Molecular Medicine

Human Immunodeficiency Virus (HIV) Latency: The Major Hurdle in HIV Eradication

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Failure of highly active antiretroviral therapy to eradicate the human immunodeficiency virus (HIV), even in patients who suppress the virus to undetectable levels for many years, underscores the problems associated with fighting this infection. The existence of persistent infection in certain cellular and anatomical reservoirs appears to be the major hurdle in HIV eradication. The development of therapeutic interventions that eliminate or limit the latent viral pools or prevent the reemergence of the viruses from producing cells will therefore be required to enhance the effectiveness of current antiretroviral strategies. To achieve this goal, there is a pressing need to understand HIV latency at the molecular level to design novel and improved therapies to either eradicate HIV or find a functional cure in which patients could maintain a manageable viral pool without AIDS in the absence of antiretroviral therapy. The integrated proviral genome remains transcriptionally silent for a long period in certain subsets of T cells. This ability to infect cells latently helps HIV to establish a persistent infection despite strong humoral and cellular immune responses against the viral proteins. The main purpose of this report is to provide a general overview of the HIV latency. We will describe the hurdles being faced in eradicating latent HIV proviruses. We will also briefly discuss the ongoing strategies aimed toward curing HIV infection.

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BACKGROUND

The human immunodeficiency virus (HIV) was first discovered in 1981, and in the last 30 years, >30 million people have died from HIV infection and progression to acquired immunodeficiency syndrome (AIDS). Sub-Saharan Africa is home to the majority of people carrying HIV, and roughly 25 million people are living with HIV, representing around 7% of the total sub-Saharan population or two-thirds of globally infected HIV individuals (1). Besides the prevalence of poverty, the inefficient distribution of anti-HIV drugs was found to be the main root cause of widespread HIV infection. Nevertheless, because of various international initiatives along with remarkable scientific efforts in developing more potent anti-HIV drugs, the onset of AIDS in the HIV-infected population has been greatly delayed, and both mortality and morbidity due to AIDS has been significantly reduced (2).

AIDS is the ultimate consequence of unchecked HIV infection, where the virus kills a sufficient amount of $CD4^+$ T cells. This depletion impairs the immune system and, because of lack of proper immune defense, any opportunistic infection could become fatal to the host. Fortunately, present anti-HIV therapy, namely highly active antiretroviral therapy (HAART) or combination antiretroviral therapy (cART), proved to be successful in controlling HIV infection and significantly prolongs the lifespan of infected individuals (3,4). Sadly, antiretroviral drugs are still not available to every infected patient in developing countries, but more and more programs have been introduced in recent years to make sure that no HIV infection goes without treatment. Especially since 2004, great strides have been achieved primarily by increasing the access to anti-HIV drugs, improving the awareness regarding HIV and introducing various preventive measures. These efforts produced clear results in the form of a reduced number of AIDS-related deaths and a proportional decrease in new infections in many parts of the world (World Health Organization report 2010 [5]).

Owing to the success of anti-HIV therapy, the median lifespan of HIV patients has improved significantly. The biggest challenge in tackling HIV is the inability of HAART to eradicate the virus. Even when the virus is suppressed to undetectable levels for many years, it quickly

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reemerges if the treatment is stopped. Two main reasons for this dreaded feature of HIV infection are replication of the virus in immunoprivileged sites, with limited access to drugs (for example, brain) and the ability of the virus to establish latent infection. Macrophages may be important contributors to continuous viral replication in the presence of HAART, since HIV is less cytopathic to macrophages, and anti-HIV drugs are also comparatively less effective in these cells (6–11). However, the main reservoirs of latent HIV are memory $CD4^+$ T lymphocytes. These cells harbor a set of integrated proviruses that are unable to complete their lifecycle because of the lack of suitable conditions and are called latent or "transiently silent" proviruses. Latent proviruses, because of their silent nature, are well protected from antiretroviral therapies, which target actively replicating viruses, and from the host's immune system, which is unable to differentiate the infected cells from uninfected cells because of the lack of any viral activity in latently infected cells. These hibernating latent proviruses are just waiting for the favorable conditions and consequently are the everlasting source of replication-competent viruses (12–14). HIV-infected memory T cells have an extremely long lifespan, exceeding many years, and are effective HIV producers on activation (13,15–23). Although a small fraction of the T-cell population carries latent proviruses (~1 in 10^6 cells), it creates a stable reservoir of virus that can be reactivated to produce a rebound of the viral load, even after successful antiviral treatment (24,25).

