) DNA.

However, the interaction between integrase and LEDGF is not an absolute requirement for HIV integration and, in a fraction of integration events, HIV integrates in a more random manner into the genome. These random events can lead to integration within a repressive chromatin environment such as heterochromatin, thereby promoting HIV latency. In the Jurkat cell J-Lat model of HIV-1 latency, the site of viral integration into the genome appears to be dominant in determining whether a provirus becomes latent, as integration at different genomic sites leads to different levels of basal HIV promoter activity (16). In a small fraction of latently infected cell lines, latent HIV-1 was found integrated into alphoid centromeric repeats, a clear and well-documented example of a heterochromatin transcriptionally repressive environment (17, 18). Moreover, investigators found that across a small number of genomic loci, the location of integrated provirus determined the variability (noise) in HIV-1 gene expression (19). Dar et al. (20) extended this finding by monitoring the proviral transcriptional activity of an HIV-derived lentiviral vector at multiple integration sites using time-lapse fluorescence microscopy. Here, the authors showed significant variability in transcriptional noise (both burst size and frequency) across ~8,000 unique genomic loci. Taken together, these studies demonstrate that HIV integration-site variability can result in substantial differences in proviral transcriptional output, which may play an important role in driving HIV latency.

Another source of heterogeneity in HIV-1 transcription is the orientation of the provirus relative to the host gene. Generally, parallel orientations increase transcriptional interference from the RNA polymerase in the host gene and antiparallel orientations decrease this interference (21). However, the conclusive role of proviral orientation in HIV transcription remains unclear. Studies in J-Lat clones, primary $CD4^+$ T cells, and other in vitro models suggest that orientation-dependent interference represses HIV transcription (18, 22–24). Conversely, a study in latently

infected CD4+ T cells derived from patients did not reveal a bias in orientation (25). Thus, the orientation in which the provirus integrates may significantly affect HIV-1 transcription; however, more work is needed to fully understand this phenomenon.

To investigate the relationship between HIV integration and transcription in a more systematic way, Sherrill-Mix et al. (8) recently performed a meta-analysis of five well-characterized models of HIV latency so as to determine the overlap between the site of integration and known transcriptional regulatory mechanisms. In their analysis, they correlated latency with 135 genomic and epigenetic features in CD4+ T cells (e.g., gene density and expression, histone posttranslational modifications, chromatin accessibility). Strikingly, the authors found that these features did not correlate with latency, either individually or in combination. Another study using a double-labeled latency model showed that features of integration sites and/or provirus orientation did not distinguish between productively and latently infected cells (26). Thus, these conflicting findings imply that the relationship between HIV-1 integration site and transcription is not easily defined, and that a more complex type of correlation—which integrates other properties—likely exists.

Although genomic features on their own may not predict HIV latency, integration of genomic factors and the availability of TFs may provide insight into this process. For example, chromatin accessibility at HIV integration sites predicted the cellular threshold of nuclear factor (NF)-κB RelA/p65 needed to activate HIV gene expression (27). This study suggests that multiple factors (i.e., chromatin accessibility and NF-κB levels) work together to control HIV transcription, but further work is needed to link this process to establishing HIV latency. The studies mentioned in this section support the hypothesis that the site and orientation of integrated provirus may influence HIV transcription, but these effects are not universally observed across HIV latency models.

Genomic Architecture

The eukaryotic nucleus is structurally and spatially heterogeneous. The nuclear core contains transcriptionally active and structurally relaxed euchromatin, whereas the periphery contains transcriptionally inactive and structurally condensed heterochromatin. At the boundaries of the nuclear lamina, a number of highly transcribed genes are associated with the nuclear pore complex (reviewed in 28, 29). Additionally, the nucleus contains many types of nuclear bodies—distinct subdomains that are classified by their function and composition. For example, transcription factories and splicing speckles are located near machinery that facilitates high levels of gene transcription. In contrast, promyelocytic leukemia (PML) and polycomb bodies are composed of PML protein (TRIM19) and polycomb machinery, respectively, and repress gene transcription (reviewed in 30).

