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Recent progress in understanding HIV reservoirs

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Abstract

Purpose of review—Reservoirs of HIV-1-infected cells persist long-term despite highly effective antiretroviral suppression therapy and represent the main barrier against a cure for HIV-1. This review summarizes recent advances in understanding the complexity and diversity of viral reservoir cells.

Recent findings—Latently-infected memory CD4 T cells are the predominant cell compartment responsible for viral persistence, but some studies suggest that myeloid cells, and possibly hematopoietic progenitors, can also serve as long-term viral reservoirs. Specific phenotypic markers, including T cell activation and exhaustion molecules, may denote CD4 T cells enriched for replication-competent-proviruses. Clonal proliferation of infected CD4 T cells *in vivo* represents an important mechanisms responsible for the remarkable long-term stability of the viral reservoir. Multiple new assays, including near full-genome proviral sequencing and simplified versions of viral outgrowth assays, are being developed to analyze and quantify persisting reservoirs of HIV-1-infected cells.

Summary—Recent technological advances allow to profile the molecular structure and composition of viral reservoir cells in great detail. Continuous progress in understanding phenotypic and functional properties of viral reservoir cells provide clues for novel clinical interventions to destabilize viral persistence during antiretroviral therapy.

Keywords

HIV; reservoirs; viral persistence

Introduction

The introduction of potent antiretroviral combination therapy (ART) has transformed HIV-1 infection into a manageable chronic disease that remains associated with increased immune activation and other immunological abnormalities, but in many cases does not compromise life quality or expectancy. Nevertheless, ART does not eliminate all HIV-infected cells from

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the human body, meaning that lifelong suppressive therapy is the only therapeutic option for infected individuals. Characterizing residual reservoirs of virally-infected cells that persist despite treatment represents a major focus area within the HIV research community and is frequently considered as an essential prerequisite for efforts to induce a long-lasting, drug-free remission or cure of HIV-1 infection. Current concepts suggest that these viral reservoirs mainly consist of latently-infected CD4 T cells that encode for sequence-intact, replication-competent proviruses but exhibit minimal or no active viral gene expression during continuous antiretroviral therapy. However, once ART is interrupted, these latently-infected cells can fuel rebound viral replication and lead to high-level viremia, frequently within as few as two weeks. The remarkable stability of this viral reservoir, calculated to have a half-life of about 44 months, remains a perplexing aspect of HIV disease pathogenesis. Increasingly, it is recognized that maintenance of this viral reservoir does not simply reflect a protracted or delayed decay of a fixed number of virally infected cells, but represents a highly dynamic process that takes advantage and exploits complex immune mechanisms designed to sustain life-long persistence of cellular immune memory. In this chapter, we review recent progress in understanding the complexity and diversity of cells that can serve as viral reservoirs, discuss proposed mechanisms and molecular pathways supporting the extreme durability of viral reservoir cells, highlight novel technological approaches to profile and quantify latently-infected cells, and provide a perspective for ongoing clinical interventions to target residual disease reservoirs.

Cellular and tissue reservoirs

Many studies concur that memory CD4 T cells contain the vast majority of persisting proviruses in ART-treated HIV-1 patients, arguably because these cells are susceptible to HIV infection, while being able to flexibly fine-tune the level of viral gene expression, possibly as part of a “bet-hedging” strategy [1]. For instance, during the transition from an activated to a resting state, these cells initiate a transcriptional program that enables viral pre-integration steps but restricts active viral gene expression [2], and for that reason is particularly conducive to supporting viral latency; this may in part explain the preference for memory T cells as long-term viral reservoirs. Nevertheless, the overall frequency of latently infected cells within the memory CD4 T cell pool is extremely small, frequently estimated at around 1 in a million cells, and the ability to support viral persistence seems to vary considerably among different subsets of the CD4 T memory cell pool. In a provocative recent study, Descours et al. [3] compared the transcriptome of HIV-1-infected and -uninfected CD4 T cells in a model of HIV-1 latency based on *in vitro* infection of resting CD4 T cells after experimental downregulation of the HIV-1 restriction factor SAMHD1. Out of 103 differentially expressed genes, the authors identified the gene encoding for CD32a, a member of the Fc Receptor family, as a marker that strongly distinguished infected and uninfected cells. Quantitative viral outgrowth assay suggested that CD32a+ CD4 T cells from ART-treated patients were highly enriched for inducible replication-competent proviruses, by a factor of up to 3,000-fold compared to total CD4 T cells. Notably, several of the CD32a-positive CD4 T cells appeared to harbor more than one provirus. Interestingly, a strong enrichment (up to 8-fold) with chromosomally-integrated HIV-1 proviruses was also noticed for cells expressing a combination of the exhaustion markers PD-1, LAG-3 and

TIGIT [4]. If confirmed in future studies, these studies may provide useful biomarkers for analyzing, monitoring and possibly therapeutically targeting viral reservoirs.

