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Proviral Latency, Persistent Human Immunodeficiency Virus Infection, and the Development of Latency Reversing Agents

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Quiescent proviral genomes that persist during human immunodeficiency virus type 1 (HIV-1) infection despite effective antiretroviral therapy (ART) can fuel rebound viremia after ART interruption and is a central obstacle to the cure of HIV infection. The induction of quiescent provirus is the goal of a new class of potential therapeutics, latency reversing agents (LRAs). The discovery, development, and testing of HIV LRAs is a key part of current efforts to develop latency reversal and viral clearance strategies to eradicate established HIV infection. The development of LRAs is burdened by many uncertainties that make drug discovery difficult. The biology of HIV latency is complex and incompletely understood. Potential targets for LRAs are host factors, and the potential toxicities of host-directed therapies in individuals that are otherwise clinically stable may be unacceptable. Assays to measure latency reversal and assess the effectiveness of potential therapeutics are complex and incompletely validated. Despite these obstacles, novel LRAs are under development and beginning to enter combination testing with viral clearance strategies. It is hoped that the steady advances in the development of LRAs now being paired with emerging immunotherapeutics to clear persistently infected cells will soon allow measurable clinical advances toward an HIV cure.

Keywords. HIV; Latency; Latency Reversing Agents.

HUMAN IMMUNODEFICIENCY VIRUS CURE AND THE CHALLENGE OF PERSISTENT HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The pandemic of human immunodeficiency virus type 1 (HIV-1) infection has swept across societies with devastating effect over the last decades. In response, the scientific community has met the emergence of this zoonosis with remarkable force and innovation. An unprecedented collaboration with the communities affected by HIV and the pharmaceutical industry has led to modern antiretroviral therapy (ART) capable of conferring lifelong control of disease and stable health [1].

The implementation of ART across the world appears to have begun to blunt the spread of new cases of HIV infection, and there is evidence that the expansion of ART as prevention may have even greater impact [2]. However, HIV infection can persist for decades despite clinically successful ART, due at least to the persistence of latent HIV infection within long-lived resting memory CD4+ T cells [3–6]. These latent reservoirs are impervious to ART and unaffected by the immune system. Therefore, most agree that treatment approaches that allow HIV eradication or drug-free remission would add a critical element to the efforts to resolve the HIV pandemic. Despite the best efforts of preventive and vaccine strategies, it is likely that there will be large populations of HIV-infected people across the world for decades to come. Focused strategies that could eradicate established infection could relieve the societal and personal burden of decades of chronic medical care and suppressive, lifelong antiviral therapy for many of these people.

Latent infection is established within days of infection and, in fact, appears to be established in a small fraction of the very first cells that become infected [7]. Latency decays very slowly with a half-life of 40–44 months, necessitating lifelong ART to suppress reignition of infection [8]. Although early administration of ART limits HIV replication and is associated with lower total and integrated HIV DNA and lower frequency of latent infection [9, 10], this is a clinically challenging maneuver to implement broadly.

Therefore a detailed understanding of the phenomena that allows HIV infection to persist for years despite potent ART is needed to guide the development of strategies that might someday safely and efficiently clear HIV infection. Proviral latency, the persistence of quiescent but replication-competent provirus in resting CD4+ T lymphocytes and to a lesser extent in other cell populations, is a central problem that must be addressed by any effort to clear persistent HIV infection [11].

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Host cell molecular mechanisms maintain the quiescence of HIV gene expression in infected resting CD4+ T lymphocytes and could therefore serve as therapeutic targets for disrupting latency. The recognition of this latent reservoir and its cellular regulation led initially to attempts to target persistent HIV infection through latency reversal using small molecules capable of inducing expression of the HIV provirus [12, 13]

Although several groups have demonstrated successful reversal of latency in vivo, as measured by increases in cell-associated HIV RNA expression, these successes have not been marked by a parallel and persistent reduction in replication-competent HIV or other measures of persistent infection [14–17]. This has led to the current clearance strategy model, invoking the use of latency reversing agents (LRAs) in parallel with immune-mediated clearance of infected cells.

