REVIEW

OPEN

HIV persistence in lymph nodes

Riddhima Banga, Olivia Munoz, and Matthieu Perreau

Purpose of review
HIV persists in distinct cellular and anatomical compartments in the body including blood, Central nervous system, and lymphoid tissues (spleen, lymph nodes [LNs], gut-associated lymphoid tissue) by diverse mechanisms despite antiretroviral therapy. Within LNs, human and animal studies have highlighted that a specific CD4 T cell subset - called T follicular helper cells locating in B cell follicles is enriched in cells containing replication-competent HIV as compared to extra-follicular CD4 T cells. Therefore, the objective of the present review is to focus on the potential mechanisms allowing HIV to persist within LN microenvironment.

Recent findings
The combination of factors that might be involved in the regulation of HIV persistence within LNs remain to be fully identified but may include - the level of activation, antiretroviral drug concentrations, presence of cytolytic mechanisms and/or regulatory cells, in addition to cell survival and proliferation propensity which would ultimately determine the fate of HIV-infected cells within LN tissue areas.

Summary
HIV persistence in blood and distinct body compartments despite long-standing and potent therapy is one of the major barriers to a cure. Given that the HIV reservoir is established early and is highly complex based on composition, viral diversity, distribution, replication competence, migration dynamics across the human body and possible compartmentalization in specific tissues, combinatorial therapeutic approaches are needed that may synergize to target multiple viral reservoirs to achieve a cure for HIV infection.

Keywords
HIV persistence, HIV reservoir, HIV transcription, lymph node, replication-competent HIV, T follicular helper

INTRODUCTION

The development and availability of potent antiretroviral therapy (ART) have revolutionized the ability to control HIV replication, reduce HIV-associated mortality and morbidity [1] as well as viral transmission [2] and has thus contributed to lower the number of newly infected individuals [2]. However, most of ART-treated HIV-infected individuals interrupting therapy experience HIV viremia rebound within 2–3 weeks [3], demonstrating that ART does not cure HIV infection and replication-competent HIV persists despite ART [4–6]. Therefore, identifying specific cell and tissue compartments harboring replication-competent HIV is a priority for the eradication of HIV-infected cells without damaging surrounding cells and tissues.

Tremendous efforts have been dedicated during the last 15 years in HIV cure research to underscore the cellular locations for HIV persistence in the face of ART. In particular, pioneering studies focused on the identification and characterization of cell subsets harboring replication-competent virus in blood because of (1) the relative ease of collection and (2) the assumption that blood would be representative of the frequencies and phenotype of infected cell subsets in lymph node (LNs) given the physiologic free exchange of cells between the two compartments [4–6]. Most of these studies applied flow cytometry combined with polymerase chain reaction-based and histopathological assays and demonstrated the presence of HIV-integrated deoxyribonuceleic acid (DNA) or inducible replication-competent virus within various blood CD4 T cell subsets including resting memory CD4 T cells (HLA-DR$^-$CD25$^-$CD69$^-$) [5,7] central memory (CM; defined by CD45RA$^-$ CCR7$^+$CD27$^+$) and transitional memory (TM;

Divisions of Immunology and Allergy, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland

Correspondence to Matthieu Perreau, PhD, Division of Immunology and Allergy, Lausanne University Hospital, Lausanne, Switzerland.
Tel: +41 213141061; e-mail: Matthieu.Perreau@chuv.ch

Curr Opin HIV AIDS 2021, 16:209–214

DOI:10.1097/COH.0000000000000686

KEY POINTS

- Animal models and human studies have demonstrated that B cell follicle resident Tfh CD4 T cells are enriched in HIV/SIV-infected cells as compared to other CD4 T cells in blood and LNs. However, the underlying mechanisms for the enrichment of the viral reservoir within these cells remain unclear.
- Several factors may account for the enrichment of HIV/SIV infected cells within Tfh cells as compared to other blood/LN CD4 T cell subsets, including a longer intrinsic life span of Tfh cells, preferential clonal expansion of Tfh cells that harbor HIV/SIV or a shielding of Tfh cells from cytolytic elimination and/or regulation by modulatory dendritic cells that reside in extra-follicular areas.
- Another possibility for enrichment of cells containing replication-competent virus within Tfh compartment stems from the possibility of the presence of low-level ongoing viral replication in B cell follicles under sub-optimal drug concentrations. It is likely that the genetic signatures of recently infected cells that would provide evidence of ongoing replication in Tfh cells during ART are missed due to the sampling depth used in current studies, and therefore warrants the development of more sensitive assays/longitudinal assessments within the same patient to address this phenomenon.

