Published in final edited form as:

Curr Opin HIV AIDS. 2016 July ; 11(4): 383–387. doi:10.1097/COH.0000000000000281.

Diversity of HIV-1 reservoirs in CD4 T cell subsets

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Abstract

Purpose of review—HIV-1 is able to create long-lasting reservoirs of virally infected cells that persist life-long and are extremely difficult to eradicate, thus necessitating indefinite antiretroviral therapy. A large number of studies suggest that CD4 T cells represent the major, and possibly the only cell type supporting HIV-1 long-term persistence. However, the ability to serve as long-term viral reservoirs may be confined to certain subpopulations of CD4 T cells with specific functional and developmental characteristics that HIV-1 can selectively exploit to propagate long-term viral survival within the host. Identification of CD4 T cell subtypes that serve as hotspots for viral persistence may be critical for designing strategies to purge the immune system of persisting viral reservoirs.

Recent findings—Developmentally immature, long-lasting CD4 memory T cell populations seem to contain the majority of latently HIV-1 infected cells that persist despite antiretroviral therapy in the peripheral blood. Emerging data suggest that functional polarization towards a Th17, a T follicular helper cell or a regulatory T cell lineage may also be associated with an increased ability to serve as a viral reservoir site. Atypical T cells such as $\gamma\delta$ CD4 T cells or tissue-resident memory CD4 T cells may be predestined to serve as sites for HIV-1 persistence in specific tissues, but will require additional exploration in future studies.

Summary—Recent advances have increased awareness for the profound diversity and complexity of CD4 T cell subpopulations serving as sites for HIV-1 persistence. Continuous technological and methodological improvements to interrogate viral reservoirs in distinct CD4 T cell subpopulations may allow to define a more complete landscape of the HIV-1 reservoir composition in different T cell subpopulations.

Keywords

HIV-1; reservoirs; persistence; CD4 T cells

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Conflicts of interest

The authors declare that financial conflicts relevant to this manuscript do not exist.

Introduction

The ability to establish a long-lasting viral reservoir in CD4 T cells that harbor replication-competent virus and can initiate rebound viremia during antiretroviral treatment interruption represents a distinct aspect of HIV-1 pathogenesis, and a critical factor contributing to our current inability to cure HIV-1 infection. Viral persistence in CD4 T cells during antiretroviral therapy is mostly attributed to latently infected CD4 T cells, which contain a transcriptionally silent form of HIV-1 that is not susceptible to antiretroviral drug effects or immune factors, and remains remarkably stable over prolonged periods of suppressive antiretroviral therapy [1]. Indeed, longitudinal viral outgrowth assays demonstrated that the half-life of latently infected resting cells containing replication-competent virus is approximately 40 months [2-4]. These findings have been corroborated by studies showing that the number of HIV-1-infected cells, as determined by HIV-1 DNA levels in CD4 T cells, typically declines during the initial 1-3 years of antiretroviral therapy, and then reaches a stable level that does not further decline during subsequent treatment [5-6]. Traditionally, resting CD4 T memory cells have been regarded as the main T cell population harboring latent HIV-1; these cells are presumably infected at the time of activation during antigen encounter, and viral latency occurs subsequently when a small proportion of these activated cells reverts to a resting condition during the contraction phase of the T cell response. However, such a pattern has recently been challenged by experiments showing that viral latency is not limited to resting and quiescent CD4 T cells, but can also be established in activated and proliferating cells [7], suggesting that the number and type of CD4 T cells able to support viral latency is larger than initially assumed. Indeed, immense progress has been made over the last years in discovering a vast functional and phenotypic heterogeneity within CD4 T cells, in particular the memory CD4 T cells pool [8-10], and it is likely that these different T cell populations differ in their ability to serve as long-term viral reservoirs. The identification of CD4 T cells that serve as hotspots for viral long-term persistence during antiretroviral therapy represents an area of active investigation, and may have direct consequences for designing strategies to reduce residual reservoirs of HIV-1 infected cells in ART-treated patients.

