Impact of Chronic HIV/SIV Infection on T Follicular Helper Cell Subsets and Germinal Center Homeostasis

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The discovery of broad and potent HIV-1 neutralizing antibodies (bNAbs) has renewed optimism for developing an effective vaccine against HIV-1. The generation of most bNAbs requires multiple rounds of B cell receptor affinity maturation, suggesting a crucial role of follicular helper T (Tfh) cells in their production. However, less than 1% of HIV-infected patients develop bNAbs that arise late in the course of infection, indicating probable Tfh and B cell dysfunctions in this context. Since the last few years, many studies have characterized Tfh cells from lymph nodes and spleen of HIV-infected individuals and SIV-infected macaques. Various lymphoid Tfh cell subsets have been identified, including precursor Tfh (pTfh), germinal center Tfh (GC Tfh), and the regulatory counterpart of Tfh cells, the follicular regulatory T cells. The latter have been reported to play a crucial role in the control of T and B cell crosstalk and GC reactions. More recently, circulating Tfh-like cells (cTfh) have been identified. Meanwhile, advances in single-cell technologies have made possible to analyze the transcriptional profiles of low abundant cells, such as Tfh populations. Using transcriptional signatures, we review here the impact of chronic SIV/HIV infection on Tfh, GC Tfh, pTfh, and cTfh differentiation and helper T cell functions with regard to their capacity to induce efficient B cell maturation. We will explore some hypothesis to explain the increased proportion of Tfh cells reported in chronically infected individuals and the impact on HIV pathogenesis.

Keywords: HIV, SIV, Tfh cell differentiation, Tfh cell dynamics, germinal center reaction

INTRODUCTION

In germinal centers (GC), T follicular helper (Tfh) cells deliver helper signals and cytokines required for B cell affinity maturation and B cell differentiation into long-lived plasma cells. Optimal Tfh and B cell crosstalk is a prerequisite for the induction of efficient humoral immunity to pathogens. By providing survival and differentiation signals, Tfh cells control multiple steps of B cell maturation and antibody (Ab) production.

In addition to the cognate antigen interaction with B cells, Tfh cells express costimulatory molecules, such as CD40L, ICOS, and OX40. Tfh cells secrete high levels of interleukin-21 (IL-21) and IL-4, which are necessary for GC formation and B cell differentiation into long-lived plasma cells, respectively (1–3).

Among tissue-resident Tfh cell subsets, early committed precursor Tfh (pTfh) and germinal center Tfh (GC Tfh) represent two different stages of the Tfh cell maturation. Follicular regulatory
T (Tfr) cells are identified as the regulatory counterpart of Tfh cells. Tfr cells control T and B cell crosstalk and GC reactions. Blood circulating Tfh cells (cTfhs) have been recently identified as a memory compartment of tissue-resident Tfh cells. Like tissue-resident Tfh cells, cTfhs are endowed with the capacity to produce IL-21 and to provide B cell help (4).

Since the last 5 years, Tfh cells have been extensively studied in the lymph nodes (LNs) and spleens of individuals with chronic HIV/SIV infection. HIV infection is associated with an altered B cell differentiation (5) and Tfh isolated from LNs of HIV-infected (HIV+) individuals provide inadequate B cell help in vitro (6). As lymphoid tissue-resident Tfh cells are targeted by HIV/SIV early after infection, they constitute a major compartment for HIV infection, replication, and production of viral particles in LNs of viremic individuals (7–9), even though in vivo production of viral particles by Tfh cells remains to be demonstrated. Likewise, in blood, within central memory CD4T cells, cTfh cells serve as HIV reservoir in chronic HIV-infected individuals under antiretroviral therapy (10). Very recently, in natural HIV controllers, study of HIV infection in various CM4 T cell subsets demonstrates various mechanism of HIV persistence according to the CD4 T cell compartment (11). LN-resident helper T cells (Tfh and non-Tfh) showed replicative virus, while clonally expanded blood CD4 T cells harbor inducible provirus (11). However, despite their high susceptibility to HIV/SIV infection, many studies reported an accumulation of tissue-resident or cTfh populations during the chronic phases of infection (7, 8, 12, 13). In addition, Hong et al. demonstrate that after a rapid expansion of GCs during the acute phase, slowly proliferative Tfh cells accumulate during the chronic phase of SIV infection (14).

Various hypotheses can support the higher proportions of Tfh cell subsets in the context of chronic HIV infection: (i) Tfh cells might present high proliferative or survival capacities; (ii) antigen persistence could drive CD4 T cells toward Tfh differentiation; and (iii) regulatory cells that control the Tfh/B cell crosstalk might be defective.

