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Review Series

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The multifaceted nature of HIV latency

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Although antiretroviral therapies (ARTs) potently inhibit HIV replication, they do not eradicate the virus. HIV persists in cellular and anatomical reservoirs that show minimal decay during ART. A large number of studies conducted during the past 20 years have shown that HIV persists in a small pool of cells harboring integrated and replication-competent viral genomes. The majority of these cells do not produce viral particles and constitute what is referred to as the latent reservoir of HIV infection. Therefore, although HIV is not considered as a typical latent virus, it can establish a state of nonproductive infection under rare circumstances, particularly in memory CD4$^+$ T cells, which represent the main barrier to HIV eradication. While it was originally thought that the pool of latently infected cells was largely composed of cells harboring transcriptionally silent genomes, recent evidence indicates that several blocks contribute to the nonproductive state of these cells. Here, we describe the virological and immunological factors that play a role in the establishment and persistence of the pool of latently infected cells and review the current approaches aimed at eliminating the latent HIV reservoir.

Distinguishing HIV latency and HIV persistence

More than 20 years after the discovery of combination antiretroviral therapy (ART), complete eradication of HIV infection has not yet been achieved, with the notable exception of a few rare cases (1, 2). While ART blocks new cycle of viral replication and partially restores immune functions, it is not a cure. In the vast majority of people living with HIV (PLHIV), interrupting ART leads to rapid viral load rebound, usually within a few weeks after treatment cessation (3), even if treatment was initiated during the first weeks of infection and maintained for years (4). These clinical observations indicate that HIV persists in reservoirs that are largely insensitive to antiretroviral drugs. Viral reservoirs have been defined as cell types or anatomical sites in association with which replication-competent forms of the virus persist with more stable kinetic properties than in the main pool of actively replicating virus (5, 6). This definition implies that any infected cell with a half-life of more than 2 days, which corresponds to the average half-life of productively infected cells (7, 8), may represent a reservoir for HIV. Using ultrasensitive methods to quantify and characterize traces of virus persisting in PLHIV and receiving fully effective ART, two types of viral reservoirs that could both contribute to HIV persistence have been identified (Figure 1).

A first reservoir is thought to be maintained by residual viral replication during ART, which has been attributed to the suboptimal diffusion of antiretroviral drugs in lymphoid tissues (9), allowing the virus to replicate at low levels. Whether this phenomenon occurs in the majority of individuals on ART is still controversial (10). Ongoing viral replication during ART is supported by a few studies that have demonstrated viral evolution in virally suppressed individuals (11) and perturbation in the reservoir following treatment intensification (12, 13), suggesting that ART is not fully efficient. The fact that HIV-specific CD8$^+$ T cells may not be able to access anatomical sites in which ongoing replication occurs, for example in the germinal center of second lymphoid organs (14, 15) or in immune-privileged organs such as testis (16), may also contribute to residual levels of viral replication during ART. In addition, HIV-specific cytotoxic T lymphocytes have limited killing capacity (17, 18), possibly due to their persistent exhaustion status (19) and/or to immunosuppressive environments (20), which may permit the replenishment of the HIV reservoir.

While residual levels of viral replication may occur in a fraction of individuals on ART, particularly those who received less efficient antiviral drugs and whose immune functions were not restored by ART, multiple studies failed to identify strong evidence for ongoing viral replication in individuals receiving new-generation antiviral drugs (21–24). Although the replenishment of the HIV reservoir through de novo infection during ART cannot be excluded, its demonstration is difficult, and the recent data indicating that the bulk of the reservoir is established near the time of ART initiation argue against it (25).

The second type of viral reservoir that contributes to HIV persistence is a small pool of latently infected cells that persists for decades in PLHIV receiving ART (26–28). Latently infected cells can be defined as cells harboring integrated and intact proviruses that do not actively produce infectious virions, but that have the capacity to do so upon stimulation (5). Even though HIV can establish latency, it differs from herpesviruses, which produce viral proteins that are specifically required for the establishment and maintenance of a viral latency program. The capacity of HIV to lie dormant within specific types of cells and at extremely low frequencies suggests that it can establish latency under rare circumstances. The first evidence that HIV can establish a nonproductive state of infection in CD4$^+$ T cells was demonstrated in 1995, before the implementation of combined ART in PLHIV: Chun et al. isolated resting CD4$^+$ T cells from the blood of individuals with active HIV infection and observed that a small fraction of these

