Cell Host & Microbe
Review

The Biology of the HIV-1 Latent Reservoir and Implications for Cure Strategies

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Antiretroviral therapy (ART) inhibits HIV replication but is not curative. During ART, the integrated HIV genome persists indefinitely within CD4$^+$ T cells and perhaps other cells. Here, we describe the mechanisms thought to contribute to its persistence during treatment and highlight findings from numerous recent studies describing the importance of cell proliferation in that process. Continued progress elucidating the biology will enhance our ability to develop effective curative interventions.

Although antiretroviral therapy (ART) can durably suppress HIV replication indefinitely, it is not curative and must be taken for life. Because of various challenges, many people are unable to achieve long-term viral suppression (Ndung'u et al., 2019). Despite a massive global investment to provide ART, only about half of the world's HIV-positive population is now on effective ART. Curing HIV is now a global priority.

As a retrovirus, HIV-1 integrates its proviral genome into the host genome of its target cells. In the absence of ART, HIV-1 preferentially infects activated CD4$^+$ T cells, most of which die quickly. A small proportion of these infected cells exist in a long-term resting state in which the integrated replication-competent viral genome persists indefinitely. These cells, called the latent reservoir, decay very slowly, with a half-life of approximately 44 months, implying that treatment will never be curative (Crooks et al., 2015; Finzi et al., 1999).

This conceptual model for HIV-1 persistence during ART was established in the mid-1990s (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997). Until recently, it was assumed that quiescent cells harboring an intact genome persisted indefinitely, presumably maintained by their slow turnover. With the emergence of advanced single-cell methodologies and next-generation sequencing capacities, it is now clear that the reservoir is far more dynamic, with multiple factors contributing to its maintenance.

In this Review, we discuss how the reservoir is maintained during ART; where the virus resides during treatment; how gender, age, and other parameters affect the reservoir; and finally, how knowledge of these factors might lead to effective interventions.

Clonal Expansion of Infected Cells

When the latent reservoir was first described, most attributed its apparently stability to the long lifespan of non-dividing resting memory CD4$^+$ T cells endowed with pro-survival capacities. Recent technological advances demonstrate that the persistence of the reservoir is ensured through massive and sustained clonal expansion of cells infected with both intact and defective proviruses. This cell proliferation is thought to maintain the majority of infected cells during ART and shapes the location and disposition of the provirus population (Figure 1).

Three mechanisms might contribute to the clonal expansion of infected cells: integration in or near genes associated with cell growth, homeostatic proliferation, and antigen-driven proliferation. These are not mutually exclusive, and it is likely that all mechanisms apply to varying degrees across individuals and perhaps time.

It has been proposed that proviral integration near genes that control cell division, including genes involved in cancer, promotes cellular proliferation (Maldarelli et al., 2014; Wagner et al., 2014). HIV-1 preferentially integrates into highly transcribed genes, many of which are actively involved in cell growth. Thus, it has been difficult to definitively determine whether preferential integration in such regions is a cause or consequence of cell activation and proliferation. Unlike transforming retroviruses that integrate into cancer genes and cause unrestricted cell growth, HIV-1 is not known to cause T cell cancers by integration. Nevertheless, altered gene expression induced via the introduction of a viral promoter is one possible mechanism that explains infected cell expansion.

In normal T cell homeostasis, memory T cell clones are maintained in response to cytokines, such as interleukin-7 (IL-7). These same factors contribute to the maintenance of the reservoir (Chomont et al., 2009). This homeostatic proliferation occurs in the absence of virus reactivation (Bosque et al., 2011; Vandergeeten et al., 2013), indicating that the low levels of proliferation required for normal T cell homeostasis allows the reservoir to be maintained while remaining invisible to the immune system and many immunotherapies.

Antigenic stimulation because of chronic exposure of microbial peptides may also drive expansion and maintenance of the latent reservoir. Early studies argued that the virus may be enriched in HIV-1-specific CD4$^+$ T cells (Douek et al., 2002), perhaps because such cells are more likely to be present and activated at sites of virus replication. More recent studies suggested that if there is enrichment, the effect is modest (Hey-Nguyen et al., 2019). Co-infection with viruses such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are widespread and could also lead to antigen-driven proliferation of latently infected cells. Indeed, Henrich and colleagues identified HIV-1 DNA enrichment in CMV- and EBV-specific CD4$^+$ T cells after