CD4 T cell diversity

The increased recognition of the complexity and functional diversity within the memory CD4 T cell pool represents one of the most significant advances in immunology research for the last several years. Since the early discrimination between effector-memory and central-memory CD4 T cells [11], the developmental profile of memory T cell has been increasingly well conceptualized, and is currently best characterized as a linear program during which highly immature, long-lasting memory T cell progressively transition towards more mature, differentiated and short-lived effector-memory cells [12]. The most primitive memory T cells are likely to consist of a small population of cells that express a mix of naïve and memory cell markers, maintain their pool size through homeostatic self-renewal, and can give rise to more committed memory T cells, likely in a sequential hierarchical order that includes central-memory, transitional-memory, effector-memory and terminally-differentiated T cells [13-14]. From a functional perspective, memory T cells have been classified into an array of different cell populations, each of them defined by specific

functional antimicrobial properties, distinct cytokine secretion patterns, and expression of signature transcription factors [15]. Based on these criteria, T cell subpopulations with the following functional polarizations have been distinguished: Th1 (antibacterial immune defense), Th2 (immune protection against helminths and other parasites), Th17 (immunity against extracellular bacterial and fungal infections), Th9 (regulation of allergic inflammation, anti-tumor and anti-parasitic immunity), regulatory T cells (fine-tuning, modulation and regulation of antimicrobial immune activity), and follicular T helper cells (support of humoral immunity). In addition to categorizing cells according to developmental and functional properties, certain atypical, unconventional or newly-discovered CD4 T cell populations have been identified and may be relevant for the composition and structure of cell subsets harboring HIV-1 in patients treated with suppressive antiretroviral therapy. These cells include $\gamma\delta$ T cells, which instead of the typical $\alpha\beta$ T cell receptor express a distinct pattern-recognition-like $\gamma\delta$ T cell receptor and have important functional roles for recognition of lipid antigens, without requiring antigen presentation by MHC complexes [16]. Moreover, recent discoveries highlighted the presence of distinct subsets of tissue-resident memory CD4 T cells, which have been detected within barrier tissues at interfaces with the environment, but also in non-barrier tissues such as brain, kidney and joints [17].

Long-lived memory CD4 T cell subsets

The concept that primitive and developmentally immature CD4 T cells, in particular central-memory CD4 cells, serve as a predominant site for the persistence of HIV-1 during treatment with antiretroviral therapy was first raised by Chomont et al [18]. These authors demonstrated that central-memory CD4 T cells, which frequently represent the largest fraction of the memory CD4 T cell pool, contain high levels of HIV-1 DNA, and make the most pronounced contribution to the viral reservoir in memory cells in many patients receiving suppressive antiretroviral therapy, specifically in those with normal CD4 T cells counts after treatment-related immune reconstitution. Persistence of HIV-1 infection in this cell subsets seems to be facilitated by the increased half-life of these cells, which based on in vivo ^2H -glucose and BrdU labeling studies clearly exceeds the lifespan of effector-memory T cells, and approximates the elimination rate of quiescent naïve T cells [19]. Moreover, preferential persistence of HIV-1 in central-memory cells appears to be supported by the increased ability of these cells to proliferate homeostatically, which, specifically when driven by IL-7, can amplify the number of HIV-1 infected cells by a mechanism that is independent of de-novo infection [20]. Notably, experimental in vitro models of latently-infected central-memory CD4 T cells demonstrated that clonal proliferation of central-memory T cells is not necessarily associated with viral reactivation or expression of viral antigens, suggesting that proliferative expansion of these cells is unlikely to be restricted by antiviral immune mechanisms [21]. The important role of central-memory CD4 T cells as a reservoir site for persisting virus is further supported by recent findings from patients who spontaneously maintained undetectable levels of HIV-1 replication in the absence of antiretroviral therapy, after an initial period of suppressive therapy during early stages of disease [22]. Interestingly, this remarkable condition was associated with very low levels of residual HIV-1 infected central-memory CD4 T cells, which represents a sharp contrast to the majority of patients treated with antiretroviral therapy. A cell population that also seems