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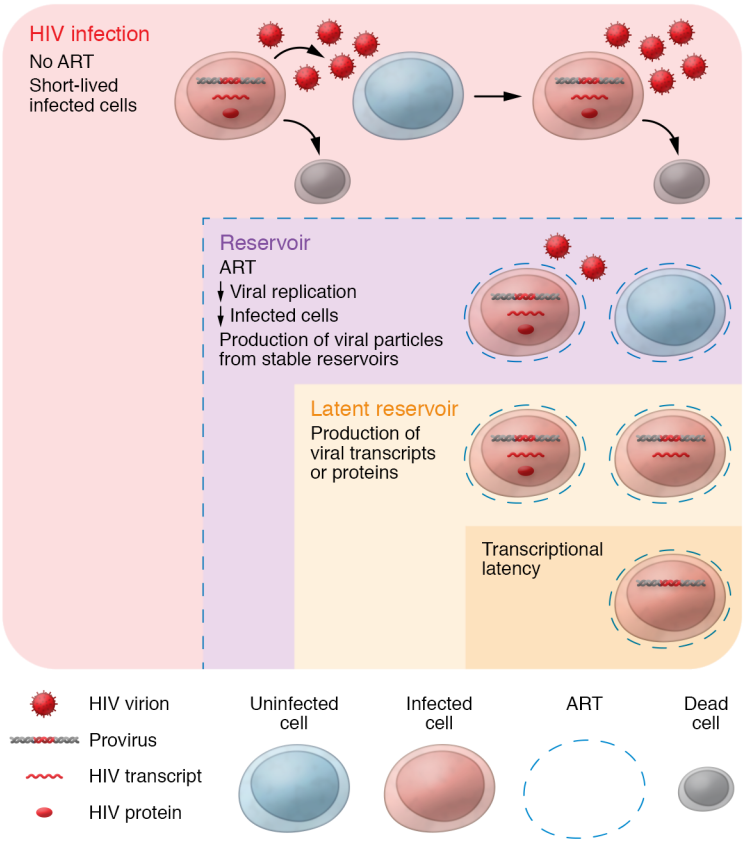

Figure 1. Distinguishing HIV persistence and HIV latency. During untreated HIV infection, the majority of infected cells are short-lived: HIV viremia is sustained by a dynamic process involving continuous rounds of de novo infection. Initiation of ART (blue dashed lines) leads to a dramatic reduction in the levels of viral replication and in the frequency of infected cells. Residual viremia persists and can originate from low levels of ongoing replication or, more likely, from the continuous production of viral particles from stable reservoirs. The majority of infected cells in PLHIV on ART do not produce viral particles and are defined as latently infected cells. Although the production of spliced transcripts or viral proteins is rare, a relatively large fraction of these cells produce short, abortive viral transcripts. Complete silencing of HIV genomes may also occur when epigenetic regulators repress the LTR transcriptional activity.

cells harbored integrated HIV genomes and could produce viral particles upon stimulation ex vivo (29). The fraction of resting $CD4^+$ T cells with integrated provirus is similarly low in blood and lymph nodes and much lower than the frequency of cells harboring unintegrated viral genomes, which are much shorter-lived (30) but can complicate the measurement of the HIV reservoir during untreated HIV infection. In 1997, the implementation of effective ART in PLHIV revealed the clinical importance of this pool of latently infected cells: three studies reported the presence of a small number of persistently infected cells harboring replication-competent HIV in individuals on suppressive ART (26–28). Although these cells are extremely rare (around 1 in 1 million resting $CD4^+$ T cells), the reservoir is long-lived, with an estimated half-life of 44 months (31, 32), indicating that ART alone will not eradicate the pool of latently infected cells in a lifetime.

Although it was rapidly apparent that this small pool of persistently infected cells would represent a formidable challenge to HIV eradication, more than 20 years later, the precise nature of the HIV reservoir remains unclear. In addition to the diversity in the tissues and cellular subsets in which HIV persists during ART (discussed below), a variety of nonproductive infection states have been described. The assessment of the transcriptional and translational status of persistent HIV proviruses in virally suppressed individuals challenges our definition of HIV latency. Whereas viral latency is often associated with transcriptional latency (i.e., the lack of transcription from the HIV promoter), an increasing number of studies indicate that complete silencing of the HIV promoter is a rare event (33, 34). Therefore, a relatively large fraction and possibly the majority of latently infected cells (as defined by cells that do not produce viral particles) may express low levels of short viral transcripts (34, 35). Although these abortive transcripts are frequently produced, they rarely elongate enough to generate complete or spliced transcripts (36). Accordingly, production of viral proteins by latently infected cells appears to be rare (37, 38). This suggests that several blocks may contribute to the inability of persistently infected cells to produce infectious viral particles, including blocks in elongation of transcription (36), nuclear export (39), multiple splicing (34), and translation, as detailed below.