The nuclear localization of an HIV provirus may dynamically influence its transcription and thereby latency. For instance, using confocal microscopy and fluorescently labeled HIV preintegration complexes, researchers observed that HIV selectively targets decondensed chromatin in the nuclear periphery (31). This finding is consistent with evidence that HIV integrates into actively transcribing genes. Specifically, within the nuclear periphery of latent J-Lat clone A1 cells (integrated in ChrXp21.1), the HIV provirus interacts in *trans* with the alphoid pericentromeric region of chromosome 12, an interaction that was lost in cells treated with the activating compound *phorbol*-12-myristate-13-acetate (PMA) (32).

Another method by which nuclear architecture may dynamically regulate HIV transcription involves PML bodies. Using 3D-ImmunoFISH in Jurkat and primary CD4+ T cell models, Lusic et al. (33) showed that latent provirus interacts with PML bodies, an interaction not seen in activated cells. This interaction causes PML to recruit histone methyl transferase G9a to potentially inactivate the HIV LTR by deposition of repressive H3K9 histone methylation (33). Interestingly,

other work suggests that PML can sequester CyclinT1 (part of the P-TEFb elongation complex), which recruits catalytically inactive, acetylated CDK9 (the kinase component of the P-TEFb elongation complex) into PML bodies, thereby preventing HIV transcriptional elongation (34). This finding supports the idea that PML bodies inactivate the P-TEFb complex and thereby repress HIV transcription.

Although we still have much to learn, these results suggest that the site of integration of HIV might influence its transcription not only via direct *cis* effects (i.e., the gene in which HIV integrates) but also via long-range *trans* effects by the local chromatin environment composed of nearby genes that might not be contiguous on the chromosome but are in close spatial 3D proximity.

Stochastic Gene Expression and HIV Latency

HIV provirus senses the T cell environment via binding sites for common immune-responsive activators of transcription that are downstream of T cell receptor (TCR) activation [e.g., NF-κB, c-Jun, c-Fos, nuclear factor of activated T cells (NFAT)]. Thus, in resting $CD4^+$ T cells, expression of immune-responsive genes and their cognate transcription factors is depleted and HIV transcription might be suppressed as a consequence. In contrast, in activated T cells, transcription factors specific to an activated cell lead to efficient HIV transcription and a productive infection.

Based on this model, it has been proposed that HIV latency is established when an activated T cell (necessary for HIV infection) returns to the resting state. However, there is little evidence for this type of deterministic model. Instead, there is growing evidence that HIV transcription might have a significant stochastic component that contributes to the two states of HIV transcription, productive versus latent (35). Indeed, within presumably homogeneous activated T cell populations, both productive and latent infections can result (17, 18, 36–44). This evidence also includes recent observations using dual-fluorescence viruses to monitor HIV transcriptional state. Using these new tools, researchers revealed high levels of both productive and latent infections in activated cells (26, 45, 46). Finally, in multiple models of HIV latency, treatment with agents that reactivate latent HIV fails to uniformly reactivate all cells within the population. This is observed not only in cell lines such as J-Lat (17) but also in latently infected $CD4^+$ T cells derived from patients. In the latter model, when initially treated with phytohemagglutinin (PHA) a proportion of cells remained latent, yet could be subsequently reactivated by repeated PHA stimulation (~25% of latent replication-competent proviruses) (10). Thus, latent HIV-1 provirus stochastically responds to full T cell activation, suggesting that a similar stochastic process may also establish latency. However, one should note that the assumption is made that cell populations in these experimental systems are truly homogeneous. This has not yet been proven and is actually unlikely in the case of $CD4^+$ T cells isolated from humans. There is therefore a possibility that HIV transcription behaves in a deterministic manner in cells that behave probabilistically in terms of their T cell activation status. Resolving this issue will require the simultaneous single-cell analysis of HIV and cellular transcriptomics.

What Is the Best Experimental System to Study HIV Latency?

A key question in the latency field is which experimental system best reflects the state of HIV latency in $CD4^+$ T cells from patients infected with HIV and on ART. Various latency models have been developed, based either in transformed lymphocytic cell lines or in primary $CD4^+$ T cells. A recent study reported on the activity of 13 stimuli, known to reactivate HIV by defined mechanisms of action, in five primary T cell models, four J-Lat cell models, and a model obtained via a viral outgrowth assay using patient-derived infected cells (9). Interestingly, no single in vitro cell model alone was able to capture accurately the ex vivo response characteristics of latently

infected T cells from patients (as defined by the outgrowth model). Protein kinase C (PKC) agonists and PHA reactivated latent HIV uniformly across models, although drugs in most other classes did not (9). The diversity of the responses of each model to different stimuli may reflect the heterogeneity of the mechanisms driving the establishment and maintenance of latency in each system.