to have an important role as a long-term reservoir for latently-infected cells consists of the recently discovered population of T memory stem cells. These cells seem to correspond to a slightly earlier developmental stage of memory T cells than central-memory CD4 T cells, and their abilities to persist long-term and proliferate homeostatically seem more pronounced [14]. Two studies [23,24] suggested that T memory stem cells may serve as an extremely long-lasting site for HIV-1 persistence in ART-treated individuals, and calculated the estimated half-life of HIV-1 DNA in these cells to reach about 277 months (as opposed to 144, 133 and 88 months for central-memory, transitional-memory and effector-memory T cells, respectively). Notably, the protracted decay of HIV-1 DNA in T memory stem cells led to a relative overrepresentation of viral reservoirs in T memory stem cells after prolonged periods of antiretroviral therapy, consistent with a progressive contraction of the viral reservoir around a remarkably stable population of long-lasting central-memory and T memory stem cells. Interestingly, recent studies suggest that T memory stem cells do not only represent a reservoir for HIV-1, but may also support persistence of alternative retroviruses such as HTLV-1, particularly in individuals developing HTLV-1-associated malignancies (such as adult T cell leukemia). Specifically, HTLV-1-infected T memory stem cells may represent a population of long-lived cancer stem cell-like cells with critical roles for maintaining and propagating HTLV-1 associated malignant disease [25].

CD4 T cell polarization

Functional polarization of CD4 T cells occurs under the influence of signature cytokines, and leads to the acquisition of effector function specializing in distinct aspects of antimicrobial immune defense. This process is regulated by master transcription factors that determine characteristic gene expression changes in each polarized cell population. A number of observations suggest that their functional polarization of T cells may affect the ability to serve as viral reservoirs during suppressive antiretroviral therapy. Most notably, some of the master transcription factors influencing T cell polarization, including Bcl-6 [26], FoxP3 [27] and Gata3 [28], are able to directly bind to the HIV-1 promoter, and by this mechanisms may affect HIV-1 gene expression and latency. In addition, it is well recognized that functional polarization of T cells is also associated with a developmental and maturational aspect that influences cellular longevity and long-term survival [29].

Most notably, several lines of evidence suggest that Th17 cells represent long-lasting lymphocytes that can proliferate in a homeostatic, stem cell-like fashion and have a high grade of developmental plasticity that allows them to serve as precursor cells for more differentiated CD4 T cells of the same or alternative polarization [29-31]. Consistent with this observation, increasing evidence suggests that Th17 cells have a more prominent role in serving as a viral reservoir site. For instance, Th17 cells seem to be intrinsically more susceptible to HIV-1 infection in ex-vivo assays [32], likely due to specific gene expression signatures resulting in a cellular microenvironment able to effectively support individual viral replication steps [33]. Moreover, in ex-vivo assays, CD4 T cells enriched for a Th17 polarization from individuals treated with suppressive antiretroviral therapy were found to harbor high levels of HIV-1 DNA with increased longitudinal stability over prolonged periods of antiretroviral therapy, while decay rates of HIV-1 DNA in Th1 cells were faster [34]. This corresponded to phylogenetic data showing higher longitudinal persistence of

identical viral sequences in Th17 cells compared to cells of alternative polarization. However, it is important to recognize that in these currently available studies, polarization of Th subsets was determined based on surface expression patterns of chemokine receptors, and not on the secretion profile of signature cytokines.

In addition to Th17 cells, T follicular helper cells are regarded as a possible cellular compartment supporting HIV-1 persistence. These cells are characterized by surface expression of CXCR5 and PD-1, reside in lymph node follicles in immediate anatomical proximity to B cells and support the germinal center reaction essential for the generation of effective humoral immunity [35]. T follicular helper cells seem highly susceptible to infection with HIV-1, and represent a major site for HIV-1 replication and production during untreated HIV-1 infection [36]. Whether these cells also represent a site of viral persistence during suppressive antiretroviral therapy is an area of active investigation. Notably, data from prior studies suggested a sharp decline of HIV-1 DNA levels in TFH within 1-2 years of suppressive antiretroviral therapy [36]; no data are currently available to determine the long-term stability of virally-infected T follicular helper cells during suppression with antiretroviral therapy. Recent studies identified circulating CXCR5+ PD-1+ cells as peripheral blood counterparts of TFH that share many of their functional properties, including the ability to secrete IL-21 and to support immunoglobulin class switching [37]. To what extent circulating CXCR5+ TFH-like cells serve as a viral reservoir site is unknown at present, although initial studies demonstrated detectable HIV-1 DNA in these cells in the majority of individuals treated with ART [34].