The continuous presence of the virus in the body necessitates the lifelong treatment with anti-HIV drugs, which results in various side effects. These side effects, along with a deregulated immune system, contribute immensely to the occurrence of various age-associated diseases in HIV patients, such as neurocognitive abnormalities, cardiovascular disease and muscle and bone disorders (26–30). Moreover, the lack of accessibility of drugs and economic constraints to supporting lifelong treatment in developing countries drastically hamper the vision of global HIV containment (31). As a result, despite global anti-HIV efforts and better availability of HAART, new HIV infections are outnumbering the patients initiating HAART (32–34).

It remains an important priority of HIV research to precisely define the molecular mechanisms that allow the establishment of latency in T cells and subsequently design therapies to achieve either complete HIV eradication or some kind of functional cure, which will allow the immune system to maintain the upper hand and keep HIV replication below threshold levels in the absence of anti-HIV drugs/therapy.

MOLECULAR BIOLOGY OF HIV LATENCY

Transcription Factors and HIV Latency

The persistence of HIV in patients, despite prolonged treatment, has prompted renewed interest in understanding the molecular mechanisms that control HIV latency. As for all retroviruses, replication of HIV provirus relies primarily on efficient transcription. HIV transcription depends on the host cell transcription machinery along with master transactivator protein of HIV, transactivator of transcription (Tat). Flaws in proviral transcription appear to be the major factor contributing to HIV latency.

HIV transcription is mainly divided into two main phases, namely the initiation phase and the elongation phase. During the initiation phase, transcription factors such as specificity protein 1 (Sp1), TATA box binding protein (TBP) and TBP-associated factors are recruited at the core long terminal repeat (LTR) promoter, a minimal essential component sufficient to sustain basal transcription. The LTR core promoter, besides TATA box and initiator sequence, also carries three Sp1 binding sites, and mutation of any of these sites individually or in combination strongly impairs both basal and Tat-dependent HIV transcription (35–39). HIV LTR also carries an enhancer sequence, which consists of the cognate overlapping binding sites for the members of both nuclear factor (NF)-κB and nuclear factor of activated T cells (NFAT) protein families (40,41). The binding of these factors strongly enhances HIV transcription, primarily by cooperating with Sp1 and recruiting histone acetyl transferases (42–46). The enhancer sequence appears to play an even more significant role during reactivation of latent proviruses (23,47,48).

HIV transcription is further divided into slower and faster, or Tat-independent and Tat-dependent, phases, respectively. The slower, Tat-independent phase encounters several restrictions primarily because of the presence of two inhibitory factors, namely the negative elongation factor (NELF) and the 5,6-dichloro-1-β-D-ribofuranoxylbenzimidazole (DRB) sensitivity-inducing factor (DSIF), which commonly restrict the transcription of several cellular genes (reviewed in [49,50]). However, once Tat protein is synthesized, it provides positive feedback to HIV transcription and enhances HIV transcription rate several hundred–fold. Thus, HIV transcription enters into the second, fast Tat-dependent phase (51,52). Therefore, HIV transcription differs from normal transcription of cellular genes, since it is autoregulated by Tat protein. In the absence of Tat, HIV transcription halts at around 60 nucleotides because of the presence of NELF, DSIF and nonprocessive RNA polymerase II (RNAP II) at HIV LTR. To overcome this obstruction and facilitate transcription elongation, HIV Tat protein binds to the nascent RNA sequences called the transactivation-responsive element (TAR). TAR is a 59-nucleotide stem-loop structure located at the 5′ end of every viral transcript. After binding to TAR sequences, Tat recruits positive transcription elongation factor b (P-TEFb; 53,54), which subsequently hyperphosphorylates the C-terminal domain of RNAP II, primarily at its serine 2 residues. The C-terminal domain hyperphosphorylation makes RNAP II highly processive (55,56). Additionally, P-TEFb also phosphorylates inhibitory

factors DSIF and NELF. These modifications eventually relieve all the restrictions, and, consequently, HIV transcription enters into the elongation phase, which enhances the rate of generation of full-length HIV transcripts (57–59).

Any defect in the process of HIV transcription (initiation or elongation phase) leads to the generation of latent proviruses. A number of cellular transcription factors known to play a critical role in HIV transcription, including NF-κB, NFAT and P-TEFb, are either lacking or present in their inactive form in resting primary T cells. As a result, HIV transcription is restricted and the virus remains silent or latent in these cells (60–63). Subsequently, this restriction translates into stochastic fluctuations in cellular Tat levels, which further dampen the prospect of effective HIV transcription (23,47,64–66).

Eventually, the fall of Tat levels below the threshold level results in the more stable maintenance phase of HIV latency (Figure 1). Thereafter, latent proviruses are unable to perform their transcription and keep waiting for the favorable conditions that eventually enhance cell metabolism, such as via T-cell receptor activation or through action of various cytokines (see Figure 1). However, once cells become metabolically active, the level of transcription factors rises. These transcription factors in turn facilitate both the initiation and elongation phases of transcription that eventually lead to the reactivation of latent HIV proviruses. For more details about HIV transcription and latency, please see recent reviews (21–23,67–69).