Even if the majority of persistently infected cells do not produce viral particles spontaneously, low levels of viremia, below the limit of detection of commercial assays, can be measured in the majority of PLHIV on ART (40, 41). The presence of residual viremia indicates that a small fraction of persistently infected cells produces viral particles at a given time. As mentioned above, residual viremia is unlikely to originate from low levels of residual replication, but rather from the production of viral particles from a stable pool of persistently infected cells. Indeed, the small population of viral particles circulating in individuals on ART is dominated by a predominant plasma clone not found in the latent reservoir, indicating that these virions are unlikely to replenish the persistent reservoir (42, 43). Residual levels of viremia show a two-phase decay, suggesting that it may arise from at least two cell compartments, one in which viral production decays over time and a second in which viral production remains stable for at least 7 years (44). The source of residual viremia remains currently unknown. The existence of this "active reservoir" is an additional challenge to the development of effective curative strategies, since it represents a likely cause of viral rebound upon ART interruption.

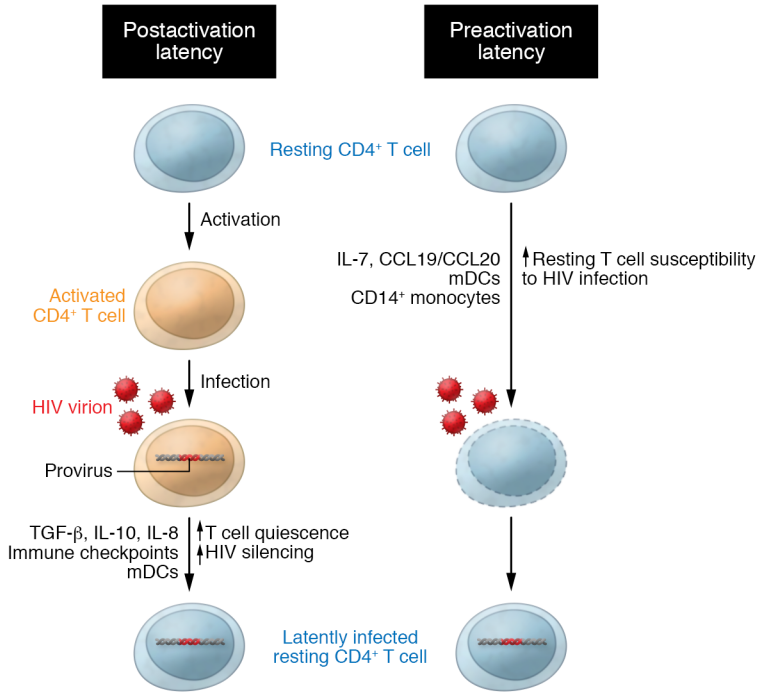

Figure 2. Models for the establishment of HIV latency. Postactivation latency refers to a phenomenon by which activated productively infected CD4+ T cells revert back to a quiescent state, which is accompanied by the silencing of the HIV promoter. In preactivation latency, resting CD4+ T cells, which are usually refractory to HIV infection, become permissive and establish latency directly (i.e., in the absence of T cell activation).

Although the HIV reservoir is usually defined as a pool of cells harboring replication-competent (and therefore intact) genomes, several lines of evidence indicate that defective proviruses, even if not capable of replication, have the ability to produce viral transcripts and viral proteins (45, 46). Albeit indirectly, these defective genomes likely contribute to sustained inflammation and T cell activation even after prolonged ART (47, 48), which may in turn contribute to HIV persistence by promoting the proliferation of infected T cells (49, 50).