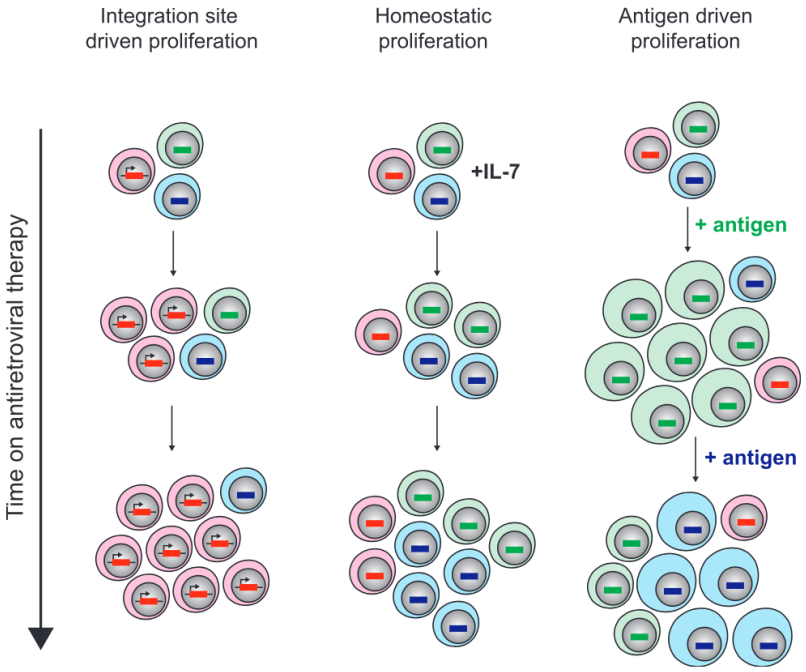

Figure 1. HIV-1 Persistence through Clonal Proliferation
Three independent mechanisms are thought to drive proliferation of latently infected cells. First, the viral integration site may provide a survival advantage allowing preferential proliferation of the infected clone. Second, homeostatic cytokines, such as IL-7, may signal latently infected cells to divide. Finally, latently infected $CD4^+$ T cells with antigen specific T cell receptors may divide in response to recurrent antigen exposure.

$CD4^+$ T cell reconstitution following chemotherapy (Henrich et al., 2017b). Recently, clones of HIV-1- and CMV-responsive $CD4^+$ T cells containing defective or intact latent proviruses were found in ART suppressed individuals (Mendoza et al., 2020). Thus, chronic or repeated exposure to antigen likely contributes to the longevity of the HIV-1 reservoir by stimulating the clonal expansion of latently infected $CD4^+$ T cells, resulting in sequential episodes of expansion and contraction in the reservoir (Wang et al., 2018) (Figure 2).

The Active Reservoir

During effective ART, a minority of HIV-1-infected cells are transcriptionally active, producing elongated HIV-1 RNA, HIV-1 proteins, and intact virions. The reservoir is hence heterogenous, with a continuum from "deep latency" (no or very little RNA produced) through a state of active virion production. Using a method to quantify unspliced HIV-1 RNA production on a per-cell basis, one group estimated that approximately 10% (with wide variability) of circulating infected cells expressed detectable levels of HIV-1 RNA (Wiegand et al., 2017). Higher levels of transcriptional activity may be evident if the early viral transcript TAR is measured (Yukl et al., 2018). The frequency of cells expressing RNA are similar in blood and lymph nodes (McManus et al., 2019) but may be relatively lower in the gut (Telwatte et al., 2018). Notably, although the frequencies of infected cells expressing viral RNA were similar in those on or off ART, the level of production was consistently very low during ART (Wiegand et al., 2017), while occasional cells from untreated individuals expressed high levels of RNA.

Simply transcribing HIV may not be sufficient to make a cell productive. In one provocative study, the main mechanisms responsible for HIV latency were not transcriptional interference or block to initiation, but rather blocks to proximal elongation, polyadenylation, and splicing (Yukl et al., 2018). Clarifying these issues will require characterizing the transcriptional profile of infected cells on a single cell basis using emerging technologies.

The active reservoir may be enriched in cells that are phenotypically distinct cells from those harboring the more quiescent latent reservoir. For example, one group found that within B cell follicles, most HIV-1 RNA-expressing cells also expressed CD32a, while cells expressing only HIV-1 RNA or CD32a were rare (Abdel-Mohsen et al., 2018). Similarly, CD30 was found to be a marker of the active (HIV-1 RNA-expressing) reservoir in blood and tissues (Hogan et al., 2018).

Most of the integrated viral genomes are defective (Bruner et al., 2016; Cohn et al., 2015; Ho et al., 2013). In one intensively studied individual, the frequency of cells expressing RNA was similar in cells harboring intact and defective genomes (Musick et al., 2019). Defective genomes can also produce viral proteins that are immunogenic (Imamichi et al., 2020; Pollack et al., 2017) and might prove to be detrimental to health, as they likely contribute to persistent inflammation during ART.

Dynamics of Infected Cells during ART

When and how the latent reservoir is established is not fully known. In simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV) infection, it is possible to initiate ART before the detection of circulating viral particles in the plasma (Whitney et al., 2014). In one study, ART started 4–5 days post-infection prevented the establishment of a permanent latent reservoir (Okoye et al., 2018). This may occur in humans receiving post-exposure prophylaxis (PEP), but once infection is clinically evident in people, a small but persistent reservoir is unavoidably established (Colby et al., 2018; Henrich et al., 2017a). A long-lived reservoir is also established during maternal-to-fetal transmission and/or at birth (Garcia-Broncano et al., 2019; Persaud et al., 2013).