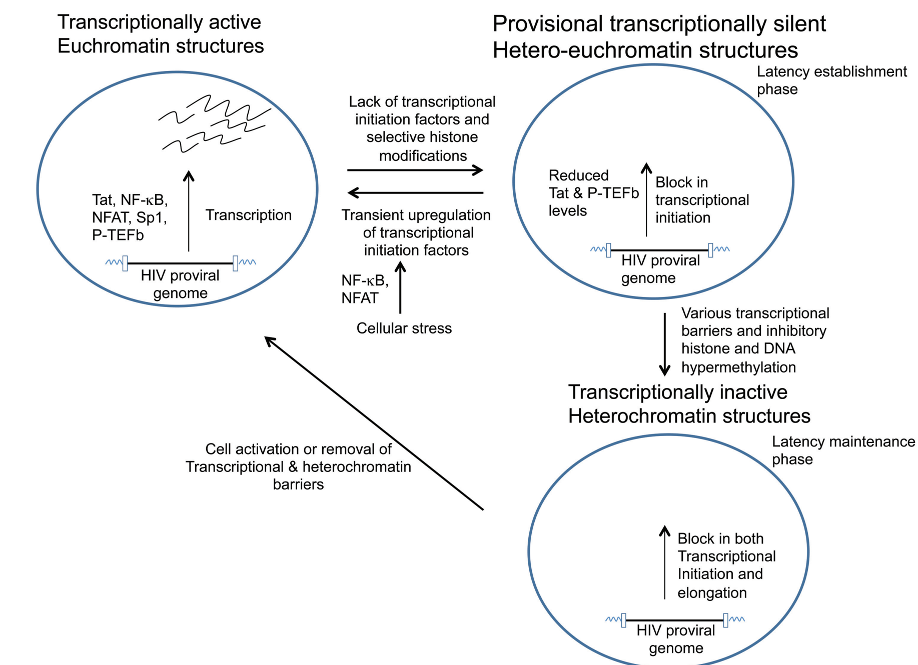

Figure 1. Factors regulating HIV provirus into latency. Lack of transcription factors in resting CD4⁺ T cells along with repressive chromatin structures impede the HIV transcriptional initiation rate, which in turn translates into reduced Tat levels that ultimately yield the entry of provirus into latency. After cell activation, the level of transcriptional factors rises and resultant Tat production reinstates HIV transcription. See details in the text.

Role of Epigenetics in Controlling HIV Transcription and Latency

The expression of every cellular gene, including integrated HIV genome, depends on specific epigenetic modifications, which define particular chromatin structures at that gene. The specific chromatin structures are characterized by their fundamental subunit, nucleosomes. A nucleosome consists of an octamer of a pair of four core histones (H3, H4, H2A and H2B), which are wrapped around by 147 base pairs of DNA. These core histones undergo various kinds of posttranslational modifications such as acetylation, methylation, sumoylation, phosphorylation, ubiquitinylation and so on. These modifications eventually define the specific nature of the chromatin structures. Chromatin structures primarily around the promoter region of a gene regulate its expression. Thus, the nature of chromatin modifications plays a decisive role in regulating the expression of a gene (70–72). The transcriptionally active, open/relaxed chromatin structures, which allow the access of transcription machinery at the promoter region of a gene, are called euchromatin. On the contrary, the transcriptionally repressive closed/compact chromatin structures, which inhibit the access of transcription machinery at the promoter region of a gene, are called heterochromatin (71,73).

After integration, the HIV genome appears to assemble into a well-defined nucleosomal structure, independent of the site of integration into the cellular genome (74,75). The epigenetic modifications at the two nucleosomes (Nuc-0 and Nuc-1) around the HIV LTR promoter play a major role in controlling HIV gene expression (75–77). Not only nucleosomal histones, but also DNA, receive epigenetic modifications, and notably the DNA methylases have also been implicated in HIV transcription and latency regulation (78–80). Interestingly, most of the enzymes that catalyze epigenetic modifications are unable to bind directly to the DNA and have to be recruited to HIV LTR by various DNA binding proteins. Proteins such as latency-C-promoter binding factor 1 (CBF-1), Yin Yang 1/late SV40 factor (YY1/LSF), P50 homodimer, activating enhancer-binding protein 4 (AP4), COUP-TF-interacting protein 2 (CTIP2) and thyroid hormone receptor recruit chromatin-modifying enzymes in the form of multiprotein complexes to HIV LTR (81–86). For example,