CD45RA$^-$CCR7$^-$CD27$^+$) CD4 T cells [8], CD4 T cell populations expressing PD-1 [9], LAG-3 [9], TIM-3 [9], CXCR3 [10], CD32 [11–15], CCR6 [16,17], CD30 [18], and/or CD20 [19]. In parallel, the application of new and improved methods such as mass cytometry, imaging platforms and fine needle biopsies – that provide a minimally invasive means of longitudinally accessing tissues has been instrumental in advancing our knowledge of tissue reservoirs. These studies have revealed that in contrast to the free exchange of infected cells theory, LNs represent distinct compartments containing phenotypically and functionally specialized cell subsets as compared to blood which may allow the persistence of HIV-infected cells and/or higher frequencies of latently infected cells capable of producing inducible replication-competent virus within these sanctuary sites [20,21]. Indeed, LNs are dynamic and highly structured tissues, consisting of strategically prepositioned LN resident cells within micro-anatomical niches and recirculating cells. The differential location of LN cell subsets within the micro-anatomical niches is associated with distinct cell phenotypes and molecular and functional signatures. In this context, NHP models have been crucial in revealing viral reservoir dynamics, especially due to the ability to perform longitudinal assessments of LN tissues. These studies showed that the frequencies of simian immunodeficiency virus (SIV) DNA containing CD4 T cells were consistently detected in cells located in LN follicular and extra-follicular areas in SIV-infected elite controller macaques [20] and ART suppressed macaques [22]. Similarly, HIV DNA containing CD4 T cells were also consistently detected in cells located in LN follicular and extra-follicular areas in of viremic and long-term ART-treated HIV-infected individuals [10,23,24]. However, transcriptionally active cells were mainly restricted to the specialized LN CD4 T cell subset called follicular helper CD4 T cells (Tfh) which preferentially localize in germinal centers (GCs), in close proximity to follicular dendritic cells (FDC) network, GC dark zone, and GC B cells [25] in HIV viremic controllers [26], SIV-infected elite controller macaques [20] and ART-treated aviremic HIV-infected individuals [21]. Notably, Tfh cells represent the major cellular compartment for HIV production and replication in viremic individuals [27] and the major CD4 T cell population for persistent HIV-1 transcription in long-term treated individuals [21] as compared to any other blood or LN memory CD4 T cell populations. In this regard, the present review focuses on the numerous studies that highlighted the multiple mechanisms which may contribute to favor HIV persistence in LN tissues (Fig. 1).

MAINTENANCE OF THE LYMPH NODE RESERVOIR BY CLONAL PROLIFERATION

A large body of evidence supports the likelihood of maintenance of HIV-infected cells containing replication-competent viruses through both long-term survival of the cells and homeostatic/antigen-driven or integration site-driven clonal proliferation, without necessarily inducing viral expression [28–34]. To address whether cell-proliferation could be one of the mechanisms favoring the enrichment of HIV-infected cells within LN cells, studies have analyzed phylogenetic and/or integration site of proviruses within distinct memory CD4 T cells in paired blood and/or LNs of elite controllers [35] and/or ART-treated HIV-infected individuals [10,36▪▪,37–39]. In principle, since HIV-1 integrates almost randomly into many sites in the human genome, finding multiple cells with exactly the same integration site is strongly indicative of these cells being descendants from a single infected cell. On this basis, recent studies indicated that (1) clonal expansion was found in both blood and LN compartments, including Tfh cells and was more prominent in chronic and ART-treated individuals and (2) a lack of compartmentalization of proliferating clones within the Tfh compartment [10,36▪▪,37].