PERSISTENCE PART I: INCOMPETENT RESERVOIRS OF FOSSILIZED HUMAN IMMUNODEFICIENCY VIRUS

As HIV enters a new target cell, it must contend with a hostile environment designed to thwart its replication. The virion will carry APOBEC enzymes, most notably APOBEC3G, packaged within the virion by the producer cell. These RNA-editing enzymes, an innate immune defense against RNA viruses, can antagonize HIV replication through cytidine deamination, resulting in hypermutation of the viral DNA genome, as well as interference with HIV reverse transcription and integration [18]. Given the additional effects of the error rates of the HIV reverse transcriptase and the degrading effects of host cell nucleases, the vast majority of HIV reverse-transcriptase products are incomplete, are circularized, fail to integrate, or are hypermutated. This predominance of HIV nucleic acid that persists in cells that were infected but are now unable to produce replication-competent HIV poses a challenge for efforts both to measure the frequency of persistent HIV infection and to assess the effects of LRAs that are under development [19, 20].

Studies have shown the establishment of HIV DNA within cells early in the course of infection despite initiation of ART during acute infection and that the burden of DNA falls and then plateaus during prolonged ART. However, such HIV DNA integrants are only a marker that the cell has been infected and that reverse transcription and integration have occurred. However, again because of the potency and efficacy of innate immunity enforced by the ApoBEC system, the vast majority of these HIV DNA genomes encode lethal mutations or deletions, and HIV DNA forms are detected approximately 300-fold more often than the frequency of recovery of replication-competent HIV [19]. Therefore, although HIV DNA levels can provide a gross overall comparative measure of past infection events in a pool of cells, such measures are blunt tools for the assessment of HIV clearance strategies after interventions. Depletions of HIV DNA have been seen following general T-cell ablative strategies, such as bone marrow transplantation [21, 22], that have depleted total T-cell populations regardless of their carriage of either defective HIV genomes or truly latent HIV. However, should future strategies be developed that selectively clear cells harboring cells carrying functional viral genomes, HIV DNA assays will persistently detect innocuous, defective HIV DNA templates.

Nevertheless, quantitative polymerase chain reaction (qPCR) measures of total or integrated HIV DNA are the most commonly used, straightforward, and tractable method to quantify durable HIV infection [23]. HIV DNA is primarily detected in 2 subsets of memory CD4+ T cells, central memory CD4+ T cells (T_{CM}) and transitional memory CD4+ T cells (T_{TM}) [24]. Transitional memory CD4+ T cells are characterized by the expression of CD27 but lack expression of the lymph node homing receptor CCR7, whereas T_{CM} CD4+ T cells express both CD27 and CCR7. However, when replication-competent HIV, rather than HIV DNA, is rigorously assessed, only the central memory CD4+ T-cell compartment has been shown to be a durable, sizable reservoir of latent but persistent HIV infection [11].

Potential foci of durable, latent infection within the T-cell compartment have been recently identified. Pallikkuth et al found that within circulating T_{CM}, the CXCR5+ subset designated peripheral T follicular helper (pTfh) cells preferentially harbor HIV DNA in virologically suppressed HIV-infected patients [25]. This CXCR5+ subset of pTfh cells appears to be highly susceptible to HIV. In HIV-infected participants, pTfh cells can be induced to express HIV p24 *Gag* protein, suggesting that frequencies of inducible HIV p24 in pTfh cells could be used to monitor HIV reservoirs in blood. However, Banga et al found higher levels of cell-associated HIV RNA in lymph node T_{fh} (LN-T_{fh}) cells than in T_{CM} subsets but detected no antigen-positive T_{fh} cells after 3 years of ART [26]. Therefore this infected cell population may decay with time, or as these effector T_{fh} cells may transition to durable circulating memory cells, such cells may become a component of the viral reservoir. The true persistence of latent infection within effector T_{fh} cells requires further definition.