CBF-1–induced repressive chromatin structures facilitate HIV latency in primary CD4$^+$ T cells (62). Recently, the Karn group demonstrated the role of EZH2, a histone methylase and a component of polycomb group corepressor (PcG) complexes, in inducing repressive chromatin structures during HIV latency (87). To further extend these studies, we found that CBF-1 is responsible for the recruitment of EZH2 and other chromatin-modifying enzymes of PcG complex to HIV LTR (M Tyagi, manuscript in preparation). PcG complexes carry multiple chromatin-modifying enzymes and thus are known to repress expression of cellular genes by inducing various layers of repressive epigenetic modifications involving both histones and DNA (88). The role of repressive epigenetic modifications in controlling HIV latency is quite evident by the fact that their removal or inhibition leads to the reactivation of latent proviruses (for details, please refer to recent reviews from the Margolis and Verdin labs [22,89,90]).

A critical role of various epigenetic modifications in regulating HIV replication and latency has been well established, but underlying molecular mechanisms that regulate these specific epigenetic changes at HIV LTR are not yet well defined. Thus, there is a pressing need to understand these mechanisms to use them therapeutically. There is an enormous potential for drugs that could manipulate chromatin structures at HIV LTR in regulating HIV latency. The search for small molecule drugs that could reactivate latent proviruses via converting heterochromatin into euchromatin structures at HIV LTR is a hot topic in the HIV latency field. However, these studies are complicated by lack of understanding regarding how specific epigenetic modifications influence each other and how they are regulated. Nevertheless, various strategies have been tried to reactivate latent proviruses, such as by activating transcription factors or targeting histone modifications, while at the same time incapacitating the reactivated viruses with HAART. This kind of strategy is usually known as the "shock and kill" strategy, where "shock" is given to reactivate latent proviruses, and the "kill" phase is used to inhibit reactivated proviruses via HAART treatment (91,92). See Ongoing Therapeutic Approaches for further discussion. For specific details about the role of epigenetic modifications in HIV gene expression, see recent reviews (21–23,90).

Besides histone modifications, nucleosomal structures are also modulated by SWItch/Sucrose NonFermentable (SWI/SNF) remodeling complexes. These complexes regulate the overall structural organization of nucleosomes via an ATP-dependent mechanism, and the resultant modulation of nucleosomal structures changes the accessibility of DNA to transcription factors (for recent literature, refer to [90,93–100]).

HIV Integration and Transcriptional Interference

Being a retrovirus, HIV integrates itself into the host cell genome. Extensive evidence has demonstrated that, most of the time, HIV integrates within the actively transcribing genes, primarily in their intronic regions. This is most likely because of the selective binding preference of lens epithelium-derived growth factor (LEDGF), a protein shown to be involved in HIV integration, toward transcriptionally active open chromatin structures (101–106).

After integration, one can envision that gene expression from the LTR promoter can affect and be affected by the direction and strength of the neighboring cellular promoters. If the cellular and proviral promoters are arranged in opposite directions, there is a chance that the RNAP II complexes of both promoters interfere with the expression of each other's gene and consequently could augment proviral latency establishment (107). Likewise, another scenario suggested by Peterlin's group can be envisioned: if the upstream cellular promoter is stronger than the LTR promoter, the upstream transcription complex may displace the transcription machinery from the LTR promoter and read through the proviral genome, messing up the HIV transcription (108). This transcriptional hindrance due to promoter occlusion can also lead to the generation of latent proviruses. Additionally, generated transcripts can act as antisense RNA and even as miRNA, which can further impede HIV transcription and stabilize HIV latency (21,108). Theoretically, transcriptional interference could play a major role in restricting HIV transcription during latency establishment. The removal of this kind of silencing of HIV transcription could present a big problem to eradication of latent proviruses. Fortunately, it was found that activation of the LTR promoter by NF-κB can overcome these transcriptional hindrances (21,109). Additionally, transcriptional interference does not seem widespread in transformed T-cell lines and in primary T cells, since, in both kinds of cells, most of the proviruses initiate transcription after integration (47,62). Nonetheless, transcriptional interference could play an important role during the maintenance phase of HIV latency.

The important role of cellular miRNAs has also been demonstrated in regulating HIV gene expression and latency. In particular, cellular miR-28, miR-125b, miR-150, miR-223 and miR-382, which are enriched in resting CD4$^+$ T lymphocytes, target HIV-1 mRNA and suppress its translation (110–112). A number of recent reports provide further details of miRNA-mediated regulation of HIV latency (113–122). Therefore, innovative methodologies designed to manipulate the action of involved miRNAs could be useful in purging latent proviruses.