Here, we propose to review recent studies based on transcriptional analysis of Tfh cell subsets and to discuss the potential consequences on GC deregulations reported in chronic HIV/SIV infection.

**POTENTIAL IMPACT OF HIV INFECTION ON Tfh CELL DIFFERENTIATION**

The signals involved in Tfh cell differentiation include TCR activation, costimulation, cytokines, and migration-associated molecules. However, the origin of Tfh cells is not well defined in humans: it is not clear whether Tfh fate is established at the time of DC priming or later. Here, we review the impact of HIV infection on Tfh cell differentiation, from the priming of CD4 T cells by DCs cells until their ultimate stage of differentiation corresponding to GC Tfh and circulating memory Tfh. Two distinct differentiation pathways have been described (Figure 1).

The linear multistage Tfh differentiation pathway implicates multiple antigen-specific interactions in secondary lymphoid organs: (i) DC priming of naïve T cells leads to the rise of pTfh cells expressing CXCR5 molecule; pTfh cells migrate toward the T/B cell border zone where they experience (ii) a second antigen-specific interaction with B cells. This interaction leads...
to the progression of pTfh cells within the B cell follicle and differentiation into Th cells. (iii) In the B cell follicle, Th cells experience multiple interactions with B cells, leading to B cell maturation and the complete differentiation of Th cells into GC Th. Thus in this model, B cells appear central in the terminal differentiation of Th cell into GC Th and reciprocally, Th are also required for B cell maturation. As with other helper T cell subsets, the stimulatory cytokines produced by DCs during the priming of naïve T cells are critical parameters of Th cell differentiation. Using monocyte-derived DCs (MoDCs), Schmitt et al. demonstrated the key role of IL-12-producing MoDCs in the induction of IL-21-producing Th-like cells (15). More recently, the same group found that TGF-β acts together with IL-12 and IL-23 to induce the expression of various molecules associated with Th functions by human naïve helper T cells, including CXCR5, ICOS, IL-21, Bcl-6, and the transcription factors BATF and c-Maf (16). However, little is known about the type of DCs responsible for inducing Th cell priming. A recent study reported that engagement of DC-SIGN by fucose-based PAMPs licenses DCs for inducing Th polarization (17). Such activated DCs produce IL-27, which is essential for Th polarization. This finding highlights the importance of adjuvants in the induction of Th cells. Interestingly, HIV particles bind DC-SIGN through Gp120, the viral envelope (18). Therefore, one can hypothesize that, under chronic HIV infection, interactions between HIV particles and DC-SIGN expressing DCs could support the Th helper cell differentiation toward a Th polarization. A recent study also showed that treatment with CpG (TLR-9 ligand) induces IL-6 production by MoDC, orientating helper T cell differentiation toward the Th-cell lineage (19). Indeed, by inducing Bcl6 early during the T cell activation, IL-6 has been shown to be critical for Th polarization (20). In the context of HIV/SIV infection, several groups reported higher plasma levels of IL-6 (13, 21). However, we and others (22) did not find any difference in the amount of secreted IL-6 between HIV-infected and -uninfected spleens upon activation (8). Once engaged into Th differentiation, the sequential differentiation model proposes that pTfh/B cell interactions dictate the fate of Th cells. Several groups highlighted the requirement of antigen presentation by B cells to induce Th cell and in turn GC reactions. In the absence of B cells, DC restricted antigen presentation initiates Th cell differentiation (into pTh) but fails to complete ultimate effector Th cell differentiation (23). In a model of bone marrow chimera, B cells deficient for the expression of MHC-II-molecules exhibit a reduced capacity to initiate T cell expansion and differentiation (24). In fact, sequential antigen-specific interactions of Th cell with DC and B cells are required to initiate Th cell and GC differentiation (25), and antigen persistence sustains Th responses and GC reactions (26). Using live multiphoton imaging, Schwickert et al. suggested that the amount of peptide–MHC (pMHC) complexes presented by antigen-specific B cells to cognate T cells, at the B-cell–T-cell border, was a limiting factor regulating the entry of B cell clones into GC (27). Furthermore, highlighting the critical role of MHC-II molecules expressed by B cells in the generation of Abs of diverse functions and of memory B-cell responses, B cells lacking MHC-II expression are unable to differentiate into memory cells and are defective in producing antigen-specific IgG (28). These results demonstrate that MHC-II-restricted antigen presentation by B cells is strictly required for B cells to receive help by antigen-specific Th cells, and thus to establish a potent humoral immune response. Therefore, Th cell differentiation and GC development require the combination of DC and B cell antigen presentation.