Similarly, in T-cell populations not distinguished by their activation status, the immune checkpoint markers LAG-3, PD-1, and TIGIT have been found to be preferentially present on cells that contain HIV DNA integrants and can express HIV RNA [27]. The stable persistence of these populations over time on ART remains to be demonstrated, as does the persistence of quiescent but replication-competent HIV within them. Further studies must define whether or not such cells comprise a population of cells that persistently carry innocuous, defective HIV DNA templates or latent viral genomes capable of sparking rebound viremia.

PERSISTENCE PART II: CELLS THAT CARRY LATENT BUT REPLICATION-COMPETENT HUMAN IMMUNODEFICIENCY VIRUS

Persistence of virus in HIV-infected patients receiving potent antiretroviral therapy was conclusively demonstrated in 1997,

when rare, integrated, replication-competent HIV was recovered from resting CD4 memory T cells [3–5]. To date this reservoir remains the most widely studied and best understood cause of viral persistence. The stability and slow decay of the reservoir has been long documented, and findings were recently precisely reproduced in a cohort studied 10 years later on more modern ART [8, 28].

The molecular mechanisms that allow the establishment of persistent but quiescent proviral infection are incompletely defined. It has been long been assumed that resting T-cell infection occurs as an activated T cell is in the process of reverting to a resting state. While reverting to a resting phase, T cells can support the early phases of virus infection, such as reverse transcription, and integration, but later steps are blunted once the cell reaches a resting state [29]. Other studies have clearly shown the ability of resting cells to be directly infected by HIV, albeit inefficiently [30]. It has also been suggested that the effects of HIV infection on other cells may induce cytokine signaling that renders resting T cells permissive for infection [31]. These mechanisms are not mutually exclusive, and indeed there are likely to be multiple pathways to the latent state. Further, although the majority of latent infections in patients who have received long-term suppressive ART exists in resting CD4 T cells, it still must be said that persistent, durable, but truly latent infection in other potential cellular reservoirs such as myeloid cells has not been completely ruled out [32].

But more recently another mechanism that may contribute to the stability of the population of cells latently infected with replication-competent proviruses has been demonstrated—that of cellular proliferation. Identical proviral sequences have been found in HIV-infected patients on long-term ART integrated at the same position in the host genome in multiple cells, consistent with the derivation of these infected cells from a progenitor clone through cellular proliferation [33, 34]. However, the replication competence of these proliferating clones remains in question. One study found that all of the 75 integrated genomes that were fully sequenced contained lethal mutations or deletions and were replication incompetent [35]. However, this finding cannot be taken as definitive, as even a tiny fraction of proliferating but replication-competent HIV genomes could contribute substantially to viral persistence. Indeed an illustrative case has already been painstakingly documented to refute this claim [36]. This issue is discussed in depth elsewhere in this supplement (Mullens et al).

Currently it is unclear whether the latent pool of infected T_{CM} derives its great stability from the longevity of the latent state itself, from proliferative forces that maintain it, or from a balance of both effects. Regardless, once durable and suppressive ART is in force, persistent HIV infection decays slowly over time, as discussed above [8, 28], suggesting that the homeostasis that is achieved between effects that deplete the latent reservoir and those that preserve it are in mild negative balance. This leads to the hypothesis that LRA strategies designed to make persistently infected cells vulnerable to clearance are required to speed the decay of the latent infection sufficiently to lead to its eradication.