Although the mechanisms controlling HIV latency are not yet clearly defined, it is now well established that it is a multifactorial phenomenon involving different mechanisms affecting HIV gene expression. These mechanisms include the lack of essential transcription factors, lack or defect in Tat transactivation, presence of transcriptional repressors, HIV transcriptional hindrance due to activation of the neighboring cellular promoter, miRNA-

induced restrictions, repressive epigenetic modifications involving both histones and DNA, integration of provirus into the heterochromatin area or unfavorable provirus orientation (40,41,47,62,78,79,81,108,122–132).

CELLULAR BIOLOGY OF HIV LATENCY

Latent and Persistent Viral Reservoirs

The persistent cellular or anatomical reservoirs allow slower but continuous viral replication, even under the optimal HAART regimen. On the other hand, proviruses in latent reservoirs, such as resting memory T cells, mostly remain silent without a productive lifecycle (reviewed in [21]).

Over the years, researchers have found that latency can exist in a range of anatomical sites and cell types. The most prominent ones are the CD4$^+$ T-cell subsets, primarily resting central memory T cells (T_{CM}) and transitional memory T cells (T_{TM}) (15,133,134). The predominant presence of latent proviruses in the resting memory CD4$^+$ T cells was confirmed by various groups via the establishment of *in vitro* primary T-cell–based model systems for HIV latency (reviewed in [67,135]). Memory CD4$^+$ T cells express CCR5, which makes them susceptible to CCR5-tropic HIV-1 variants dominating transmission (136–138). Normally, however, because of the presence of various blocks to the viral lifecycle, HIV is not able to infect quiescent T cells efficiently (139–142). Latently infected resting memory T cells thus appear to be generated primarily when HIV-infected actively replicating antigen-stimulated cells differentiate into long-lived resting memory T cells (19,21,143). This result is a normal physiological phenomenon that the immune system uses to mount a quick immune response if the same antigen appears in the system again. Direct infection of quiescent T cells can also occur, although inefficiently, when cells are treated with a selected set of chemokines (144,145). This treatment is suggested to prime these cells for infection without full reactivation (106,144). Additionally, induced signal transduction pathways following the binding of cytokines or HIV to its coreceptor also appear to make resting T cells permissive for HIV infection (146–148).

Other T-cell subsets, such as naive T cells and CD34$^+$ multipotent hematopoietic stem cells, have also been demonstrated to contain latent proviruses (133,149–151). These cells express CXCR4 but not CCR5 (152,153) and are therefore susceptible only to infection by X4 viruses. The matter of infection of CD34$^+$ hematopoietic progenitor cells is still debatable, since a recent study by the Siliciano group did not find the prevalence of HIV genomic DNA in the CD34$^+$ hematopoietic progenitor cells of HIV patients (154). The infection of naive T cells, which are quiescent in nature, could be explained by their transient partial activation, such as under the influence of cytokines or during thymopoiesis, but they eventually return back to the quiescent (G0) phenotype (149, 151,155). Another primary target of HIV infection and one of the major persistent HIV reservoirs are the cells belonging to the monocyte/macrophage lineage, where viruses are generally not completely silent but maintain a low level of replication (156,157). In contrast to T cells, HIV infection is not cytopathic to these cells; it even extends their lifespan and makes them more resistant to apoptosis (9,158–161).

An important anatomical site for the HIV reservoir is the central nervous system (CNS), where HIV-infected cells are continuously replenished by circulating infected monocytes. These monocytes cross the blood-brain barrier and differentiate into macrophages and microglial cells. These cells and the infected astrocytes are the main cause of CNS pathology. The blood-brain barrier, which restricts entry of cytotoxic T cells and does not allow free flow of anti-HIV drugs, also reduces the impact of immune response and HAART on CNS-localized HIV viruses (162–170). Moreover, protease and reverse transcriptase inhibitors, the main anti-HIV drugs, were found to be less effective in macrophage and microglial cells (11,171). CNS thus acts as a sanctuary site for the HIV virus and continuous viral replication, even during HAART, and it could be responsible for the evolution of drug-resistant viruses. However, significance of this viral reservoir is still debatable, since the overall share of CNS-derived viruses in persistent viremia appears to be negligible (172).

Another important HIV reservoir is in gut-associated lymphoid tissue (GALT), where 5–10 times more HIV RNA than in peripheral blood mononuclear cells can be recovered (173,174). This clearly suggests that there is an ongoing rapid HIV replication, even in patients undergoing effective HAART treatment. As a result, the gastrointestinal (GI) tract CD4$^+$ T-cell population takes much longer to revive under HAART than in peripheral blood and never seems to be restored up to the original levels (175).