Establishment of HIV latency

When, where, and how HIV latency is established is still the object of intensive investigations. In the absence of ART, activated CD4+ T cells represent the main target for HIV and die rapidly upon infection (7, 8). Only a minute fraction of these cells survives and enters the pool of persistent and long-lived latently infected cells (51). Although the timing of the establishment of the pool of latently infected cells in humans remains unclear, several groups recently used nonhuman primate models of HIV infection (rhesus macaques infected with SIV) to precisely determine the duration of untreated infection needed to establish persistent infection (52, 53). In these studies, ART was initiated at different times after infection, and the size of the pool of persistent SIV-infected cells during prolonged suppressive therapy was evaluated by measurement of markers of viral persistence and by analytical treatment interruption. Whitney et al. (52) reported that initiation of ART 3 days after SIV inoculation blocked the emergence of viral RNA and proviral DNA in peripheral blood. Nevertheless, after discontinuation of ART following 24 weeks of fully suppressive therapy, virus rebounded in all animals (with a moderate but statistically significant delay in comparison with animals initiating ART later). In contrast, Okoye et al. (53) observed that ART initiation at days 4 and 5 followed by suppressive therapy for 2 years led to viral control and possibly eradication of the virus, indicating that very early and prolonged therapy may either prevent the establishment or accelerate the clearance of viral reservoirs. Altogether, these observations indicate that early ART may not entirely prevent the establishment of the reservoir, but may accelerate its decay.

For obvious reasons, human studies of early reservoir establishment are much more complicated to conduct: very recently infected individuals are difficult to identify, and access to tissues from PLHIV poses logistical and ethical challenges. The unique case of an individual infected during the initiation of pre-exposure prophylaxis who initiated ART only 10 days after infection is informative: even if HIV could not be detected in blood and tissues from this individual after 2 years of therapy, he experienced viral rebound 225 days after ART cessation, suggesting that a persistent reservoir was established in less than 10 days (54). In line with this observation, initiation of ART in eight individuals at the earliest stage of diagnosable HIV infection (Fiebig I stage) drastically reduced the size of the pool of HIV-infected cells but did not prevent viral rebound upon ART initiation (4, 55). A recent and larger study of acutely treated PLHIV revealed that the pool of infected cells rapidly increases and reaches its maximal size in tissues in the first 2 weeks of infection, i.e., before seroconversion (56). Interestingly, the majority of these early targets are rapidly cleared upon ART initiation, suggesting that infected cells have a greatear ability to persist after peak viremia. This is in line with the study by Okoye et al. in nonhuman primates (53) and suggests that the early reservoir may be relatively labile. The virological and immunological mechanisms supporting this phenomenon remain to be determined.

As mentioned above, circulating cells are not the unique site of HIV replication and are unlikely to represent the most favorable environment for the establishment of HIV latency. Less than 2% of the total body T cells are found in peripheral blood. Therefore, it is not surprising that HIV-infected cells are found in multiple tissues after years of ART (57, 58), particularly in gut and lymph nodes, and at lower frequencies in the spleen, liver, lung, central nervous system, and bone marrow (59). Since gut-associated lymphoid tissue (GALT) and lymph nodes are particularly enriched in persistently infected cells during ART (56, 60), lymphoid tissues may represent a favorable environment for the establishment of viral latency.

The anatomical location and the timing of the emergence of persistently infected cells offer a glimpse into some of the mechanisms contributing to the establishment of HIV latency (Figure 2). Latently infected cells are mainly resting memory CD4+ T cells (26–28, 30, 61). The transition from an activated state to quiescence may offer a narrow window of opportunity that per-