Based on an original study showing that T cells with a T follicular helper cell profile can serve as a predominant viral reservoir cell population in lymph nodes [5,6], subsequent studies have explored how the functional polarization of T cells influences their ability to serve as a viral reservoir. Notably, regulatory T cells seem to be able to serve as a viral reservoirs, although more definitive evidence is necessary to fully appreciate their role as viral reservoirs in humans [7–9]. Th17 cells, predominantly located in mucosal surfaces and endowed with key roles for antibacterial and antifungal immune defense, seem highly susceptible to HIV, in part due to elevated expression of CCR5 and the HIV co-receptor alpha4/b7 and lower expression of RNases with antiviral activity [10,11], but their role as a site for persistence of viral reservoirs is less clear. In one study that was limited to peripheral blood lymphocytes, Th17 cells from ART-treated patients did indeed harbor intact proviruses, however, their relative frequency was lower compared to Th1 cells, and not statistically different from the number of proviruses in Th9 and Th2 cells [12]. Given that a large proportion of the viral reservoir is likely to reside in gut-associated lymphoid tissues (GALT) [13], a dedicated investigation and characterization of proviral sequences in Th17 cells from mucosal intestinal tissues in ART-treated patients is a high priority for future studies. A summary of T cell subsets serves as preferential viral reservoir sites is included in Table 1.

Viral non-CD4 T cell reservoirs

Although CD4 T cells represent the only cell compartment for which long-term persistence of HIV-1 has been unequivocally proven, there is increasing interest in defining potential non-CD4 T cell reservoirs for HIV-1. For instance, Sebastian et al [15] recently examined bone marrow derived hematopoietic stem cells from ART-suppressed patients and reported detection of near full-length HIV DNA in highly-enriched progenitor cells. Cell purity analysis demonstrated that this viral DNA was unlikely to derive from contaminating CD4 T cells. Notably, proviral HIV-1 sequences were enriched in a subset of more committed hematopoietic progenitor cells that express high levels of CD4, and seem to be able to support both CCR5 or CXCR4 tropic-infection. Phylogenetic viral sequencing studies revealed the presence of identical viral env sequences in progenitor cells and multiple more differentiated hematopoietic lineages, supporting the hypothesis that proliferation and differentiation of infected hematopoietic progenitor cells is a potential mechanism contributing to HIV-1 long-term persistence. In contrast, prior studies have failed to confirm the presence of HIV-1 sequences in more primitive hematopoietic stem cells. A high level of uncertainty exists regarding the role of monocytes and other CD4-cell expressing myeloid cells as potential viral reservoirs. To address this, Honeycutt et al used a humanized myeloid-only mice (MoM) model, in which only myeloid and B cells are systemically reconstituted with human cells since the host animal (NOD/SCID mice) cannot support human T cell development. Plasma viral load was successfully suppressed during ART treatment, but a part of the infected MoM (33%, n=3) displayed rebound viremia after treatment interruption [16]. Based on these results, the authors estimated that infected macrophages in MoM had a rather short half-life (1.05 days as compared to 2.4 days in

HIV-1-infected CD4 T cells), and that their number would decay to about 1% after 1 week of ART. The results reinforce the contribution of T cell as the predominant HIV-1 reservoir cell population during long term ART treatment, but also indicate that at least in principle, macrophage can carry intact provirus during suppressive antiretroviral therapy. To what extent viral persistence in monocytes occurs under physiological conditions in ART-infected humans remains an ongoing question for future studies.