Although the matter of HIV reactivation remains contentious, several studies clearly demonstrated that the major population of residual viruses in the plasma of patients undergoing successful HAART regimens arises primarily from reactivation of the latent provirus in resting CD4$^+$ T cells. The sequence analysis of the circulating viruses in HIV patients who are either undergoing successful anti-HIV therapy or have a rebound of the virus after HAART interruption demonstrated that very few HAART-selected mutations are present in the rebound viruses. This result suggests a very low ongoing viral replication during HAART. This notion is further supported by the observation that intensification of the HAART regimen, either by including another drug or replacing one of them with a more potent drug, does not lead to the reduction of either the residual viremia or the latent reservoir (176–182). Overall, most studies suggest that the source of the circulating viruses is the archival, pre-HAART virus present in the latent reservoir of resting CD4$^+$ T cells (21,183–188). These cells become activated either via antigenic stimulation or homeostatic proliferation (Figure 2

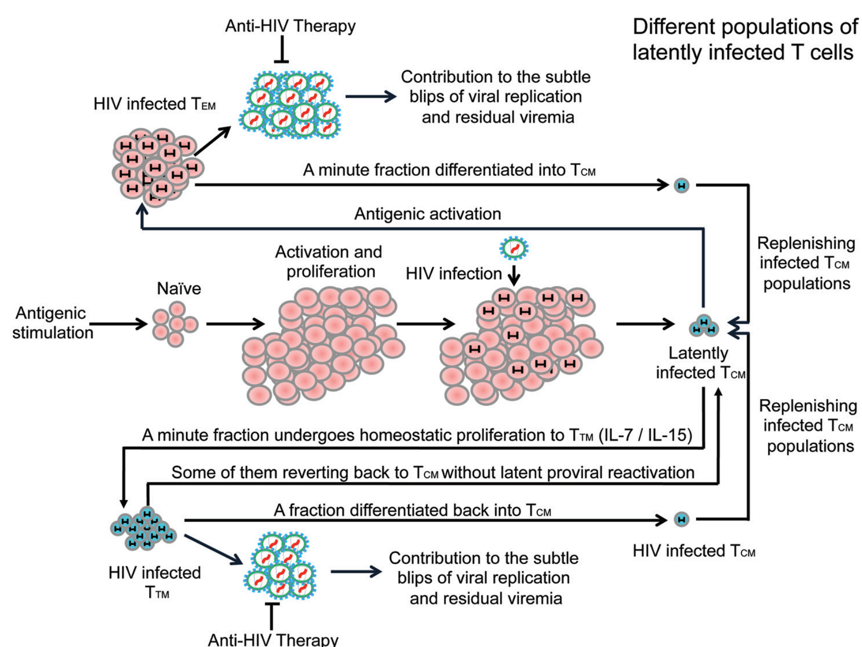

Figure 2. A schematic representation of turnover of latently infected T cells. Naive T cells get activated after antigenic stimulation and become susceptible to HIV infection. Most of infected cells die, but a minute fraction turns into TCM for the encountered antigen. Antigenic stimulation of TCM results in their activation and conversion into TEM. Their metabolically active state leads to the reactivation of integrated latent provirus that eventually contributes to the blips and viremia. Most of the T cells die off because of viral production toxicity and clearing the antigens from the system, but a tiny fraction gets differentiated into TCM specific for that antigen and replenishes the source. HIV homeostatic proliferation induced by IL-7 and IL-15 results in TCM differentiation into partially activated TTM, which support partial reactivation of latent HIV. Some of TTM revert to TCM. See details in the text.

and next section). The contribution of other reservoirs, such as infected macrophages, to residual viremia has also been documented (157,186,189–191). However, because of a comparatively shorter half-life of these cells, their significance as a persistent source of HIV is uncertain, especially in patients undergoing successful anti-HIV therapy (21,192,193).

Residual Viremia, Viral Blips and Homeostatic Proliferation

Unfortunately, latent proviruses in resting CD4$^+$ T cells do not remain silent forever. Instead, HIV patients experience transient episodes of higher viral loads, called “blips,” because of changes in the metabolic state of the cells. It has been suggested that viral blips (episodes of higher viremia) and their amplitudes depend on various factors, which include viral evolution and immunologic or drug shortcomings (for recent review, see [194]). To keep these blips in check, HIV patients have to continue taking anti-HIV drugs even when the viral levels fall below detection limits (12). As shown in Figure 2, one can anticipate that *in vivo* there could be continuous stimulation of TCM, either via antigen or homeostatic proliferation, resulting in TCM differentiation to effector memory T cells (TEM) or TTM, respectively. These events in turn lead to the reactivation of latent proviruses, either fully or partially. However, because of the continuous presence of anti-HIV drugs in the system, viremia reverts back to the controlled range. Most of the differentiated cells die off because of cytopathic effects of the virus and host immune responses, but a minute fraction of these differentiated cells revert back to the TCM phenotype with new viral sequences, albeit with limited mutations because of restricted rounds of viral replication. This result suggests that latent reservoir in TCM cells keeps replenishing itself with updated viral sequences (18,195). The same conclusion was also drawn from simian immunodeficiency virus (SIV) studies that showed that ongoing rate of viral replication defines SIV DNA sequence turnover in resting CD4$^+$ T cells (196). In sanctuary sites such as GALT, where anti-HIV drugs are found to be comparatively less effective, newly produced viruses could further infect neighboring TCM cells activated after antigenic stimulation or homeostatic proliferation. This result could be one of the factors contributing to the higher ongoing viral replication in GALT (173,174,194).