Although viruses are deposited into longer-lived memory cells early during infection (Leyre et al., 2020; Puertas et al., 2014), the reservoir during the untreated state is not stable. Most of the reservoir that persists during ART appears to be have formed just prior to treatment initiation (Abrahams et al., 2019; Brodin et al., 2016; Pankau et al., 2020). The pool of cells harboring

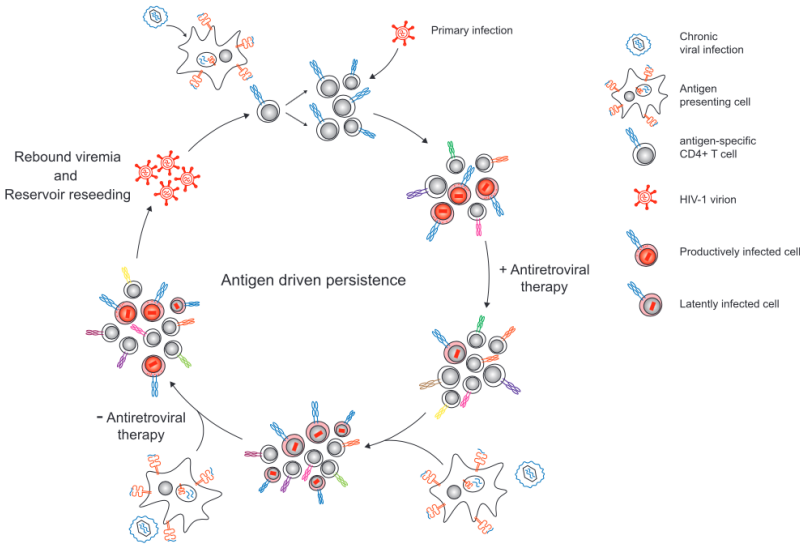

Figure 2. Antigen-Driven Viral Persistence
The presence of chronic viral infection (in blue) leads to specific activation of antigen responsive $CD4^+$ T cells. These activated T cells are targets for primary HIV-1 infection. Upon initiation of antiretroviral therapy, the majority of productively infected cells die rapidly, leaving behind latently infected cells. During repeated exposure to chronic virus, the latently infected, antigen specific cells divide, and the clones wax and wane in response to antigen exposure. If therapy is ceased, chronic viral antigen can be presented to latently infected cells which may trigger HIV-1 transcription and virus production, resulting in viral rebound and latent reservoir reseeding.

potentially replication competent HIV-1 decays more rapidly during early compared with late ART (Laanani et al., 2015; Peluso et al., 2020), presumably because of the rapid clearance of a pool of relatively short-lived infected cells (Leyre et al., 2020). The frequency of cells actively producing virus within lymphoid tissue also decays more rapidly during the first few years of ART (Banga et al., 2016).

Over a period of years to decades, the circulating reservoir becomes increasingly clonal (Cohn et al., 2015) and is often found in cells that are proliferating (Wagner et al., 2014) and more differentiated (Hiener et al., 2017). As the modern ART era is now over 25 years old, studies of individuals with decades of viral suppression are now possible. The reservoir may be qualitatively different in people treated for short compared with long period of times, and indeed, defective and intact proviruses have been shown to decay at different rates during therapy (Peluso et al., 2020).

Characteristics of Infected Cells in Blood and Tissue

Cellular Reservoirs

HIV-1 persists in all subsets of memory $CD4^+$ T cells, including the classic memory subsets (memory stem cells, central memory cells, transitional memory cells, and effector memory cells), and a variety of functional subsets (particularly T follicular helper cells, T regulatory cells, T helper 1 (Th1) cells, and Th17 cells). Although memory cells harbor the bulk of HIV proviral DNA during ART, naive cells can also contribute to HIV persistence (Roche et al., 2019; Venanzi Rullo et al., 2019). Cells that have intrinsic self-renewing capacity might prove to be the most recalcitrant source of virus during long-term ART. T memory stem and central memory $CD4^+$ T cells (defined by expression of CCR7 and CD27) are generally accepted to be important reservoirs that can differentiate into effector cells (Buzon et al., 2014; Chomont et al., 2009; Jaafoura et al., 2014), the latter of which can expand dramatically. Bone-marrow-derived $CD34^+$ hematopoietic stem cells were found to harbor HIV-1 provirus in some but not all studies (Carter et al., 2010; Durand et al., 2012).

Infected memory $CD4^+$ T cells within distinct memory subsets display different HIV transcriptional activity, proviral inducibility, and contribution to the pool of cells harboring genetically intact genomes (Grau-Expósito et al., 2019; Kulpa et al.,2019; Kwon et al., 2020; Pardons et al., 2019b). Specifically, more differentiated effector memory cells have been shown to be enriched in intact genomes compared to less differentiated central memory cells (Hiener et al. 2017). The less quiescent status of effector memory cells has also been associated with greater inducibility in most (Pardons et al., 2019b; Kulpa et al., 2019) but not all (Kwon et al., 2020) studies. Person-to-person variability in the size and distribution of the viral reservoir is substantial (Chomont et al., 2009; Eriksson et al., 2013), which complicates efforts to design a "one-size-fits-all" curative strategy.