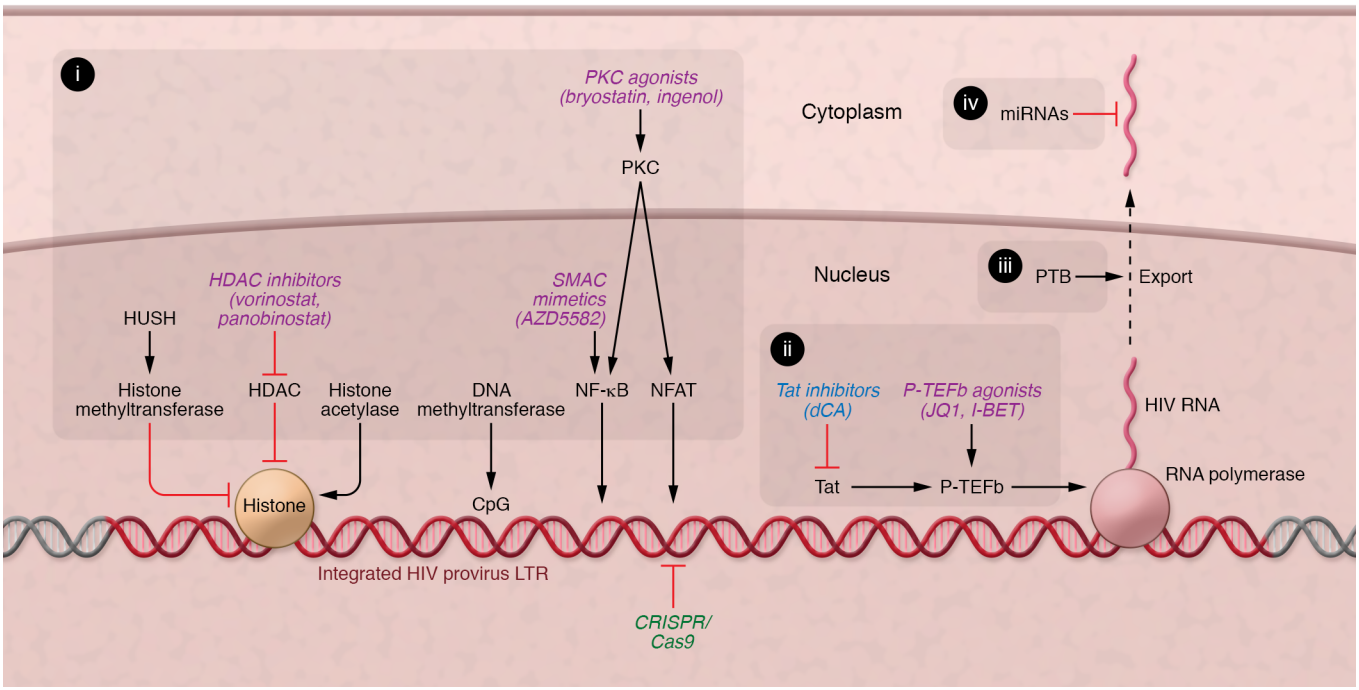

Figure 3. Mechanisms and targets of HIV latency. HIV silencing is regulated by key control elements acting on (i) HIV transcription initiation (histone acetylation/deacetylation, histone and DNA methylation, transcription factors), (ii) HIV transcription elongation (positive transcription elongation factor b [P-TEFb] and viral protein Tat), (iii) HIV RNA export (polypyrimidine tract–binding protein [PTB]), and (iv) HIV RNA degradation (miRNAs). Pathways promoting HIV expression are shown with black arrows, whereas those inhibiting HIV expression are shown in red. These pathways can be targeted in vivo (in italics) in order to reverse HIV latency (latency-reversing agents [LRAs], purple), promote HIV latency (latency-promoting agents [LPAs], blue), or edit the HIV genome (CRISPR/Cas9, green).

mits HIV silencing and persistence of the infected cells. During the contraction phase of the immune response, when the antigen load decreases and activated cells transition from an effector to a memory phenotype, a rare subset of cells are still permissive to HIV infection but also are transcriptionally programmed to become quiescent, a state that is favorable to HIV latency (62). The role of cytokines such as TGF-β, IL-10, and IL-8 in this phenomenon has been confirmed in in vitro experiments using polarized CD4$^+$ T cells (63). Additionally, immune checkpoint molecules are known to dampen T cell activation and may consequently favor HIV latency. PD-1, LAG-3, and TIGIT were initially identified as markers of HIV-infected cells during ART (64–66). Further investigations demonstrated the active role of PD-1 in silencing HIV transcription (67, 68). Interestingly, the presence of monocytes or myeloid dendritic cells (mDCs) in coculture with activated HIV-infected T cells may favor their transition to a postactivation state of latency, highlighting the role of cell-to-cell contact in the establishment of HIV latency (69, 70).

Several lines of evidence suggest that latency may also be directly established in resting CD4$^+$ T cells. Although resting CD4$^+$ T cells are refractory to productive infection as a result of numerous blocks in the HIV replication cycle, cell-cell interactions increase their susceptibility (71–73). Soluble factors are also known to increase the susceptibility of resting CD4$^+$ T cells to latent HIV infection. For instance, IL-7, a cytokine involved in T cell homeostasis, modulates the activity of the restriction factor SAMHD1 and increases the permissiveness of resting CD4$^+$ T cells to HIV infection (74, 75). Similarly, CCL19 and CCL20, two chemokines involved in the trafficking of cells to lymph nodes and GALT, enhance HIV infection of resting CD4$^+$ T cells by modifying the actin cytoskeleton, thereby increasing nuclear entry and integration of the viral DNA (76). Furthermore, the anatomical localization of resting CD4$^+$ T cells may influence their susceptibility to HIV infection: CD4$^+$ T cells isolated from lymphoid tissues (spleen and tonsil), which display an intermediate level of activation, are more permissive to HIV infection and latency (77). A model in which cell-to-cell transmission from activated, productively infected CD4$^+$ T cells to resting CD4$^+$ T cell leads to latent infection may mimic the spread of HIV infection in anatomical sites with a high density of T cells, such as germinal centers in lymph nodes (78). All these studies support an alternative model in which direct nonproductive infection of resting CD4$^+$ T cells may result in viral latency.