After antigenic stimulation or homeostatic proliferation, TCM cells lose CCR7, a homing receptor for secondary lymphoid organs (197,198). As a result, a fraction of these partially activated HIV-infected cells could leave the secondary lymphoid organs and move to the peripheral bloodstream, providing an HIV source for infection of partially activated TTM cells, thus contributing to the generation of viral blips (194,199,200). Phylogenetic analyses of blipped viruses also suggest that most of them were generated after reactivation of latently infected T cells (187,188). Taken together, these results suggest that the escape of viruses from HAART can occur during TCM to TTM conversion, especially in sanctuary sites such as GALT.

New Tools to Study HIV Latency

The failure of current therapy to eradicate HIV appears to be due to the creation of stable reservoirs of latently infected cells and/or pools of slowly replicating viruses in pharmacologically privileged sites that can be reactivated and produce a rebound of the virus after termination of successful antiviral treatment (13,24). Novel technologies are required to eradicate the HIV virus and

cure HIV infection. To develop such therapeutic interventions, we need to understand the precise molecular mechanisms responsible for the establishment and maintenance of postintegration latency and for subsequent reactivation of the latent proviruses. Because of the rare availability of latently infected cells in patients (~1 in 10^6), it is almost impossible to isolate them in sufficient numbers to be able to do biochemical studies (143). In addition, the lack of any specific marker on the surface of latently infected cells further complicates their isolation from noninfected counterparts (15,134, 201). Consequently, most of the biochemical studies concerning the molecular aspect of HIV latency until now were done by using latently infected transformed cell lines (47,77,202–205). However, the quiescent phenotype of the latently infected $CD4^+$ T cells found *in vivo* is substantially different from the replicating and constitutively activated transformed T cells. Many laboratories have therefore come up with several informative primary cell–based model systems (46,144,151,206–214).

The Siliciano laboratory used the antiapoptotic protein, B-cell lymphoma 2 (Bcl-2) to help maintain primary $CD4^+$ T cells longer *in vitro* (215,216). Although promising, this strategy raises concerns that cell transformation by overexpression of Bcl-2 might alter the cellular physiology. Nevertheless, using this system, they performed several high-throughput screenings and identified compounds that activate latent provirus, including 5-hydroxynaphthoquinone and disulfiram (216,217). In addition, via this system, they also confirmed the role of transcriptional interference in HIV-1 latency (127).

The model introduced by Planelles laboratory involves T-cell differentiation into nonpolarized cells (NP) and infection of these cells with Env-defective HIV-1 (46,78). This model has been useful in defining several signaling pathways required for HIV reactivation and also in establishing the role of DNA methylation in supporting HIV latency establishment (46,78). Using this model, they also demonstrated recently that homeostatic proliferation of TCM might not be able to effectively reactivate latent HIV proviruses (218).

Another informative model comes from the Karn lab (62). The unique ability of this latency model to provide large quantities of pure latently infected primary $CD4^+$ T cells makes it the most suitable model system for various biochemical analyses that require many cells, such as protein analysis by Western blot, and particularly to study the role of epigenetic modifications on HIV gene expression and latency. The only drawback of this model is the time needed to establish the system, since the provirus requires 5–7 wks to become latent. Using this model, we demonstrated the role of repressive chromatin modifications in restricting HIV gene expression during latency establishment in primary T cells (62). Furthermore, we also demonstrated that transcription of latent proviruses in primary T cells is restricted not only at the initiation but also at the elongation phase (62,67). These results further strengthen the notion that, to reactivate latent proviruses, various viral lifecycle steps have to be targeted simultaneously.

Although every model of HIV latency has its own advantages and limitations, these models provide an opportunity to perform HIV latency studies in physiologically relevant setups (for detailed reviews of particularities of every model, see [67,135,219]). These models open up new avenues to search for therapeutic approaches aimed at fighting HIV latency.