Thus, we currently do not know whether latency in vivo is similarly heterogeneous or whether latency as we see it in primary $CD4^+$ T cells isolated from patients is the relevant model system. The key unanswered question is the cellular source of the virus that persists under ART and is able to reseed the infection upon cessation of therapy.

IMMUNOLOGICAL AND ENVIRONMENTAL VARIABILITY IN HIV LATENCY

The mechanisms that establish and maintain HIV latency are also likely to involve many *trans*-acting immunological and environmental factors (e.g., T cell subsets, anatomical sanctuaries, the cytokine environment, and immunological synapses). As discussed below, these mechanisms further support the idea that HIV latency is a heterogeneous process.

Cellular and Anatomical HIV Reservoirs

HIV tropism is governed by expression of the entry receptor complex of CD4 and CXCR4 and/or CCR5 on the surface of target cells. Other chemokine receptors, such as CCR3 and CCR8, are also HIV coreceptors—a fact that expands the pool of cells and tissues that HIV targets. HIV predominantly targets $CD4^+$ T lymphocytes and monocytes/macrophages, although additional cell populations can also be infected. For example, dendritic cells (DCs), natural killer (NK) cells, and other specialized cell types derived from various tissue reservoirs of HIV (e.g., renal, mucosal, and cervical epithelial cells; mastocytes, astrocytes, and microglia in the central nervous system; skin fibroblasts; and bone marrow stem cells) may contribute to the viral reservoir.

The best-characterized cellular reservoir of HIV comprises resting $CD4^+$ T cells. The members of this highly heterogeneous population are distinguished by their differentiation state (reviewed in 47): naïve T cells (T_N), memory stem cells (T_{SCM}), central memory T cells (T_{CM}), transitional memory T cells (T_{TM}), and effector memory T cells (T_{EM}). T_N cells are poorly susceptible to HIV infection. T_{CM} cells survive and proliferate after activation and migrate to secondary lymphoid organs. Conversely, T_{EM} cells display immediate effector functions after antigen stimulation and migrate to peripheral tissues. T_{CM} cells differentiate into T_{EM} cells either after TCR crosslinking or, to a lesser extent, in response to homeostatic cytokines. The functional and transcriptional characteristics of T_{TM} cells are intermediate between those of T_{CM} and T_{EM} cells (47). Chomont et al. (48) showed that T_{CM} and T_{TM} cells comprise most of the latent CD4 T cell reservoir, whereas T_N and effector T cells only marginally contribute to this pool. More recently, investigators identified another subset of memory T cells—T_{SCM}—endowed with self-renewal, high proliferative potential, and homeostatic proliferation (49). Buzon et al. (50) compared T_{SCM} cells to other memory compartments and demonstrated that they have higher levels of HIV DNA per cell, slower decay, and identical HIV sequences after four to eight years, suggesting that this pool may also contribute to the long-term HIV reservoir.

HIV targets a range of tissues—including the gut-associated lymphoid tissues, genital tract, lymph nodes, central nervous system, spleen, liver, kidney, and lungs—and the HIV species targeting these sites may be biologically distinct from those in the systemic circulation. In addition, each

anatomical compartment's T cell subset has unique functional, phenotypic, and survival properties. This diversity may have a substantial impact on the long-term in vivo persistence of latent HIV infection through various mechanisms, which raises the possibility that latency is driven by different mechanisms in different anatomical compartments. This possibility has not been explored yet because most primary $CD4^+$ T cell models rely on the use of activated peripheral $CD4^+$ T cells because accessing these other sites is more challenging.

Immunological Synapses

Cell-mediated HIV infection can occur via virological or infectious synapses that form between productively infected cells and uninfected target cells, or between uninfected antigen-presenting cells (APCs) harboring HIV and target T cells, respectively. Infectious synapses occur with or without cognate antigens, a distinction that affects T cell survival rates or regenerative capacity. Independent of antigen interactions, synapse formation requires coreceptors and adhesion molecules, and weakens processes that maintain homeostasis of naïve cells (e.g., proliferation, T cell activation, and long-term survival) (2, 51, 52). One example of infectious synapses involves DCs, a particular type of APC. These cells constantly scavenge for pathogens, including HIV, in peripheral tissue before transitioning to lymphoid tissue, where they interact with immune cells. When DCs capture HIV, they confine the infectious virus in a non-degrading compartment (without becoming infected) and then transmit the virus to target cells through infectious synapses (3, 53, 54). Thus, DCs disseminate virus, through a *trans*-infection process, to sites and cells that contribute to HIV latency (53, 55–57).