A major focus of ongoing research is the precise characterization of cells harboring latent infection. If a signature phenotype can be identified and validated, then therapies can be developed to eliminate the precise cells harboring the latent reservoir. Because of the rarity of infected cells, this has proven difficult, though significant progress has been made by many independent groups. As might be expected considering the role of cell activation and proliferation in maintaining the reservoir, HIV proviruses are enriched in cells that express the canonical markers of activation HLA-DR (major histocompatibility complex [MHC] class II) (Horsburgh et al., 2020; Lee et al., 2019a), CD25 (the alpha chain of the IL-2 receptor and a constitutive marker of regulatory T cells) (Tran et al., 2008), and CD69 (a marker of tissue-resident memory T cells)(Cantero-Pérez et al., 2019). Therefore, subsets of cells that express HLA-DR, CD25, or CD69, and hence do not fulfill the classical definition of resting $CD4^+$ T cells, contribute to the long-term persistence of HIV during ART. These studies highlight the importance of studying total $CD4^+$ T cells rather than resting $CD4^+$ T cells to comprehensively address the issues of HIV persistence. In addition, HIV-1 proviral DNA has in most (but not all) studies been found to be modestly enriched in cells expressing immune checkpoint receptors, programmed cell death-1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T cell immunoglobulin, and ITIM domain (TIGIT), lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin and mucin 3 (TIM-3), and CD160 (Chew et al., 2016; Fromentin et al., 2016; Pardons et al., 2019a). Importantly,

the level of enrichment increased as more receptors were expressed (Chew et al., 2016; Fromentin et al., 2016).

Several CD4$^+$ T cell subsets endowed with specific immune functions are enriched in persistent HIV. In tissues, the B cell follicle is a major viral reservoir, particularly during the first few years of ART. HIV-1 proviruses are highly enriched in CD4$^+$ T follicular helper (Tfh) cells. When these cells circulate, they upregulate expression of the chemokine receptor CXCR3. As expected, such cells tend to harbor more replication-competent HIV-1 provirus (Banga et al., 2018). Several groups have argued that tissue-based Th17 cells are enriched for HIV-1 DNA, presumably because they largely reside in the gut—a preferred site for high levels of virus replication (Pardons et al., 2019a; Wacleche et al., 2016). In another study, clonal expansions of intact genomes were preferentially observed in cells displaying Th1 functions (Lee et al., 2017), a subset that encompasses most virus-specific cells, including HIV-specific and CMV-specific CD4$^+$ T cells. Tissue resident memory CD4$^+$ T cells can harbor HIV, as expected, and may be difficult to characterize given that they primarily reside in difficult-to-access tissues (Cantero-Pérez et al., 2019).

The role of non-CD4$^+$ T cells as a reservoir is controversial. Although HIV-1 can infect macrophages and may be important in the establishment of chronic infection at the site of viral exposure, it is unclear whether infected macrophages persist during ART (Calantone et al., 2014). In a humanized myeloid-only mouse (MoM) model, macrophages harbored replication-competent HIV-1, but these cells had a limited half-life *in vivo* and hence would not be expected to be a major reservoir during long-term ART (Honeycutt et al., 2017). As most macrophages exist in hard-to-access tissues, their relevance as a reservoir during ART remains largely undefined.

Tissue Reservoirs

The geographic distribution of HIV-1 within the body is now being mapped by multiple groups. Tissues that are highly enriched with lymphoid structures, particularly lymph nodes and the gut mucosa, contain the largest amount of virus and highest frequency of infected cells (Banga et al., 2016; Chun et al., 2008; Yukl et al., 2010). Precise mapping of the tissue reservoir in living people is not possible. Non-human primate studies and, more recently, human autopsy studies are better suited to address this issue. In a comprehensive assessment of the reservoir in SIV-infected macaques on effective ART, the vast majority (>98%) of the total body reservoir was found in the gut (Estes et al., 2017). The gut is also likely the largest reservoir of HIV-1 in people (Chun et al., 2008; Estes et al., 2017). In a recent prospectively designed rapid autopsy study (the "Last Gift" cohort), HIV proviruses were detected in all 28 tissues analyzed (Chaillon et al., 2020). Importantly, lineage phylogenetic analyses revealed that the blood and lymphoid tissue act as the main vehicle for virus dissemination throughout the body. The degree to which low levels of intact HIV-1 persists in the brain remains controversial, though all six donors from the above study had detectable HIV DNA in this organ.

The B cell follicle within lymphoid structures has unique properties that make it ideal to support HIV-1 replication and persistence. In states of inflammation—directly and indirectly induced by HIV-1 infection—germinal centers containing activated Tfh cells develop. To maintain normal B cell function, CD8$^+$ T cells and other effector cells are actively excluded from the follicle, thus providing the virus with an immune sanctuary (Connick et al., 2014; Fukazawa et al., 2015). It has also been argued that antiretroviral drug penetration into the lymph nodes is suboptimal (Fletcher et al., 2014), although the mechanisms for this effect is unknown and the data are inconsistent (Burgunder et al., 2019).

The reproductive tracts are important and poorly understood tissue reservoirs. These tissues tend to be rich in macrophages and might be potential immune sanctuaries. Antiretroviral drug penetration into these tissues may be suboptimal. Recent findings suggest that macrophages within the male genital tract may be an important reservoir (Ganor et al., 2019). Additionally, tissue resident memory CD4$^+$ T cells in the female genital tract (particularly the cervix) are highly enriched with HIV-1 DNA (Cantero-Pérez et al., 2019).