Taken together, these observations suggested the possibility of infection of a progenitor cell, clonal

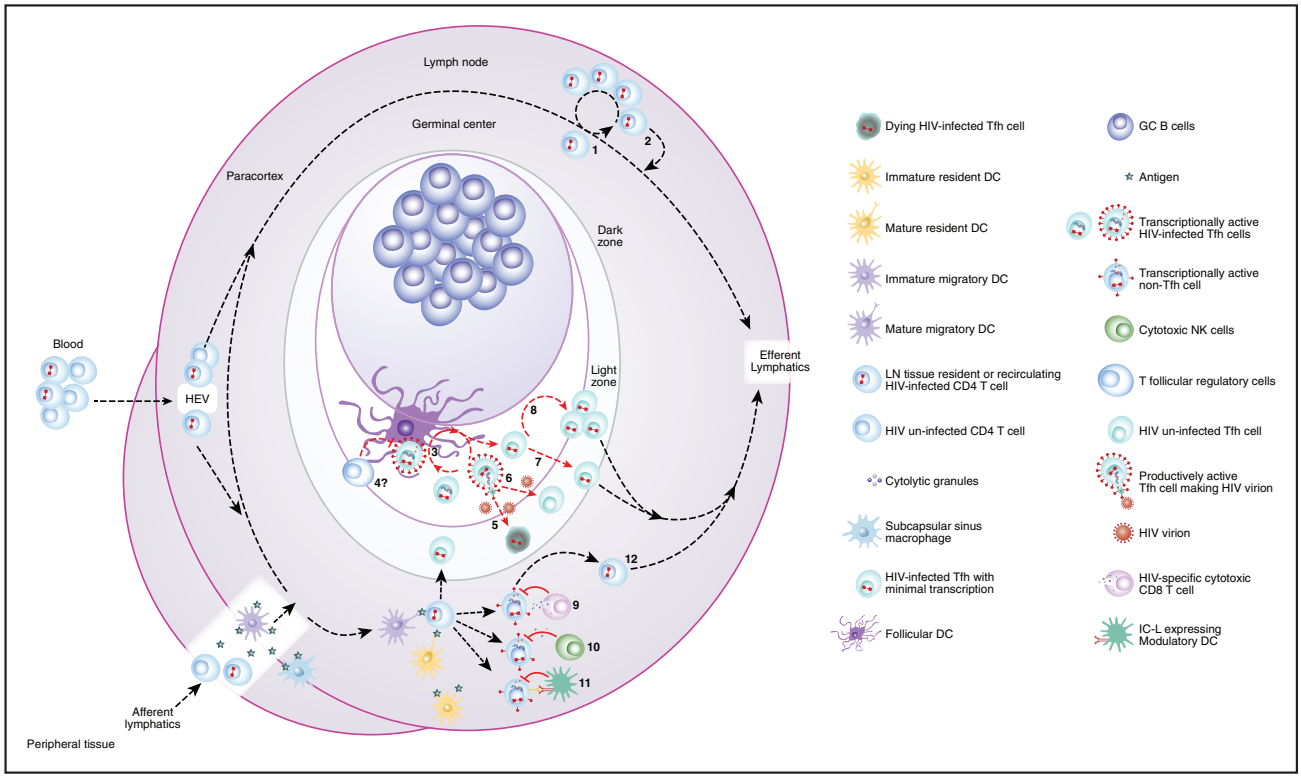

FIGURE 1. Schematic representation of potential mechanisms allowing HIV-persistence within lymph nodes: CD4 T cells trafficking in lymph node (LN) *via* the afferent lymphatics or from HEVs are referred to as 're-circulating' cells. Distinct possibilities allowing HIV-persistence within LNs include: (1) LN resident or recirculating HIV-infected CD4 T cells persist by long-term survival; (2) LN resident or recirculating HIV-infected CD4 T cells persist by homeostatic proliferation; LN resident or recirculating HIV-infected CD4 T cells are activated in the paracortex by antigen-loaded mature migratory dendritic cells (DCs) or LN mature resident DCs, and differentiate into either Tfh cells which enter germinal center (GC) or into non-Tfh cells that locate in the paracortex. HIV-infected Tfh cells may have distinct fate in GCs based on differential drug concentrations and cytopathic elimination [3–8]. In the paracortex, HIV-infected CD4 T cells may have distinct fate that might be influenced or not by cytopathic elimination and regulatory cells [9–12]. Within GCs, (3) HIV-infected Tfh cells are transiently transcriptionally active and not eliminated; (4) The role of Tfr cells in regulating HIV transcription remains to be determined; (5) HIV-infected Tfh cells are productively infected and are eliminated by viral cytopathic effects; (6) productively infected Tfh cells *de novo* infect un-infected Tfh cells under suboptimal drug concentration; (7) HIV-infected Tfh cells persist by long-term survival with minimal viral transcription; (8) HIV-infected Tfh cells persist by homeostatic proliferation with minimal viral transcription. Tfh cells reverting to memory state leave the GCs and may recirculate in blood. Within paracortex, (9) HIV-infected transcriptionally active CD4 T cells may be recognized and eliminated by HIV-specific CD8 T cells; (10) HIV-infected transcriptionally active CD4 T cells may be recognized and eliminated by cytotoxic NK cells; (11) HIV-infected transcriptionally active CD4 T cells may be modulated by immune checkpoint ligand (IC-L) expressing migratory DCs; (12) HIV-infected transcriptionally active CD4 T cells escaping cytopathic elimination may revert to latency and may or may not leave the LNs.