Recent data also suggest that the strength of the T cell receptor engagement could influence the transcriptionally active versus latent status of the provirus. Intermediate and low signals predispose cells toward latent infections that are refractory to reversal (79). Evans et al. demonstrated that interactions between antigen-presenting cells (APCs) and resting CD4$^+$ T cells favor latency establishment (80). Signals from mDCs induce downregulation of the NF-κB pathways and upregulation of Krüppel-like factor 6 (KLF6) in CD4$^+$ T cells, thereby promoting T cell quiescence. In vitro, only mDCs and CD14$^+$ monocytes expressing specific cell surface molecules involved in cell-cell interaction have the ability to induce HIV latency in CD4$^+$ T cells (69), suggesting that cell-cell

interactions modify the transcription network of CD4+ T cells to establish a prolatency environment in a cell-specific manner.

Locations of HIV reservoirs

In addition to blood, persistently infected cells are found in the lymph nodes (64), gut (81–83), central nervous system (84), lungs (85), bone marrow (86, 87), and genital tract (16, 88–90). Recently, a study performed with tissues collected from six PLHIV postmortem revealed the presence of HIV DNA in all 28 tissues analyzed (58). If HIV DNA can be easily detected in most parts of the body at relatively high frequencies, these measures overestimate the size of the reservoir. In the blood, replication-competent proviruses are present in only 1 in 1 million CD4+ T cells (26, 27), whereas HIV DNA quantification typically measures frequencies that are two to three orders of magnitude higher. This difference is due to a large proportion of HIV genomes presenting various defects preventing them from replicating (91). In the blood, defective proviruses represent 95%–98% of the viral genomes (92), and these defects accumulate rapidly in the course of infection (93). Only a few studies have investigated the presence of intact or replication-competent genomes in tissues from PLHIV (30, 58, 64, 94). Therefore, the exact contribution of these anatomical reservoirs to the pool of persistently infected cells remains largely unknown.

It is generally accepted that memory CD4+ T cells represent the major cellular reservoir for HIV, at least quantitatively (30, 61). To a lesser extent, cellular reservoirs may also comprise myeloid cells such as monocytes, tissue-resident macrophages, and follicular dendritic cells (reviewed in refs. 95, 96), although whether these cells represent long-lived reservoirs for the virus after prolonged ART remains unclear (97–99). The CD4+ T cell compartment can be divided into subsets endowed with different functional, proliferative, and survival capacities. Several studies have shown that the less differentiated memory subsets, including stem cell memory (Tscm) and central memory (Tcm), are particularly enriched in HIV genomes during ART (61, 100, 101). These cells possess a high proliferation potential and, following antigen stimulation, can give rise to effector memory T (Tem) cells (102, 103). While Tcm cells have been shown to highly contribute to the pool of cells harboring integrated HIV DNA (61), the Tem subset may encompass the majority of intact and potentially replication-competent proviruses (104). In addition to these memory subsets, specific functionally polarized subsets of CD4+ T cells have also been shown to be enriched in persistent HIV: blood- and gut-derived Th17 cells are enriched in HIV proviruses (105), and a large portion of replication-competent viral genomes are found within the Th1 and T follicular helper cell (Tfh) subsets (64, 92). HIV also preferentially persists in tissue-resident memory T cells (106). The diversity of the cellular reservoirs for HIV, which are characterized by distinct mechanisms of maintenance, adds another level of complexity to the development of HIV eradication strategies (38, 107, 108).

Molecular mechanisms of HIV latency

Unlike herpesviruses, HIV does not encode for proteins specific for a latency program. Nonetheless, HIV expression is under the control of molecular components of the infected cell that can induce and/or maintain a nonproductive state of infection (Figure 3). A first mechanism that governs HIV expression is linked to the location of the integration site. HIV proviruses are more frequently integrated in introns of actively transcribed gene (109, 110). Integration in the same orientation of actively transcribed genes can impair HIV expression through transcriptional interference (111). HIV integration site may also influence the degree of latency, with deeply latent proviruses being mostly located in non-genic regions (112).