Population-Specific Characteristics of the Reservoir

Most studies of HIV-1 persistence have been performed in middle-aged and older men who live in resource rich areas, were infected with subtype-B virus, and who started ART during chronic infection. Data from such studies are not fully generalizable as age, sex, HIV subtype, duration of ART, and timing of ART initiation are now known to impact the size, distribution, and/or activity of the reservoir.

Biological Sex

In the absence of therapy, women generally have lower plasma viral loads than men, particularly in early-stage disease (Gandhi et al., 2002). In the presence of ART, reproductive-aged women have similar levels of cell-associated HIV-1 DNA to well-matched men but lower levels of cell-associated RNA (in isolated CD4+ T cells) and residual plasma HIV-1 RNA levels (Scully et al., 2019). In Uganda, the size of the replication-competent reservoir that could be activated *ex vivo* was lower in women than men. These sex-dependent effects may be because of the activity of estrogen and estrogen resceptor-1, which inhibit HIV-1 transcription and blunt the activity of latency reversal agents (LRAs) *in vitro* (Das et al., 2018).

Age

The biology of HIV-1 replication is also age dependent. The neonatal and infant immune system are characterized by an abundance of naive CD4$^+$ T cells and a limited capacity to generate antigen-specific memory cells; thus, it is to be expected that the distribution and activity of the reservoir will be unique, but such studies are difficult to perform. When ART is initiated during this period, the reservoir is exceedingly small and the immune system apparently protected (Garcia-Broncano et al., 2019). The latent reservoir in perinatal infection may be slower to reactivate and of lower magnitude compared to adult infection, independent of proviral load (Dhummakupt et al., 2020). Whether this difference in the inducibility of the latent reservoir between adults and children is attributed to differential epigenetic regulations of the provirus or to the different location of the reservoir in subsets that are differentially prone to reactivate HIV remains to be determined. In children, the reservoir size is highly dependent on when ART was initiated, with a dose response toward higher levels if therapy was delayed until after the age of one and even more so after the age of 5 years (Persaud et al., 2014).

The impact of advanced aging on the reservoir is not known. As advance age is associated with profound changes in T cell dynamics (Thome et al., 2014), it is reasonable to assume that the latent reservoir will also evolve over time. In blood, the immune system in the elderly is characterized by lower levels of naive cells and massive expansion of effector cells, many of which are likely driven by prevalent antigens, particularly CMV. As people and their immune system age, the reservoir is likely to become increasingly clonal and concentrated in these cells. Data on this issue are sparse, but a recent study showed the a clonal virus population can be readily detected in CMV-specific cells from most people (Mendoza et al., 2020). Notably, older age has been associated with slower decays in the reservoir size, as estimated using HIV-1 DNA measurements (Golob et al., 2018)

HIV Subtype

Defining the impact of HIV-1 subtype would require a careful comparison of the latent reservoir in people infected with subtype B (the focus of most studies) and non-B (more prevalent globally). Such studies might prove impossible to perform carefully, as multiple confounders might affect such a comparison (people living in resource-rich areas versus resource-poor regions in terms of ART exposure, co-infections, and genetic backgrounds). In one comparative study, the latent reservoir was approximately 3-fold lower in people infected with HIV-1 living in Uganda compared with those living in Baltimore, MD (Prodger et al., 2017), which may have been due to differences in viral subtype, although multiple other factors differed between these two groups. A higher reservoir in subtype B versus other subtypes was noted in a separate cohort and mechanistically attributed to a greater activity of the HIV-1 Nef protein, particularly with regard to the downregulation of human leukocyte antigen (HLA) class I (which, in turn, prevents immune clearance) (Omondi et al., 2019).

Timing of ART Initiation

The impact of the duration of ART on the reservoir differs among those treated early versus late. A more rapid and sustained decay in the reservoir size has been observed among individuals treated during acute compared with chronic infection (Chun et al., 2007; Hocqueloux et al., 2013). The mechanism for this potential more rapid decay in those treated very early is not known but might include the preservation of more effective HIV-specific immunity and/or prevention of rapid escape mutations (Lee et al., 2019b; Leyre et al., 2020; Ndhlovu et al., 2019; Takata et al., 2017). It has also been suggested that longer-lived cells are less activated during acute infection, and hence less likely to become infected, resulting in a reservoir that is enriched in shorter-lived cells (Chéret et al., 2015; Leyre et al., 2020).

Co-infections and Inflammation

HIV-1 induces a chronic inflammatory state that persists during ART. HIV-1-associated harm to the gut mucosa results in chronic systemic exposure to microbial products and more inflammation (Brenchley et al., 2006). HIV-1-related immunodeficiency increases the burden of CMV and other viruses, also contributing to chronic inflammation (Hunt et al., 2011). Although there are exceptions (Gandhi et al., 2017), most studies to date suggest that the inflammatory environment shapes the distribution and reservoir in a complex manner, with more activation leading to higher reservoir sizes (Banga et al., 2016; Chomont et al., 2009; Fromentin et al., 2019). HIV and SIV DNA have been found to be enriched in cells that express markers related to immune activation (e.g., HLA-DR, CCR5, PD-1, CD30) (Lee et al., 2019a; McGary et al., 2017; Pardons et al., 2019a; Thornhill et al., 2019). The inflammatory environment of the lymphoid tissues is widely assumed to cause immune dysfunction and reduced immunity to HIV-1.