Importantly, these synapses may change the biology of HIV-infected cells to keep HIV silent. Indeed, a recent study shows that co-culturing productively infected resting $CD4^+$ T cells with DCs increases HIV latency rates in $CD4^+$ T cells (56). However, this is independent of DC-to-T-cell transfer of HIV, suggesting that DCs can transform productive infection into latent infection, regardless of T cell activation, through signaling during contact with $CD4^+$ T cells.

Cytokine Environments

HIV infection causes a potent inflammatory response that produces excess proinflammatory cytokines, including interferons, interleukins, chemokines, and tumor necrosis factor alpha (TNFα). Several of these cytokines regulate viral replication, and innate and adaptive immune responses, and may also be involved in establishing and maintaining a viral reservoir.

When HIV infection transitions from the acute to the chronic phase, an anti-inflammatory response produces immunosuppressive cytokines such as interleukin (IL)–10 and transforming growth factor (TGF)–β. IL-10 reduces Th1 type responses and antigen presentation by down-regulating major histocompatibility complex (MHC) class II at the surface of APCs (58). IL-10 also inhibits activation of $CD4^+$ T cells by directly upregulating suppressors of T cell activation, such as programmed cell death protein (PD)–1, discussed below (59). Similarly, TGF-β reduces T cell activation by upregulating suppressors of cytotoxic T lymphocyte antigen (CTLA)–4, also discussed below, and inhibiting IL-2 production (60, 61). By modulating T cell activation, these cytokines may therefore regulate the entry of HIV into latency and its persistence.

There is also evidence for a role of specific chemokines (CCL19, CCL20, CCL21, CXCL9, CXCL10) and gamma-chain (γc) cytokines (IL-2, IL-4, IL-7, IL-15) in latency. These cytokines may allow resting $CD4^+$ T cells, which are poorly permissive to HIV infection (62), to become infected by HIV in vitro without T cell activation (63–66).

Once a viral reservoir is established, cytokines may also regulate the maintenance of the reservoir. First, during HIV progression, cytokines that reactivate latent HIV may promote the replenishment of the HIV reservoir. For example, the combination of TNF, IL-2, and IL-6 reactivates viral production in resting $CD4^+$ T cells isolated from virally suppressed patients (67). Additionally, IL-18, which exists at high levels in the serum of chronically infected patients, increases HIV replication in infected $CD4^+$ T cells (68). Second, homeostatic proliferation of memory T cells, the major pool of latently infected cells, may also contribute to the maintenance of the viral reservoir. For example, IL-7 promotes the survival and basal homeostatic proliferation of memory T cells via the AKT/FOXO3a pathway (69, 70) and stabilizes the size and genetic diversity of the HIV reservoir (48).

Negative Regulatory Molecules

During chronic HIV infection, the persistence of the virus and cytokines induces a state of chronic activation of the immune system. As a result, T cells become exhausted, gradually lose their ability to proliferate, and become hyporesponsive to antigen-specific stimulation. T cell exhaustion is associated with the upregulation of negative costimulatory molecules on the cell surface, such as PD-1, CTLA-4, T cell immunoglobulin and mucin protein (TIM)-3, lymphocyte activation gene (LAG)-3, TNF-related apoptosis-inducing ligand (TRAIL), and others (reviewed in 71). These inhibitory receptors regulate distinct cell functions that establish and maintain peripheral tolerance and abortive T cell responses.