LATENCY REVERSAL: THE RATIONALE FOR LATENCY REVERSING AGENTS

The precise accounting that adds up to the persistence of replication-competent HIV capable of fueling rebound viremia following the interruption of ART is hotly debated. Factors that may drive viral persistence above and beyond the longevity of quiescent, integrated, replication-competent HIV provirus include only the following:

1. The long-term persistence of an unknown, long-lived cell population that chronically expresses replication-competent HIV;
2. Cryptic, ongoing rounds of infection of cells—despite the presence of fully suppressive, clinically successful ART—resulting in the de novo integration with fully replication-competent provirus; and
3. Homeostatic or dysregulated proliferation of infected cells encoding quiescent, integrated, replication-competent HIV provirus.

Without extensively reiterating the controversies in the literature, it can be said that the possibility of durable reservoirs of active HIV production [37] has thus far been most strongly rebuffed [38–40]. The potential presence of cryptic replication and ART sanctuaries [41] has been challenged by methodological report from the same laboratory and other findings [42, 43]. A further report on cryptic replication has recently captured attention [44], but more recent rebuttals are now under peer review. However, the phenomenon of proliferation of cells encoding HIV proviruses has been clearly demonstrated [33, 34], and although the proportion of these genomes that are replication competent requires further definition [35, 36], it seems most likely that cellular proliferation may contribute to persistent proviral infection.

Regardless of the final adjudication of these controversies, given the slow decay of persistent HIV infection on current ART [8, 28], it would seem logical that persistent HIV infection could be extinguished by the development and implementation of sufficiently safe and effective LRAs capable of enforcing HIV antigen expression within the latent HIV reservoir such that these viral sanctuaries are vulnerable to clearance by antiviral effector mechanisms, be they natural or engineered. The development of such LRAs and their careful pairing with appropriate and effective viral clearance strategies defines the core challenges of current HIV cure research.

LRAs have been conceptualized as agents that specifically target host cell mechanisms that either (1) restrain proviral expression and allow or enforce proviral latency or (2) establish or

enforce latency as they are required for proviral expression and exist in relative deficiency or limiting concentration in latently infected cells. Because T_{CM} that do not display significant cell surface levels of cell activation markers ("resting CD4+ T cells") have been defined as the most predominant and most durable reservoir of latent HIV infection, it is within this cellular milieu that the relevant host cell mechanisms to be targeted by LRAs should be first defined.

Epigenetic silencing of HIV transcription is thus far the best-validated target for development of clinically testable LRAs. The "histone code" hypothesis holds that combinations of distinct modifications occurring at particular sites on the histone tail direct which proteins are capable of interacting with histone–DNA complexes and determine gene activity [45, 46]. Already >50 enzymes are known that selectively modify the histone tail, thus providing the means to make a combinatorial histone code. Multiple signaling pathways result in enzymatic covalent modifications (eg, acetylation, phosphorylation, methylation) of specific amino acids in histone tail domains. These modifications do not simply make chromatin more or less accessible but inscribe biophysical marks on gene regions, signals for the ordered recruitment of complexes of regulatory factors that up- or downregulate gene expression. However, it must be remembered that host enzymes that regulate the modification of histone proteins are also capable of modifying the activity of other cell proteins, so the cellular phenotypes that result after the activity of such enzymes is altered may be exceedingly complex. For example, histone acetylases act to allow the transcriptional machinery access to the DNA template and compete with histone deacetylases that blunt transcription by reducing accessibility of DNA templates. But the acetylation or deacetylation of other host proteins can affect cytoskeletal structure or transcription factor complex formation and thus may affect other steps of proviral expression or virion production [47].

The role of histone deacetylases (HDACs) in the maintenance of stable, nonproductive HIV infection has been well documented [48]. Consistent with a major role for HDACs in establishing HIV latency, many drugs that inhibit HDAC activity, such as trichostatin A and valproic acid [49, 50], are effective inducers of HIV transcription in latently infected cells.