These observations collectively argue that any chronic inflammatory state will alter the relationship between the host immune system and the latent reservoir. Therefore, regional differences driven by prevalent co-infections or microbial products may have important implications for any future cure strategy. Intensive investigations of these issues are needed.

Therapeutic versus Immunologic Control

There are ample data indicating that sustained immunologic control of HIV and SIV is possible. A small proportion of individuals naturally control HIV replication in the absence of therapy ("elite controllers"). A recently appreciated subset of elite controllers have particularly low reservoir sizes and a normal immune system; cohorts of these "exceptional controllers" are now being assembled because they may prove useful as a model for an ideal remission or cure (Canouï et al., 2017; Casado et al., 2020; Mendoza et al., 2012). A small proportion of individuals present with high levels of viremia, go on effective ART for years, interrupt therapy, and subsequently maintain durable virus control ("post-treatment controllers") (Namazi et al., 2018; Sáez-Cirión et al., 2013). Finally, in the modern era of cure studies, a number of promising immune therapies have generated sustained control of SIV in non-human primates (Borducchi et al., 2016; Borducchi et al., 2018; Hansen et al., 2013) and perhaps in people (Mendoza et al., 2018; Niessl et al., 2020). In all four of these unique phenotypes, control is likely mediated in large part by virus-specific T cells that are functional and target vulnerable and conserved regions, although other likely pathways are involved.

The circulating reservoir in those controlling HIV via a sustained host response is often much smaller than in typical person on ART (Hatano et al., 2009; Sáez-Cirión et al., 2013; Sharaf et al., 2018). This small reservoir may be both a cause and a consequence of sustained control (Conway and Perelson, 2015). In contrast to those on ART, the virus population in most elite controllers replicates and evolves (Mens et al., 2010). Some (Miura et al., 2009) but not all (Salgado et al., 2014) studies have found that the virus population in controllers is less fit than that in non-controllers. Finally, even though the replicating population in blood is low in controllers, it may remain high in tissues, particularly in the B cell follicle, an immune-privileged region lacking in $CD8^+$ T cells (Boritz et al., 2016; Fukazawa et al., 2015). As many immunotherapies aim to recapitulate exceptional and/or post-treatment control, more studies detailing the mechanisms associated with these unique clinical phenotypes are warranted.

Reservoir Eradication and Control Strategies in the Clinic

Knowledge regarding how the reservoir is maintained, how it evolves, and where it resides has direct implications for the HIV-1 cure agenda (Figure 3).

Antiretroviral Therapy

Given the slow rate of virus decay during ART, current therapies are unlikely to be curative. As some people have unusually low reservoirs and others have rapid decay rates (Leyre et al.,

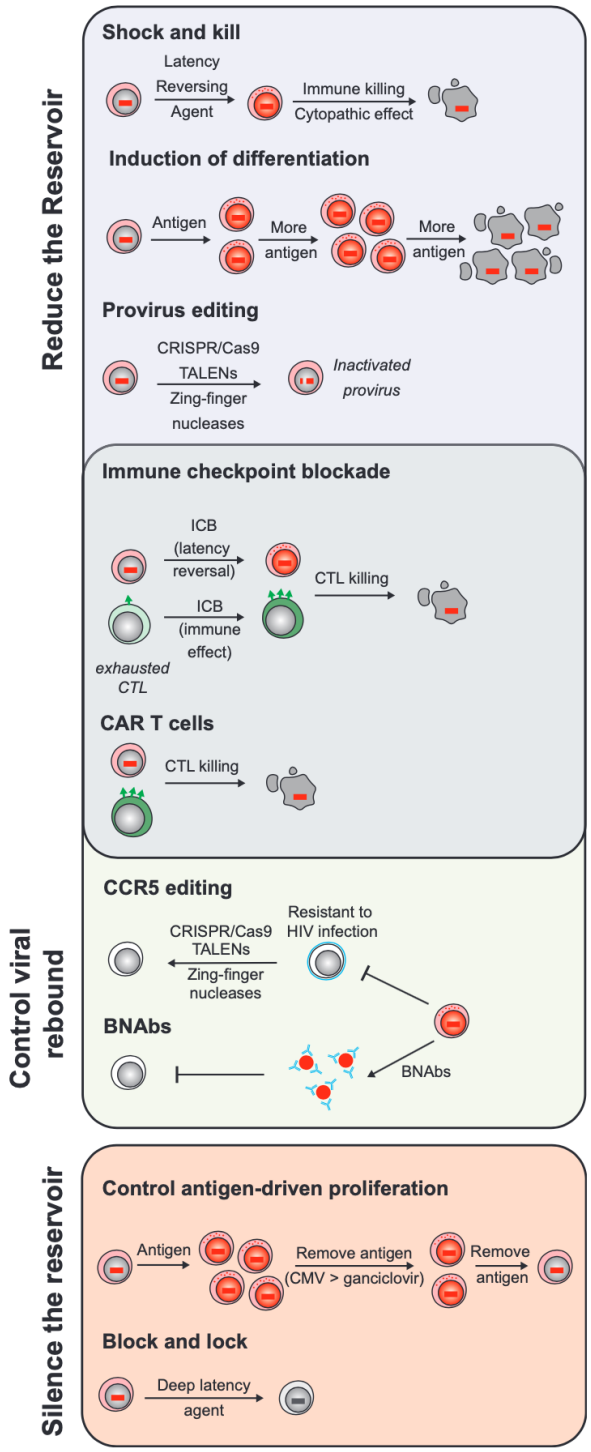

Figure 3. Clinical Strategies for Eradication
Strategies are divided into those which aim to reduce the size of the reservoir, control viral rebound, or silence the reservoir.