CD4 T cells polarized towards a regulatory phenotype express FoxP3 as a master transcription factor and are known for a dichotomous role in HIV-1 pathogenesis that may reduce pathologic HIV-1 immune activation and simultaneously inhibit beneficial antiviral immunity. Interestingly, in one prior study, regulatory CD4 T cells from ART-treated patients were shown to contain more abundant HIV-1 DNA levels compared to alternative CD4 T cells, and the half-life of these HIV-1-infected regulatory T cells was estimated to last about 20 months [38]. Whether these cells contain replication-competent virus that can contribute to rebound viremia is currently unknown.

Alternative, non-conventional T cells

Progress over the recent years has demonstrated that HIV-1 may also be able to reside in atypical CD4 T cells that were not included in initial assessments of viral reservoirs. These include $\gamma\delta$ T cells, which constitutively express low levels of the CD4 receptor and may for that reason represent less preferred target cells for HIV-1. Hypothesizing that elevated immune activation during chronic HIV-1 infection may increase CD4 expression intensities in $\gamma\delta$ T cells to render these cells susceptible to HIV, a recent study found that peripheral blood $\gamma\delta$ T cells from ART-treated patients contain HIV-1 DNA in quantities that clearly exceed those of resting CD4 T cells [39]. Moreover, replication-competent virus was retrieved from the majority of these cells in viral outgrowth assays, suggesting that these cells may indeed contribute to persistence of fully-functional virus despite antiretroviral therapy. Tissue-resident memory CD4 T cells represent a newly-defined subset of CD4 T cells that includes a large portion of lymphocyte populations in diverse peripheral tissue

sites, including mucosal tissues, barrier surfaces, and in other non-lymphoid and lymphoid sites. Whether these cells can serve as viral reservoirs remains to be more definitely investigated in future studies, but currently available data show that at least in adipose tissues, tissue-resident memory CD4 T cells are able to represent a site for viral persistence [40-41].

Conclusion

Identifying cellular subsets that contribute to the persistence of HIV-1 in patients treated with effective antiretroviral therapy is one of the chief prerequisites for developing strategies to completely eradicate or cure HIV-1 infection. The growing understanding of the full diversity of the CD4 memory T cell compartment has led to significant advances in characterizing cellular populations and subpopulations in which HIV-1 persists during treatment. Many of these cell subsets are very rare, which poses unique methodological challenges for investigating the role that these cells may play as a viral reservoir site. The use of novel technologies for studying viral sequence integrity and replication-competence [42] in very small cell subsets may pave a promising way to obtain a more complete and detailed understanding of the heterogeneity of HIV-1 reservoir cell subsets in future studies. Moreover, improvements in techniques to analyze clonal expansion of HIV-1 infected cells through next-generation sequencing of viral integration sites [43-45] in distinct subsets of T cells would be highly informative. Finally, the investigation of viral reservoirs in tissue-based CD4 T cell subsets represents a particular difficulty in humans, due to the limitations in tissue material that can be collected in biopsies; therefore studies related to tissue reservoir cells may to a large extent rely on animal models in the foreseeable future.

Acknowledgments

Financial support and sponsorship

M. L. is supported by NIH grants AI120008, AI114235, AI 106468, AI098487, AI117841.

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Key points

- CD4 T cells represent the predominant, and possibly the only cell type serving as a long-term HIV-1 reservoir
- The ability to serve as a site for viral long-term persistence may be limited to subpopulations of CD4 T cells with specific developmental and functional characteristics
- CD4 central-memory and T memory stem cells seem to represent the most durable sites for HIV-1 persistence in peripheral blood
- Increasing data suggest that Th17 cells and T follicular helper cells represent important viral reservoirs, specifically in lymphoid tissues.