proliferation followed by the dissemination of proliferating clones within the two compartments. Recently developed assays including - simultaneous detection of matched integration site analysis and near-full-length proviral sequencing assays (MIP-Seq) [40] and/or microfluidic method to sequence entire proviruses in their native integration site (SIP-seq) [41] may further provide an in-depth characterization of the integrated viral reservoir that is necessary to address the contribution of clonal expansion to HIV persistence and viral rebound [42]. These initial observations also point to alternate mechanisms such as the epigenetic landscape of Tfh cells that could be associated with the higher inducibility of replication-competent virus within Tfh cells in viral outgrowth assays, that remains to be determined in future studies.

ROLE OF ONGOING VIRAL REPLICATION

Accumulating pharmacokinetic evidence of commonly used antiretrovirals has showed that the LN concentrations of some drugs were much lower compared to the concentrations achieved in blood

[43–45], raising the possibility of viral production and low-level viral replication to contribute to HIV persistence in LNs [43,46,47]. To address the contribution of ongoing viral replication to HIV persistence in LNs, multiple studies assessed the attributes of genetic evolution in proviral DNA sequences isolated from LN CD4 T cells compared to ancestral viruses obtained from pretherapy plasma, the presence of which would reflect cycles of error-prone reverse transcription, production and replication [46,48,49]. Although these studies revealed lack of viral evolution in LNs under therapy [37,50▪], it is difficult to formally rule out the possibility of infrequent replication events based on the currently available data generated through phylogenetic techniques of sampling tissues at only one time-point under ART. In this context, longitudinal assessment within the same patient, of viral evolution of proviral DNA sequences within sorted LN CD4 T cell subsets at distinctly spaced time-points under therapy and upon intermittent breaks in therapy might further support or not these conclusions. In addition, it is also likely that virus genetic signatures of recently infected cells that would provide evidence of ongoing replication during ART are easily missed due to the sampling depth of these studies that rely either on unfractionated cells, few sorted cells directly *ex vivo*, or on narrowly defined circulating cell-counterparts assumed to be found in blood. Furthermore, a study based on mathematical modeling also proposed the possibility that ongoing replication might be - at least in part –fuelled by cell-to-cell spread of infections transmitting virion numbers much in excess of what is required to infect a cell in the absence of or at low drug concentrations within LNs [49]. Taken together, whether or not viral production and low-level replication occur needs to be further investigated [44].