2020; Peluso et al., 2020), it remains possible that some will be cured after decades of ART. If, as proposed by some (Buzón et al., 2010; Fletcher et al., 2014; Lorenzo-Redondo et al., 2016), ongoing virus spread and reseeding of the reservoir continues during otherwise effective ART, then more potent drugs with the capacity to penetrate all tissue reservoirs might accelerate that decay for the virus—in some leading eventually to a cure. As any residual virus spread is assumed to be local, definitive studies on this issue will be challenging, even as more potent drugs emerge.

The transition from the untreated to the treated state provides unique opportunities for curative interventions. During long-term ART, much of the reservoir apparently derives from cells that were infected just prior to treatment initiation, arguing that in untreated disease, the putative reservoir is unstable (Abrahams et al., 2019; Brodin et al., 2016). This implies that during early ART, the rapidly changing immune environment shifts the balance toward a state in which latency can be achieved. Presumably, the massive reductions in HIV-1-associated inflammation and T cell activation reduces the turnover of the reservoir, leading to generation of longer-lived cells harboring intact genomes. Immune stimulation under the cover of ART (preferably with co-administration of a therapy that induces killing of infected cells) might work best during this window of opportunity.

Latency Reversal

The molecular mechanisms maintaining latency are now being actively targeted with the goal of reversing transcriptional silencing ("latency reversal"), thus inducing cells to produce viral RNA and proteins, which in turn makes the cell recognizable to the immune system or immunotherapies ("shock-and-kill" strategy). The first generation of LRAs successfully induced RNA production, but only some drugs (and in only some studies) induced the production of proteins and viral particles. None of these interventions caused a reduction in the reservoir size, presumably because the degree of latency reversal was limited and/or the immune system was not primed to clear antigen-expressing cells (Kim et al., 2018).

The next generations of LRAs are more promising. Pre-clinical data suggest that small-molecule inhibitor of apoptosis antagonists, particularly the SMAC mimetic compounds, induces reversal of latency (Pache et al., 2015). Importantly, these drugs have demonstrated potent and consistent activities in multiple animal models and by independent teams (Nixon et al., 2020). Studies in people have not yet started.

Immune stimulating approaches that activate $CD4^+$ T cells might also prove useful. Toll-like receptor (TLR)-7 agonists have demonstrated direct latency reversing activity in non-human primates (Lim et al., 2018), but the effect was not confirmed in subsequent studies (Del Prete et al., 2019). When used in combination with other immunotherapies in non-human primates, TLR-7 agonists induced a state of remission and perhaps even a complete cure (Borducchi et al., 2016; Borducchi et al., 2018); the mechanism for this effect may have been via their immune-enhancing activities rather than as latency reversing agents. Other TLR agonists and cytokines (particularly IL-15) are being actively studied.

Latency Silencing

If the virus cannot be fully induced and cleared, can it be permanently silenced? Deep and irreversible latency of HIV-1 would

not be unprecedented. A substantial proportion of the human genome consists of ancient retrovirus DNA that is not transcribed, except under rare circumstances. These human endogenous retroviruses (HERVs) are silenced through epigenetic mechanisms, including DNA methylation (Schulz et al., 2006). Although not definitely proven, it is likely that latent HIV proviruses are regulated similarly to HERVs. Indeed, there are emerging data that through multiple mechanisms, over time the HIV genome becomes preferentially enriched in intergenic regions and/or become hypermethylated, resulting in less expression (Einkauf et al., 2019). This evolution of proviral distribution likely reflects the survival of cells with defective viruses and viruses in regions of the genome that allow for "deep latency".

Identifying therapeutics that accelerate this process is a high priority. Proof-of-concept studies involved didehydro-cortistatin A (dCA), an inhibitor of the viral transcriptional activator Tat (Mousseau et al., 2015). In humanized mice, dCA administration during ART reduced reservoir activation post treatment, although the effect was modest (Kessing et al., 2017). In a large screen of other potential latency silencing approaches, inhibitors of mammalian target of rapamycin (mTOR) were identified as promising candidates for block and lock strategies (Besnard et al., 2016). This approach is being studied in the clinic.

Immunotherapy

There is a robust and rapidly expanding pre-clinical and early clinical research program aimed at inducing a sustained HIV-1-specific immune response that can effectively clear infected cells during ART and/or control any persistently infected cells post-ART cessation.