Mechanisms of persistence

Considerable progress has been made recently in understanding the mechanisms that enable and drive the long-term persistence of viral reservoirs. Lorenzo-Redondo et al. [17] proposed that ongoing viral replication in lymphoid tissues with insufficient antiretroviral drug penetration may support viral persistence, based on viral sequencing results in three individuals analyzed during the first six months of antiretroviral therapy; however, such short-term treatment durations might be insufficient to investigate the role of ongoing viral replication for persistence of viral reservoirs [18,19]. Using a variety of different approaches, a series of recent studies have shown that clonal proliferation of CD4 T cells, a mechanism during which identical proviral copies are passed on from dividing cells to daughter cells, plays a critical role for preserving and amplifying viral reservoirs. For instance, Hosmane et al. [20] and Bui et al. [21] identified identical viral RNA sequences in independent viral outgrowth assays from the same patients, consistent with the presence of identical, replication-competent proviral sequences in distinct CD4 T cells within the same individual. Such clusters of identical sequences accounted for roughly 50–60% of all detected sequences, and highly likely derive from clonal proliferation of HIV-1-infected CD4 T cells in vivo. Evidence for clonal expansion of T follicular helper cells encoding for replication-competent HIV-1 was also found in individuals who naturally control HIV-1 in the absence of antiretroviral therapy [14]. Remarkably consistent with these results, Lee et al. employed a near full-length next-generation viral sequencing approach to identify clusters of intact proviruses that were completely identical over the entire near full-genome sequence [12]. Such intact, clonally-expanded sequences also accounted for approximately 50–60% of all intact sequences, were longitudinally detectable for a duration of up to five years, were preferentially observed in Th1 cells, and were fully replication- and infection-competent. Together, these studies show that viral reservoirs do not consist of a fixed number of virally infected cells, but are replenished or expanded by ongoing proliferation of infected T cells. Whether proliferation of such T cell occurs in response to antigenic stimuli, i. e. as a by-product of physiologic antigen-specific T cell proliferation, as a consequence of homeostatic, TCR-independent proliferative signals or through other mechanisms is not fully known. Notably, it is conceivable that the chromosomal integration of intact proviruses in genes governing and driving T cell proliferation may result in selective expansions of the respective cell clone, as suggested by some experimental findings [22].

Clonal proliferation is known to be a highly vulnerable step in a cell's life cycle, suggesting that specific molecular mechanisms protect and safeguard HIV-1-encoding CD4 T cells during this process. In this context, several studies suggest that expression of anti-apoptosis molecules, previously mostly recognized for their role in maintaining reservoirs of treatment-resistant cancer cells in malignant diseases, are involved in preserving survival of

viral reservoirs. Most recently, Cummins et al. [23] demonstrated that Casp8p41, resulting from cleavage of procaspase 8 through enzymatic activity of HIV-1 protease, can form biophysical interactions with BCL-2, a member of the inhibitor of apoptosis family of proteins. Blockade of BCL-2 in combination with viral latency reversal resulted in accelerated elimination of virally infected cells in vitro, suggesting that BCL-2 has a role in maintaining the survival of these cells. Clearly, a more detailed analysis of how anti-apoptosis molecules support viral persistence is an area of high interest for future research projects.

An ongoing question is the relevance of antiviral immune responses for shaping and structuring the viral reservoir, and for influencing its size and durability. Studies by Takata et al. suggested that cellular immune responses recruited during very early stages of infection were associated with faster HIV-1 viral load reduction after antiretroviral treatment initiation, and with more limited viral reservoir sizes during the subsequent disease process [24]. An influence of cellular immune responses on the composition of the viral reservoirs was also proposed by Pollack et al. [25], who showed that viral proteins encoded by defective proviral sequences can be recognized by HIV-1-specific T cells. Notably, an impact of virus-specific CD8 T cell responses on the viral reservoir was also suggested by CD8 T cell depletion studies in ART-treated SIV-infected rhesus macaques [26], and by associations between nef-specific CD8 T cells and proviral DNA in HIV-1-infected humans [27].

Methods for quantifying viral reservoirs

All approaches for quantifying viral reservoirs are heavily influenced by the technologies to assess HIV-1-infected cells in ART-treated patients. Traditional viral outgrowth assays (VOA) are regarded as the “gold standard” for quantitative viral reservoir analysis, but are complex, labor-intensive and require several weeks to complete. Recently, several technical modifications have been suggested that may improve the feasibility and practicability of this assay, through simplification of procedures to isolate CD4 T cells [28], the use of specific reporter cell lines [29] and the introduction of ultrasensitive techniques for scoring p24 production in reactivated CD4 T cells [30,31]. Nevertheless, it remains clear that this assay underestimates the true viral reservoir, given the stochastic response of latently infected cells to viral reactivation stimuli [32]. As an alternative assay for viral reservoir quantification, Bruner et al [33] have pioneered the use of PCR-based quantification of intact, near full-length viral sequences. This approach is in many cases more informative, more sensitive and more precise than traditional viral outgrowth assays and arguably represents one of the most helpful assays for deconstructing the true complexity of the viral reservoirs, specifically when adjusted for use in combination with next-generation viral sequencing technologies, as developed by Lee et al. [12] and Hiener et al [34]. In contrast to VOA, it allows to quantify the relative proportion of intact and defective proviruses, can identify clusters of clonally-expanded proviruses, and also provides some information about immune- or antiretroviral drug-driven viral sequence variation, although its rather costly and labor-intensive nature is a concern. Detection of cell-associated HIV-1 RNA by in situ hybridization, followed by flow cytometric visualization, was most comprehensively demonstrated by Baxter et al. [35] but also explored by others [36,37], and may provide for an elegant approach to analyze cells

expressing viral RNA after latency reversal. To what extent cellular expression of viral RNA detected by this approach reflects the presence of replication-competent viral sequences needs to be studied in future work.