Histone deacetylase inhibitors (HDAC*i*s) have been the most extensively tested in the clinic for their activity as HIV LRAs. The potent HDAC inhibitor vorinostat (VOR) induces HIV chromatin acetylation and promoter expression in cell lines and elicits virus production ex vivo from the resting $CD4^{+}$ T cells of HIV-infected patients on suppressive ART. Importantly, this effect is achieved without cellular activation, upregulation of HIV coreceptors, or de novo HIV infection [51, 52]. Direct proof of concept of latency reversal has also been achieved in clinical studies, where a significant increase in cell-associated HIV RNA production was observed following in vivo administration of VOR to ART-suppressed patients [14]. This finding has been confirmed in several other studies using VOR as well as the HDAC*i*s panobinostat and romidepsin as LRAs [14–17].

Another epigenetic LRA target that is under investigation is the methylation of histones [53–58]. Although DNA methylation does not appear to play a critical role in HIV silencing in vivo [59], histone methyltransferase inhibitors (HMT*i*s) and HDAC*i*s are reported to synergistically induce the expression of latent HIV, but studies have largely been performed in cell lines. These studies [60, 61] suggest that different levels of latency exist: "inducible latency," in which provirus is largely restricted by deacetylated histones, and "locked latency," in which additional modification such as histone and DNA methylation further restrict proviral expression. Histone deacetylase inhibition alone may not be sufficient to disrupt locked latency. In a primary resting T-cell model of HIV latency, a potent and selective EZH2/EZH1 (enhancer of zeste 2 Polycomb repressive complex 2 subunit 2 or 1) inhibitor, GSK343, reduced histone 3 trimethylation at lysine 27 (H3K27) at the HIV provirus in resting cells. Remarkably, this epigenetic change was not associated with increased proviral expression in latently infected resting cells. However, following the reduction in H3K27 at the HIV long terminal repeat, the expression of the provirus appeared sensitized to subsequent exposure of the HDACi VOR, and both HIV gag RNA and HIV p24 antigen production was induced up to 2.5-fold greater by combined exposure than by VOR alone. It remains to be proven that such true mechanistic synergy in the reversal of HIV latency can be achieved in human studies by the combination of HMT*i*s and HDAC*i*s.

The bromodomain and extraterminal domain (BET) family of proteins has also emerged as a potential target of LRAs, although mechanisms by which BET inhibitors act to induce HIV transcriptional reactivation may be multimodal. BET inhibitors, in development in the context of oncology and more recently immune inflammatory disease [62, 63], serve to block the ability of BET proteins to recruit and form multiprotein complexes with many roles in host gene and viral expression. BET inhibitors promote chromatin reorganization by inducing the expression of histone acetytransferases, HDACs, and histone demethylases, while suppressing histone methyltransferases and multiple T-cell activation genes. Such inhibitors may also influence the activity of the HIV Tat transactivator protein [64]. Such agents have been examined in animal models but are yet to undergo testing in HIV-infected people.

Beyond epigenetic targets of LRAs, small molecules or signaling molecules that alter metabolism of resting CD4 cells may be needed to effectively reverse HIV [65] latency. Because resting cells express relatively low levels of the coactivating factors NF-κB or NFAT, their induction may allow resumption of transcription by latent HIV proviruses [65, 66]. The critical viral transactivator, Tat, recruits a transcription complex that contains novel components, including the transcription elongation factor P-TEFb [67, 68], and upregulation of the level of

the P-TEFb component CycT1 [69] or release of the P-TEFb complex from its regulatory complex may allow effective latency reversal [70]. Agents that act as protein kinase C agonists can mediate such cellular alterations. These were among the first LRAs proposed, when Dean Hamer first promulgated the "shock and kill" hypothesis in 2004 [12]. Recently, the use of a novel pathway to induce NFkB signaling has been proposed [71]. Although there have been several studies reporting the LRA activity of protein kinase C agonists, both alone and in combination with other LRAs, few animal model studies have been reported, and a single human study was thwarted because effective levels of bryostatin could not be achieved [72].