PD-1 is associated with T cell exhaustion in HIV infection (72), and its upregulation correlates with the size of the HIV cellular reservoir (73). Engagement of PD-1 by its ligand PD-L1/2 (broadly expressed on APCs and non-APCs) inhibits HIV production in primary $CD4^+$ T cells isolated from viremic and virally suppressed patients (74). Interestingly, PD-1 binds to its ligands only when it is engaged at the same time as the TCR. Binding of PD-1 to its ligands induces the phosphorylation of its cytoplasmic domain, which then recruits SH2 domain-containing tyrosine phosphatase (SHP)-1 and -2. SHP-1 and -2 then dephosphorylate molecules involved in TCR proximal signaling, such as zeta-chain-associated protein kinase 70 (ZAP70), protein kinase C (PKC)–θ, and CD3ζ. This modification reduces phosphatidylinositide 3-kinase (PI3K) activity, Akt phosphorylation, and glucose metabolism and inhibits T cell activation (75). Similarly, CTLA-4 expression negatively regulates T cell activation and IL-2 production by interacting with its ligands, CD80 (B7-1) and CD86 (B7-2), on APCs (76). In HIV-infected patients, CTLA-4 expression negatively correlates with $CD4^+$ T cell counts, becomes upregulated on $CD4^+$ T cells, and may inhibit HIV replication and promote latency (77). Both PD-1 and CTLA-4 inhibit signaling through different molecular mechanisms that involve Akt, but PD-1 more effectively suppresses T cell–specific transcription than does CTLA-4 (75).

Other studies have also indicated that exhausted T cells represent a unique state of T cell differentiation (78) characterized by changes in the expression of unique transcription factors such as basic leucine zipper transcription factor *ATF*-like (BATF), B lymphocyte–induced maturation protein 1 (Blimp-1), T-box expressed in T cells (T-bet) protein, and many others (reviewed in 71). BATF, for example, is induced by PD-1 ligation and forms dimers with the TF c-Jun. This displaces the TF c-Fos, thereby inhibiting canonical transcription mediated by AP-1 (79). AP-1 is a potential key factor in establishing and maintaining HIV-1 latency (80).

Thus, co-expression of negative regulatory molecules is associated with T cell exhaustion and rapid HIV disease progression, making them potential targets of therapeutics in HIV infection. Blocking inhibitory molecules would favor antigen-specific TCR activation and downstream

signals that are needed for proliferation, effector function, and HIV replication, therefore reactivating latent provirus.

T Cell Quiescence

Human T lymphocytes, both naïve and memory, remain quiescent over long periods of time. This state is actively maintained by specific transcription factors and is characterized by decreased cell metabolism, RNA synthesis, and cell size. Because quiescent T cells comprise most of the HIV reservoir, we need to understand the mechanisms that drive and maintain T cells in this state. Many of these mechanisms are still under investigation, but several transcription factors have already been shown to regulate this process. These include FOXO3a, signal transducer and activator of transcription 5A (STAT5A); Lung Krupple–like factor (KLF2); and Tob (81) (reviewed in 82). It is likely that some of these factors participate actively in maintaining HIV latency. In particular, FOXO3a regulates cell cycle, differentiation, apoptosis, stress resistance, and metabolism of resting T cells. Recent studies suggest that FOXO3 promotes the survival of T_{CM} cells during HIV infection. Specifically, T_{CM} cells express high levels of inactive phosphorylated forms of FOXO3, which repress expression of proapoptotic genes, such as Fas ligand (FasL) and Bim (83), and, consequently, favor their persistence over time (69).

THERAPEUTIC OUTLOOK

Three recent isolated instances of cure for HIV infection—the "Berlin patient" Timothy Brown (84), the French VISCONTI cohort (85), and the Mississippi and California babies (86)—have fueled the notion that a cure for HIV is possible. Importantly, however, rebounded HIV was recently observed in the Mississippi and California infants previously thought to have been cured.