Immunotherapies that directly target cells expressing the envelop protein for killing are particularly attractive. A number of broadly neutralizing antibodies (bNAbs) that target the envelope glycoprotein suppress virus replication *in vivo* (Bar et al., 2016; Mendoza et al., 2018), presumably because of their ability to block virus entry and virus spread. Theoretically, bNAbs might also bind envelope proteins expressed on the cell surface and trigger host-mediated cytotoxicity, which could reduce the active reservoir. Whether cell killing happens *in vivo* remains unproven, although indirect evidence suggests it may have some effect (Lu et al., 2016). Similar to bNAbs, emerging HIV-specific chimeric antigen receptor (CAR)-T cells target envelope on the cell surface, which might lead to reservoir reduction during ART or virus-control post-ART. Several approaches are moving toward the clinic.

Antibodies and CAR-T cells share the same potential limitations: both require that the epitope be expressed on the cell surface at high densities and both target the highly variable envelope glycoprotein. In a recent pre-clinical series of studies involving a novel HIV-1-specific CAR-T cell approach, efficacy was largely dependent of the levels of envelope expression in the targeted cells (Herzig et al., 2019). These therapies may only work when used in combination with latency reversing agents or during a treatment interruption.

A number of strategies aimed at enhancing control are undergoing evaluation in early proof-of-concept studies. Even if bNAbs are unable to clear reservoir cells directly, they have been postulated to do so for highly immunogenic antibody-antigen responses, which in might stimulate potent HIV-specific immune responses and post-ART control (Niessl et al., 2020). Leveraging advances made in the management of cancer, several groups are studying immune checkpoint blockade. This effect is justified in part by the enrichment for HIV proviruses in $CD4^+$ T cells expressing immune checkpoint molecules(Chew et al., 2016; Fromentin et al., 2016), by the ability of antibodies against PD-1 and CTLA-4 to enhance latency reversal (Evans et al., 2018; Fromentin et al., 2019), and by the well-known capacity of these approaches to boost antigen-specific T cell responses. Although immune checkpoint blockade led to a marked decrease in markers of HIV persistence in case report studies (Fromentin et al., 2019; Guihot et al., 2018), two NIH-sponsored clinical trials were prematurely terminated because of unacceptable toxicity (Gay et al., 2017), indicating that the dose of immune checkpoint blockers will need to be adjusted if additional studies are to be conducted.

Clonal Proliferation

The reservoir is largely maintained by the proliferation and expansion of memory $CD4^+$ T cells. Treatment with IL-7—a key cytokine driving T cell homeostasis—increases the proliferation and expansion of memory T cells, and the reservoir size increases accordingly (Vandergeeten et al., 2013). Any cytoreductive intervention that reduces the total $CD4^+$ T cell count would have the opposite effect, as demonstrated in the most extreme cases with ablative chemotherapy followed by allogenic stem transplants, but ultimately, a small reservoir of infected progenitor cells persists, providing a self-renewing source for continued clonal expansions as the immune system is reconstituted (Henrich et al., 2017b). If proliferation is to be targeted, the ideal intervention would selectively block infected over uninfected cells from expanding, thus allowing the potential for the total memory population to be slowly enriched for uninfected cells. Alternatively, should some chronic antigen be found to be driving the continued proliferation of memory cells harboring intact HIV-1, then targeting this antigen might reduce the reservoir. CMV, gut microbes, and other persistent antigens that are enhanced by HIV-1 might be targeted (Figure 3).

Irrespective of the strategy to be used to either induce or limit T cell expansions in the reservoir, the core of the reservoir is likely to lie in stem and central memory cells (Buzon et al., 2014; Jaafoura et al., 2014) that have a unique capacity to self-renew upon antigen stimulation. Even if most of the "visible" reservoir of intact genomes is seen in cells displaying a more differentiated phenotype following clonal expansions, a small frequency of poorly differentiated parent cells with high survival capacities may represent long-lived sources of infected cells during ART.

Gene Editing

Once the cellular and tissue reservoirs of HIV are carefully characterized, it may be possible to design therapies that can deliver gene editing technologies (e.g., Zinc-finger nucleases or CRISPR-Cas9) that disrupt the provirus (Dash et al., 2019). To be curative, all infected cells need to be treated, which will be challenging because of the rarity and the diversity of integrated proviruses even within a single individual. Disruption of the virus co-receptor CCR5 and other pathways needed for virus replication might be easier to achieve; in these cases, the gene edits might not need to be 100% effective, as simply reducing the number of susceptible targets blunt the massive amplification of the virus population that occurs when a

systemic infection is established (Davenport et al., 2019; Hataye et al., 2019).

Conclusions

Despite progress in both the prevention and treatment, developing an effective and scalable cure for HIV-1 remains is now a global public health priority (Deeks et al., 2016). Antiretroviral therapy fails to cure individuals because of a long-lived latent reservoir of infected cells. Since its initial description in 1997, advances in technology have enabled immense progress in understanding the biological characteristics of this reservoir. Continued progress elucidating the biology will enhance our ability to develop effective curative interventions. Further study is required to uncover the contribution of infected cells in the periphery to viral rebound, and a method to assess the whole-body virus burden in people living with HIV-1 is urgently needed. It is also possible that a sterilizing cure may not be achievable. In this case, additional insight into immune control of HIV-1, possibly through the study of individuals who spontaneously control HIV-1 infection, will prove to be important. Finally, as new therapies emerge, it is critically important that funding, existing networks and infrastructure, and education provide access to all communities across the globe.

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