Other molecular mechanisms that establish or enforce HIV latency have been reported. These include impaired HIV mRNA export in resting T cells [73], miRNAs that may impede HIV mRNA expression or translation, transcriptional interference mediated by active upstream host promoters, and limiting amounts of other cellular kinases that may be critical to HIV transcriptional expression and induction [74]. However, therapeutics to target these cellular events and serve as LRAs have not yet been defined.

Finally, LRAs whose mechanism of action is less clear have been reported. Toll-like receptor agonists [75, 76], thought to reverse latency in vivo through cellular signaling, are under investigation. Several high-throughput screens have been performed and reported in industry research programs and academic collaborations. Some have yielded potential LRAs of either defined or unknown mechanisms of action, but none have yet been fully validated and are ready to enter human clinical studies.

LATENCY REVERSING AGENTS: DEVELOPMENTAL CHALLENGES

The development and testing of HIV LRAs is fraught with several unique challenges. The process of drug discovery involves the identification of a therapeutic target, followed by the synthesis, characterization, screening, and assessment of lead compounds that act on this target. Therapeutic advancement of LRAs is daunting because (1) few targets are well-validated, (2) effective LRA therapy may require agents with activity against multiple targets, (3) screening criteria that define effective in vivo activity are incompletely understood, and (4) assays to study and validate latency reversal in vivo are incompletely developed.

As discussed in the section above, there are several promising targets against which LRAs may be developed. However, because latency reversal is not a clinical or therapeutic end in and of itself, truly effective LRAs may only be defined when they can by themselves also induce measureable clearance of persistent HIV infection or, more likely, when they can be appropriately paired with viral clearance strategies that result in depletion of latency. As an intermediate step, it is rational to define LRAs as agents that can reverse latency as defined by upregulation of HIV expression in the best available model of in vitro latency, followed by validation of this activity in vivo in an animal model of HIV latency such as the HIV-infected humanized BLT mouse or the simian immunodeficiency virus–infected macaque. Ultimately, LRAs must be validated in human studies.

Initially, the reversal of latency was defined by upregulation of HIV expression in chronically infected T-cell lines such as ACH2 and U1 [77, 78]. However, these early models displayed restricted HIV expression due to defects in the Tat/TAR axis and were later replaced by improved models in Jurkat CD4$^+$ T cells such as JLat and J89 [79–81].

Further, and most critically, the many mechanisms that restrict HIV gene expression appear to act largely independently. This was elegantly illustrated in the work of the Verdin laboratory [80, 81], wherein different clonal isolates of the Jurkat CD4$^+$ T-cell line model of latency were examined. Infected with the same HIV laboratory clone but with viral integrants in various genomic sites, these models of HIV latency presented a spectrum of responsiveness or lack thereof to different signals such as HDAC inhibition or the viral activator Tat.

Seeking to better represent the biology of latent proviral infection in an in vitro model system, a variety of primary cell models have been developed. As reviewed by Yang [82], a variety of methods for in vitro infection of primary CD4$^+$ T cells allow infected cells to return to the resting state. These models allow for the laboratory study of primary, untransformed cells that have been infected in a polyclonal fashion by a variety of viral clones.

Seeking to assess the responsiveness of a number of these primary cell models of HIV latency to a variety of pathways and signals known to disrupt HIV latency, Spina, Planelles, and colleagues in the Martin Delaney CARE consortium performed a comprehensive comparison of 5 primary cell models and 4 J-Lat clones to the responses obtained in quantitative viral outgrowth assays in resting CD4$^+$ T cells obtained from aviremic, ART-treated, HIV-positive donors [83]. A panel of 13 stimuli known to reactivate HIV by defined mechanisms of action was selected. Disappointingly, no single in vitro cell model alone reflected precisely the ex vivo response characteristics of latently infected T cells derived from HIV-positive individuals. Rather, it seemed that the diversity of the biology of latency could only be encompassed by a diverse set of model systems. In a follow-up study, the role of integration site was analyzed across 5 primary cell models, and similarly, although the site of proviral integration appeared to contribute to HIV silencing, the location of a given proviral integrant did not by itself determine latency phenotype [84]. In summary, although the use of primary cell models of latency may be useful to better reflect the response of latent HIV to putative LRAs, there is currently no single in vitro model that accurately and comprehensively reflects the biological diversity of HIV latency.