As for transcriptionally active cellular genes, the HIV genome needs to be accessible to the transcriptional machinery, and this access is epigenetically controlled. The HIV promoter is wrapped around nucleosomes nuc-0 and nuc-1 (113). These histone complexes are posttranslationally modulated by epigenetic marks to induce HIV transcription through acetylation (114, 115), or silencing through deacetylation (116, 117) and methylation (118). In addition, two CpG islands found in the HIV long terminal repeat (LTR) can be methylated and consequently maintain viral repression (119). The protein complex HUSH also contributes to the epigenetic control of HIV transcription by silencing proviruses harboring H3K9me3 methylation marks when the viral proteins vpr and vpx are not expressed (120, 121).

In addition, initiation of HIV transcription requires the binding of the cellular transcription factor NF-κB to the viral promoter (122). Notably, binding sites for the ubiquitous transcription factors Sp1 and AP-1 and for the immune cell–specific NFAT (123) are also present in the LTR. The elongation of HIV transcripts is relatively inefficient in latently infected cells: a considerable amount of short, abortive transcripts lacking the poly(A) tail are detected in latently infected cells from PLHIV on ART (36). NF-κB plays an additional role by recruiting the elongation factor P-TEFb (124). However, this recruitment is mostly accomplished by the viral protein Tat once it has been translated (125). In addition to transcriptional regulation, the lack of viral production by infected cells can also be attributed to post-transcriptional blocks. Unspliced viral RNA and as many as 40 different singly and multiply spliced RNAs can be transcribed from a single genome (126). A defect in splicing may contribute to the absence of viral proteins, particularly Tat, which is required for expression of all viral transcripts (34). Furthermore, viral RNAs accumulate in the nucleus of latently infected cells, and this defect in RNA export can be reverted by overexpressing the polypyrimidine tract–binding protein (PTB) in resting cells (39). Finally, microRNA can prevent the translation of coding viral RNA by complementary homology. HIV infection is known to impact the miRNA profile of the cell (127). Cellular miRNA, such as miR-132 (128), can promote HIV replication indirectly by silencing proteins involved in transcriptional regulation. Conversely, latency can be maintained by miRNAs directly targeting the 3′ end of HIV transcripts (miR-28, miR-125b, miR-150, miR-223, miR-382) (129) or the HIV *nef* RNA (miR-29a) (130). Altogether, these studies highlight the multiplicity of the cellular mechanisms that contribute to the nonproductive state of HIV-infected cells during ART.

Maintenance of latently infected cells by cell proliferation

As discussed above, the majority of persistently infected cells do not produce viral particles during ART. These latently infected cells are maintained through both cell survival signals, preventing HIV-infected cells from death, and cell division signals, promoting

the expansion of HIV-infected clones (61, 131). Several studies over the past 10 years have clearly demonstrated that clonal expansions occur in the persistent HIV reservoir, as shown by the duplication of partial and near-full-length HIV genomes and/or integration sites (92, 109, 110, 112, 132–139). These infected clones wax and wane during ART, resulting in a dynamic pool of infected cells over time (133, 137, 140, 141). Several mechanisms contribute to the dynamics of the HIV reservoir (142): (a) antigen-driven proliferation (134, 143, 144), (b) homeostatic proliferation (61, 137, 145), and/or (c) HIV integration–induced proliferation (109, 110, 146). Although antigen-driven proliferation has been proposed as the major driver of HIV persistence, all three phenomena likely coexist (142, 147).

Targeting HIV latency in vivo

The ultimate objective of an HIV cure is to eradicate all infected cells from the body or to induce durable immune control of the HIV reservoir (reviewed in ref. 148). Here, we will focus on strategies that target the pool of latently infected cells and that consist of either depletion of these cells or permanent silencing of the integrated proviruses.

The shock-and-kill strategy combines the reactivation of latent proviruses ("shock") and the elimination of the resulting productively infected cells ("kill"), with the hypothesis that viral cytopathic effects or clearance of virus-expressing cells by the immune system will reduce the size of the latent reservoir (149, 150). To induce proviral expression, a large number of latency-reversing agents (LRAs) have been identified (151). These agents need to be potent enough to reactivate efficiently most HIV proviruses without inducing a cytokine release syndrome, which would result in major adverse events. This fine balance is a major obstacle to the success of these strategies: the administration of an anti-CD3 antibody together with IL-2, although efficient at reactivating HIV, was highly toxic in PLHIV on ART (152).