Recent efforts toward widespread curative solutions for HIV infection have focused on the shock-and-kill approach. This approach is based on the early discovery that HIV can be reactivated from latency by treatment with histone deacetylase (HDAC) inhibitors (87). Although initial efforts to reduce the size of the latent reservoir were unimpressive (88–90), recent studies with more potent compounds are promising. For example, a small clinical trial demonstrated that a single dose of the HDAC inhibitor vorinostat, also known as suberanilohydroxamic acid (SAHA), induced cell-associated HIV Gag RNA in some patients (91). Drug discovery and clinical efforts have focused on compounds that do not activate T cells, such as HDAC inhibitors, PKC agonists, and bromodomain inhibitors (reviewed in 92). Surprisingly, a recent study of popular latency-reactivating agents tested in a novel viral outgrowth assay suggested that only drugs that activate T cells (PMA + ionomycin) actually induce de novo viral transcription and synthesis (9, 93). Non–T cell–activating compounds (vorinostat, romidepsin, panobinostat, disulfram, JQ1, bryostatin-1) failed to induce viral outgrowth. Furthermore, vorinostat induced cell-associated HIV Gag RNA was shown to be the result of host-gene readthrough transcription rather than bona fide HIV transcription (93). These results were also independently confirmed by another recent study that observed vorinostat-induced reactivation of only 0.079% of proviruses in patient-derived resting $CD4^+$ T cells, despite anti-CD3/CD28-induced reactivation of 1.5% of proviruses in the same cells (94). The study also noted a significant positive correlation between unspliced cellular HIV RNA and virion synthesis following anti-CD3/CD28 treatment but not SAHA treatment (94). These studies suggest that non–T cell–activating compounds inefficiently reactivate latent HIV and that alternative compounds must be discovered.

A number of significant hurdles must be cleared to find a successful shock-and-kill therapy: (*a*) the need for non–T cell–activating compounds that induce viral outgrowth in patient cells (93, 94), (*b*) a latent reservoir that may be 60-fold larger than previously thought (10), (*c*) stochastic latency reactivation despite maximal T cell activation (10), and (*d*) the lack of effective small molecules that induce the killing of cells in which HIV has reactivated (95). Many of the aspects of the heterogeneity of HIV latency discussed in this review further complicate these hurdles. Indeed, all of these issues exemplify the complexity of HIV latency and of our attempts to eradicate HIV.

It is important to note, however, that we still have only a rudimentary understanding of the molecular mechanisms that drive HIV latency, and that much more work needs to be done to identify the factors that contribute to HIV latency and its reactivation. More specifically, we need to study latency with systems-level approaches rather than evaluating specific mechanisms individually. The studies highlighted in this review indicate that many redundant factors, both cellular and viral, contribute to establishing, maintaining, and reactivating latent HIV. The emerging picture is that HIV latency is multifactorial, a circumstance likely to complicate the identification of small molecules that reactivate all latent HIV.

Permanent HIV Suppression

Endogenous retroviruses make up ~8% of the human genome and may be the benign legacy of exogenous retroviruses that were once pathogenic (reviewed in 96). The nonpathogenic nature of permanently suppressed, endogenous retroviruses may provide an alternative strategy for HIV therapy. Given the mounting barriers to a successful shock-and-kill approach, this strategy is becoming increasingly attractive. Thus, a viable alternative to latency-purging strategies may involve therapies designed to mimic a transcriptionally silent endogenized state or, instead, actually accelerate HIV endogenization. Although the idea of permanently suppressing latency is not novel, little research has been published in this area.

A number of possible targets for permanent HIV suppression have been identified. They include p-TEFb (CDK9/Cyclin T1), whose activity affects HIV transcriptional elongation (97, 98); PIM-1, a "gatekeeper" kinase needed to reactivate latency (99); and Hsp90, a heat-shock protein that activates the NF-κB pathway driving HIV transcription in response to T cell activation (100). Of these targets, the p-TEFb inhibitors 5,6-di-chloro-1-β-D-ribofuranosyl-benzimidazole (DRB), flavopiridol, and seliciclib, as well as the Hsp90 inhibitor AUY922, have already been evaluated in clinical trials as cancer chemotherapeutics. However, their clinical effectiveness in HIV suppression remains unknown. Supplementary to these efforts are suppression/silencing strategies based on DNA editing. Recently, both CRISPR and TALEN genome editing technologies have been used to "cut out," or otherwise inactivate/permanently suppress, latent proviruses (101, 102). Although these studies are only proof-of-concept results, they offer an important new approach to solving HIV latency.

CONCLUSION

During the past 30 years, highly effective ART has saved the lives of millions of people worldwide, yet HIV latency prevents eradication of the virus in most infected patients. Much remains to be learned about HIV latency, and especially the multifactorial and probabilistic nature of establishing, maintaining, and reactivating HIV latency. Systems-level studies will be needed to design and test model systems that fully recapitulate the dynamics and complexity of HIV latency in vivo. Moveover, it seems prudent for the field to move beyond an almost exclusive focus on shock-and-kill strategies and explore alternative therapeutic approaches, e.g., permanent HIV suppression, that may be more feasible and efficacious.

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