Ongoing Therapeutic Approaches

An approach to tackle HIV latency is to make anti-HIV therapy eradicate HIV completely. In that approach, anti-HIV therapy needs to inhibit the establishment of latent proviruses, and it should also either reactivate or permanently silence every latent provirus. Because a single remaining virus could repopulate the reservoirs, successful anti-HIV therapy has to eradicate every single infected cell. This step is a huge challenge. As mentioned above and depicted in Figure 2, the intensification of HAART was not able to reduce the residual viremia in patients, largely because of the intrinsic capability of latently infected resting memory T cells to replenish their population (176,220,221). Consequently, researchers have come up with an alternative approach involving reactivation of latent proviruses to purge latent viral pools (12,222). As mentioned earlier, this "shock and kill" strategy involves reactivation of latent proviruses via a shock stimulation and killing of infected cells by viral cytopathic effects and cytolytic immune responses, while keeping new infections under control by HAART (91,92). For this strategy to be effective in purging latent proviral pools, the shock phase needs to reactivate and inactivate all latent proviruses, and all infected cells have to be killed. Additionally, target specificity of the shock stimulus is another critical factor that has to be taken into serious consideration.

Several activation strategies and various stimulants have already been tried *in vitro* and in clinical trials, such as interleukin (IL)-2, IL-7, anti-CD3 antibodies, inhibitors of histone deacetylases and agonists of protein kinase C (22,25,223–231). These strategies obtained limited success but proved the concept. Because HIV latency involves various mechanisms, a cocktail of several drugs along with HAART has to be used to reactivate latent proviruses, which have stuck at different stages of their lifecycle. In addition, immune responses of HIV-infected patients may be inadequate to eliminate reactivated HIV-infected T cells (232); hence, treatments stimulating CTL response or targeting toxins to infected cells should be added to the therapy regimens. Interestingly, IL-7 was found to boost antiviral CTL response against SIV in rhesus macaques (233).

However, a lesson can be learned from a recent case, where an HIV patient (famously known as "the Berlin patient") had received a bone marrow transfer from an individual carrying a mutation in the chemokine receptor CCR5, the main coreceptor for HIV. This bone mar-

row transfer has allowed him to control the rebound HIV virus without taking anti-HIV drugs, for about 4 years now (234,235). Thus, this HIV patient appears to be cured from AIDS, despite maintaining a certain number of infected cells. This approach suggests the possibility of a functional cure, where a patient could maintain a manageable viral pool without AIDS, in the absence of antiretroviral therapy. In that regard, some groups have started the preliminary work needed to use gene therapy approaches to delete or mutate CCR5 on $CD34^+$ hematopoietic progenitor cells of HIV patients, either *in situ* or *in vitro*, and later put the cells back into the patients. As a result of such treatment, all the lineages originating from these cells will be resistant to HIV because of the lack of functional CCR5 on their surface (236–238). Consequently, the loss of $CD4^+$ cells because of HIV infection could be controlled, and a healthy, HIV-resistant immune system could be reconstituted. Although we have a successful example of this approach and translation of this concept seems to be quite straightforward, it carries the limitations intrinsic to gene therapy. Moreover, additional care has to be taken to prevent these approaches from altering the homeostatic balance of the host immune system, since these modifications are expected to enhance the availability of free chemokines in the system. Of note, these CCR5-modified T cells remain susceptible to CXCR4-tropic strains of HIV-1; the same approach can also be used to remove the CXCR4 receptor (239). Overall, these findings demonstrate that the goal of curing AIDS is feasible and may be within our reach.

CONCLUSIONS

Because of a very slow decay rate of latently infected $CD4^+$ T cells with a half-life of around 44 months, and considering the occasional blips of proviral reactivation that further facilitate new rounds of viral infection, it is now accepted that complete eradication might not be possible with current anti-HIV therapies (13). Careful analysis of cells harboring replication-competent latent proviruses appears to show a rare but highly stable population that is resistant, even to intensive HAART regimens (15,176,240). These infected cells can undergo homeostatic proliferation, a process further contributing to the maintenance of the latent reservoirs (133). Thus, in the present scenario, latently infected resting $CD4^+$ T cells seem to be the major obstacle in eradicating HIV proviruses.

To target latent cells, a "shock and kill" strategy appears to be promising. The fact that HIV latency is a multifactorial phenomenon involving various mechanisms suggests that multifaceted approaches are required to activate latent proviruses, where different steps of the viral lifecycle are targeted simultaneously by a cocktail of drugs, along with the HAART regimen to keep new infections in check. The drugs should also be able to target viruses present in sanctuary sites such as CNS and GALT. Additionally, anti-HIV CTL responses should be boosted or other means of killing reactivated HIV-infected cells should be introduced. Before this can be accomplished, however, we have to better understand the underlying molecular mechanisms that support the existence and maintenance of latent proviruses and latently infected T cells. Consequently, complete eradication of HIV appears to be quite distant, but prospects for a functional cure seem more in the realistic range.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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