The complexity of the biology of HIV latency leads to a corollary challenge in the development of LRAs. Although the mechanisms that establish HIV latency and maintain proviral quiescence within the many biologically diverse cellular milieu that comprise the latent HIV reservoir are not fully understood, it is clear that effective reversal of latency will be a complex endeavor. Although the transcriptional activity of an individual provirus, in vivo or in any model system, is typically assessed in a snapshot of time, the regulation of HIV gene expression is complex and dynamic. A cell that appears to be latently infected, lacking viral expression at one moment, may be active below the threshold of detection of the assay being used or may become active at a later moment in time. Indeed, the "noise" of basal HIV promoter expression and stochastic fluctuations in this level of expression have been elegantly described [85, 86]. Therefore, because of the dynamic state of the latent reservoir, latency reversal strategies should be evaluated in complex systems that can assess changes and responses in cells over time and following multiple rounds of single or combinatorial stimuli. Obviously, the number of possible conditions and combinations may be too great to allow the experimental assessment of every possibility, and typical high-throughput screening approaches may have to give way to rationally designed screens, informed by our current understanding of mechanisms of cell and viral regulation.

Given the challenges of the available model systems, methods to allow the direct assessment of LRA activity in peripheral blood cells donated by ART-suppressed, HIV-infected people have been used. Archin et al first validated the activity of VOR by measuring changes in cell-associated unspliced HIV RNA message in multiple pools of circulating, resting $CD4^+$ T cells. Multiple other assays of cell-associated HIV RNA have since been promulgated [87–89]. Comparison of these assays is an ongoing effort. Although such assays provide definitive proof of the induction of HIV transcription, at least in the cell population used in the assay, they are resource intensive and not suitable for high-throughput testing.

However, as reemphasized by the work of Ho and colleagues, the majority of integrated HIV genomes within the latent reservoir fail to express HIV RNA that can lead to virion production [20]. Therefore, because the induction of HIV RNA expression does not ensure that HIV proteins are properly translated and presented as viral antigens that might be recognized and cleared, novel assays have recently been presented that seek to detect cells presenting HIV proteins or antigen [90]. The challenges of measuring the frequency of latent, persistent HIV infection are discussed in greater depth elsewhere in this supplement (T. Henrich, S. Deeks, this issue).

Finally, our group has developed latency clearance assays using HIV-positive participants' autologous CD4+ cells, viral isolates, and effector cells to demonstrate that LRAs act to induce sufficient viral antigen to allow for clearance of infection by effector mechanisms and to simultaneously demonstrate—at least in vitro—that effector mechanisms can operate effectively in the presence of LRAs [91, 92]. Looking forward, the effect of LRAs on immune effector function will be an important metric to consider as latency reversal and viral clearance strategies are paired in animal model and human clinical testing. Already, groups have reported disparate effects of HDACis, for example, on the immune response, likely because of methodological differences [91, 93, 94]. These effects will have to be carefully considered as LRA development moves forward.

CONCLUSIONS

The difficult work of attacking the latent reservoir of persistent HIV infection has begun. Much is to be learned about the biology of HIV latency, but numerous promising directions for the development of LRAs have already emerged from our current, imperfect knowledge of HIV persistence. An array of novel assays has been developed and implemented in the past several years to allow the discovery and testing of LRAs, and improvements in these tools will greatly accelerate the development of effective therapeutics. As both preclinical and clinical studies advance and tools and model systems are improved, progress should accelerate toward the goal of pairing safe and effective LRAs with immunotherapeutic strategies to clear persistent infection. It is a goal worthy of the effort that will be needed.

Notes

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