ROLE OF LYMPH NODE MICROENVIRONMENT

The presence of cells expressing HIV genes in LNs could also be a consequence of a reactivation event due to the lower degree of restriction of viral expression in B cell follicles. In this context, the physiological homing properties of Tfh cells via the chemokine receptor CXCR5 [27,51,52] - within B cell follicles in LNs might provide them a transient and a relative privilege that could favor viral transcription in these cells particularly within these areas as compared to extra-follicular CD4 T cells. This privilege might result from the fact that B cell follicular microenvironment is a unique environment where (1) the specific cytokine enrichment - such as IL-10 enrichment contributes to induction of Tfh differentiation, thus providing increased targets for HIV infection [53▪▪] and (2) FDC-bound/retained HIV during therapy, could be a source of virions to Tfh cells [3,54–56]. In addition to providing infectious virus, FDCs may contribute to the increase HIV production, contributing to a tissue microenvironment that is highly conducive to HIV transmission and expression through the secretion of TNF-α [57]. This may in-turn increase Tfh cell susceptibility to FDC-bound HIV-IC-mediated infection as compared to extra-follicular CD4 T cells, favoring their increased infection frequencies [57].

The second main feature of GCs is that they are more prone to cell activation and may therefore favor HIV transcription compared to extra-follicular regions. This is supported by recent studies that demonstrated the relative enrichment of immune checkpoint ligand (IC-L) expressing modulatory dendritic cells (DCs) that could efficiently suppress T cell receptor-induced HIV transcription and production, in extra-follicular regions as compared to GC areas [58▪▪]. Moreover, the presence of relatively infrequent polyfunctional HIV/SIV-specific CD8 T cells and NK cells within B cell follicles of monkeys [59], viremic and treated HIV-infected individuals may also contribute to the compromised antiviral clearance in these structures, thereby allowing HIV-infected transcriptionally active cells to persist [20,60–62]. Notably, regulatory T cells and DCs expressing immunomodulatory cytokines such as TGF-β and IL-10 [63] and plasmacytoid DCs secreting type I interferon may also contribute to the suppression of CD4 T cell activation/function and consequently promote viral latency in the LN paracortex [53▪▪,64]. Of note, the regulatory and/or viral control potential of the few (<1%) regulatory CD4 T cells found in the follicles called - T follicular regulatory (Tfr) cells [65] and the few antigen-specific cytotoxic CD8 T cells that locate within the follicles still remains to be confirmed [61,66].

CONCLUSION

In conclusion, a large amount of evidence generated through the use of NHP models or with human samples indicates that LNs represent major HIV tissue reservoirs, in which HIV may persist using multiple nonmutually exclusive mechanisms. The highly specialized cellular organization and compartmentalization create a microenvironment that may facilitate the spread of HIV infection during the viremic phase and may allow viral persistence in sanctuary sites during therapy. A growing body of evidence has revealed that among the various cell types locating in LNs, Tfh cells, a highly differentiated CD4 T cell population localized in

immunological sanctuary sites, i.e., LN GCs serves as a major T cell reservoir of HIV. However, the fact that rebound viremia occurring postanalytical treatment interruption has been shown to arise at multiple sites within LNs with genetically diverse populations of virions is consistent with reactivation from distinct HIV-infected cell subsets in LNs [3]. In this context, the contribution of the less well characterized extra-follicular resident TRegs was recently proposed using SIV-infected macaque model, which highlighted the presence of replication-competent virus within CTLA-4$^+$/PD-1$^-$ memory CD4 T cells and linked their ability to support viral persistence to increased potential of survival through high Bcl-2 expression and homeostatic proliferation [67]. Similarly, the proposition of infection of non-CD4 T cell subsets such as myeloid cells encompassing macrophages [68] and DCs [69] in different body tissues during ART warrants a deeper and broader evaluation of these potential cellular reservoirs in the LN compartment as well. Ultimately, if heterogeneous cellular reservoirs persist in LNs, HIV cure strategies might need to be adapted accordingly to purge these distinct viral reservoirs to achieve a cure for HIV infection.

Acknowledgements

We would like to thank Aaron Weddle for his assistance in figure preparation.

Financial support and sponsorship

This work was supported by Swiss National Science Foundation Grants 320030_173071 and 320030_200912 to Prof. Matthieu Perreau and by Swiss life and Foundation Machaon to Dr Riddhima Banga.

Conflicts of interest

There are no conflicts of interest.

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