LRAs can be divided in several pharmacological classes and can be used alone or in combinations: histone deacetylase inhibitors (HDACis [refs. 117, 153–156]), protein kinase C (PKC) agonists (157–160), P-TEFb agonists (161, 162), second mitochondria-derived activator of caspase (SMAC) mimetics (163–165), and Toll-like receptor (TLR) agonists (166, 167). These agents target different cellular pathways, all of which are nonspecific to the induction of HIV transcription. In fact, most of these LRAs were originally developed for other indications. For instance, the HDACi suberanilohydroxamic acid had been shown to be active against leukemia and breast cancer cell lines (168, 169). Similarly, the fusion inhibitor maraviroc, which was originally used as an antiretroviral drug, has been shown to induce HIV transcription through the NF-κB pathway in vivo and may also be used as an LRA (170).

The majority of clinical trials conducted so far mostly used HDACis such as valproic acid (171–175), vorinostat (176–178), panobinostat (179), and romidepsin (180), and demonstrated a transient increase in cell-associated HIV RNA and/or residual plasma viremia in virally suppressed PLHIV. Low doses of the PKC agonist bryostatin were also recently tested in a phase I clinical trial and did not result in an induction of HIV transcription in vivo (181). Disappointingly, none of these studies resulted in a significant decrease in the size of the HIV reservoir in individuals on ART. Several factors may explain these negative results: (a) LRAs may have differential effects between tissues (177); (b) LRAs are not equally efficient in all populations of $CD4^+$ T cells, suggesting that they may be largely ineffective in some cellular reservoirs (107, 108); (c) effector cells may be lacking at sites of active viral production such as lymph nodes (14, 15, 17); (d) the persistent immune exhaustion of $CD8^+$ T cells may not allow the efficient elimination of productively infected cells (182); (e) some LRAs, particularly HDACis, have been shown to impair cytotoxic effector responses (183) and antigen presentation by APCs (184), which could hamper viral reservoir elimination; and (f) productively infected cells may be inherently resistant to immune-mediated killing through the expression of prosurvival factors (185).

Several improvements have been proposed to increase the efficacy of LRAs at reactivating latent HIV proviruses in individuals on ART, including the use of LRA combinations, development of new pharmacological classes of LRAs, and enhancement of cytotoxic immune responses (186). Overall, a better understanding of the effects of LRAs in vivo is needed to improve future shock-and-kill strategies.

The opposite of shock-and-kill strategies, block-and-lock strategies are aimed at promoting permanent silencing of HIV proviruses using latency-promoting agents (LPAs). This approach is based on the hypothesis that HIV latency can be induced in an irreversible way, which would prevent viral rebound upon ART cessation. These LPAs act by inhibiting viral or cellular proteins involved in HIV transcription (187). The best-described LPA is didehydro-cortistatin A (dCA), a Tat inhibitor (188, 189) shown to decrease viral RNA in cells and tissues and delay viral rebound upon ART cessation in a humanized mouse model (189). The efficacy of LPAs at preventing viral rebound in HIV-infected individuals has not been investigated yet.

Another approach to reduce the reservoir of latently infected cells is to permanently inactivate the integrated proviruses. Given the recent development of the CRISPR/Cas9 system, the excision or inactivation of the HIV genome is now theoretically possible (190). Inactivation of the provirus by targeting of different conserved HIV sequences was tested in several in vitro models. Although the approach was originally promising (191, 192), it resulted in a long-term escape caused by HIV DNA mutations in the targeted sequences, preventing single-guide RNA recognition (193–196). Nonetheless, HIV proviral genome excision by CRISPR/Cas9 in a humanized mouse model resulted in promising observations (197). Future studies are warranted to determine whether the combination of multiple-guide RNAs targeting different HIV variants and/or genome sites could prevent viral escape.

Conclusions

In individuals on ART, HIV persists at low levels in several tissues and multiple cellular subsets and displays different states of transcriptional and translational activities. The multifaceted aspect of these HIV reservoirs complicates their analysis and the development of efficient therapeutic strategies to target them. The relative contributions of these reservoirs, from cells that continuously produce low levels of viral particles during ART to the extreme case of fully transcriptionally silent genomes, remain unclear. More importantly, the clinically relevant reservoir that causes viral rebound upon ART cessation has not been identified yet.

Given the large diversity of the cells in which HIV persists and the multiple molecular mechanisms contributing to viral persistence, studies conducted on bulk populations of cells are unlikely to reveal targetable mechanisms to cure HIV infection. The recent development of single-cell approaches to study the transcriptome and proteome of individual HIV-infected cells (38, 198, 199) will certainly help in this endeavor.

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