Clinical approaches for viral reservoir reduction

Most clinical efforts to reduce viral reservoirs focus on the shock and kill strategy, which is based on the assumption that pharmacological reactivation of viral gene expression results in immune-mediated elimination of virally-infected cells; recent studies do suggest that triggering of viral antigen expression through latency-reversing agents is indeed sufficient in quantity and duration to allow recognition and clearance of such cells, at least in *in vitro* assays [38]. Several recently-conducted clinical studies have shown that induction of proviral gene expression by histone deacetylase inhibitors [39] and TLR9 ligands [40] is possible without inducing generalized immune activation [41], however such efforts have failed so far to translate into pronounced reductions of viral reservoir size, even when combined with therapeutic vaccines designed to activate HIV-1-specific T cells [42]. Notably, *in vivo* studies demonstrated that reactivation of viral gene expression from latency by pharmacological inducers critically depends on the chromosomal position of integrated proviruses [43], implying that development of a “one-size-fits-all”-type of latency reversing agent may not be feasible; instead, combinations of different LRAs may be required to mobilize the entire spectrum of integrated proviruses. Interestingly, initial cell culture assays demonstrated that pharmaceutical inhibitors of LEDGF/p75, a host factor responsible for driving HIV-1 integration into actively transcribed regions of the genome, may allow to steer viral genomes into chromosomal segments that do not effectively support viral gene transcription [44]. Such an approach, specifically when combined with pharmacological inhibitors of the viral transactivation protein Tat that can silence HIV-1 gene expression [45], may allow to induce a state of “deep latency” that may enable some degree of spontaneous ART-free viral control. Notably, unselective killing of CD4 T cells during cytotoxic chemotherapy, was paradoxically associated with increased levels of CD4 T cell-associated HIV-1 DNA and RNA, possibly as a result of clonal expansion of HIV-1-infected CMV- or EBV-specific CD4 T cells [46]. In contrast, a relatively long remission of HIV-1 replication during an analytical treatment interruption was noticed in an HIV-1-infected recipient of an allogeneic bone marrow transplant [47]; this and other similar cases [48] suggest that such a therapeutic approach is likely to induce more complex changes in the reservoir structure, and deserve further investigation.

Conclusion

The remarkable ability of HIV-1 to persist in the human body despite decades of highly effective antiretroviral therapy arguably represents the most perplexing and irritating aspect of HIV disease pathogenesis. Recent studies emphasize the complexity of the viral reservoir, and highlight that viral eradication or a “sterilizing cure” of HIV-1 infection remains a daunting task. Nevertheless, there is undeniable progress in understanding the cell compartments and the biological mechanisms contributing to persistence of long-lived viral reservoirs. The authors of this article remain hopeful that increased insight into the structure

and composition of viral reservoir cells will ultimately allow to transform HIV-1 infection into a curable disease.

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Key points

- Specific subsets of CD4 T cells seem highly enriched with proviral HIV-1 DNA encoding for replication-competent HIV-1.
- Clonal expansion of CD4 T cells harboring intact proviral DNA is an important mechanism to stabilize long-term persistence of viral reservoir cells.
- Increasing evidence suggests that non-CD4 T cells can also serve as niches for persistence of HIV reservoirs.
- No interventions are currently available to reliably reduce the number of viral reservoir cells, but multiple different approaches are being evaluated in proof-of-concept clinical trials.

Table 1

Recently-described CD4 T cell populations serving as preferential sites for HIV-1 persistence.

Phenotypic/functional marker	Cell population	Reference
PD-1, CXCR5; IL-21 secretion	T follicular helper cells	5, 13
CCR5, CXCR3; IFN- γ secretion	Th1-polarized CD4 T cells	11
FoxP3, CTLA-4	Regulatory T cells	7,8
CD32a	Activated T cells?	3
PD-1/LAG-3/TIGIT	Exhausted T cells	4