As DC during T cell priming, B cells also provide additional signals to Th cells, contributing to their helper functions and maintenance. These signals include CD40L/CD40, OX40/OX40L, signaling lymphocyte activation molecule (SLAM) family members, and adhesion molecules that strengthen GC Th/ GC B cell interaction. Interaction between ICOS and ICOS ligand (ICOSL) as well as IL-21 production has been implicated in GC formation (29, 30). PD-1/PD-1 ligand interactions also control Th and GC B cell differentiation (1, 31). Murine Th cells also express the nutrient transporter folate R 4 (FR4) and CD73 (32) although their functional relevance for Th cell differentiation and B cell help has not yet been uncovered. In sum, the sequential differentiation proposes that combined interactions with DC and B cell dictate the fate of Th cells.

The alternative “post effector” developmental pathway proposes that Th-like cells may develop either from the memory CD4 T cell lineage (33, 34) or from effector T helper cell subsets (35, 36), rather than arising from pTh cells. It has been shown that Th and central memory T cells (TCM) are similar in their developmental pathway, including the requirement of Bcl6 and low levels of IL-2 signaling (37). In line with this, Th and TCM gene programs can co-initiate from effector Th1 cells upon increased Bcl-6 expression in response to a decrease of IL-2, resulting in a “Th1/TCM-like” population. IL-7 signaling also acts as a negative feedback that downregulates the differentiation of Th1 into Th-like cells (38). Interestingly, in spleens from HIV-infected individuals with a high proportion of Th cells, we reported a markedly reduced expression of the IL-7r encoding gene in all CD4 T cell populations (8). Taken together these observations support the hypothesis that, in addition to Th cells, other T helper populations may contribute to B cell maturation into long-lived plasma cells. Hence, in humans, the precursors of Th cells might be composed of heterogeneous cell populations, which have the ability to differentiate into distinct types of Th cells. The latter keep some functional imprint from the parental T cell subtype. Of note, in this alternative pathway, interactions with antigen-presenting B cells are still a key event of the Th cell orientation thus raising the question of the impact of Thf infection by HIV on the B cell compartments.

Germinat center Th cells have long been considered as the terminal stage of tissue-resident Th cell differentiation. Newly identified, memory Th cells are preferentially located in secondary lymphoid organs and bone marrow although they can recirculate in the blood. These cTh involve several subsets that differentially support Ab secretion (4) and are related to lymphoid-tissue-resident Th cells by their gene expression profile, cytokine production, and functional properties (39). Recently, adding to the CXCR5 and PD-1 canonical markers, Schultz et al. proposed that cTh can be identified by their ability to produce IL-21, the cardinal Th cytokine (40). Interestingly, activated memory B cells induce rapid re-expression of Bcl6 by
memory Tfh cells (41), reinforcing the concept that many features of Tfh cells are highly linked with those of the B cells. Thanks to their accessibility and relative high frequencies, cTfh cell dynamics and features are the focus of growing interest in the context of infection and vaccination.

**THE FREQUENCY AND FUNCTIONS OF Tfh CELLS ARE TIGHTLY CONTROLLED**

Follicular helper T cell homeostasis is critical to the induction of high affinity Ab responses that are devoid of self-reactivity. Indeed, optimal Tfh cell frequency imposes competition between B cells, thus favoring survival of high affinity B cell clones. Several cell populations maintain Tfh cell homeostasis, including regulatory T (Treg) cells, Tfr cells, CD8 regulatory cells, and plasma cells (42). Tfr cells are identified as the main T cell subset implicated in the control of Tfh cells. They migrate into follicles and directly control GC reaction (43, 44). Hence, many studies have demonstrated increased GC and T cell responses in the absence of Tfr (43, 45, 46). Tfr cells co-express Bcl6 and Blimp-1 (43) that is known to negatively regulate Tfh cell differentiation pathway (47). Indeed, Blimp-1 represses Bcl-6 and reciprocally, which might explain the lower expression of Bcl-6 in Tfr cells as compared to Tfh cells (43). Tfr cells express CTLA-4 and produce high amounts of IL-10. They have been shown to arise from Foxp3+ precursors that highjack the Tfh differentiation pathway. However, a recent study showed that, using an appropriate vaccine adjuvant, Tfr cells can derive directly from naïve CD4 T cells (48). Alteration of Tfr cells functionality might contribute to higher proportions of Tfh cells during HIV-infection. Our results indicated that HIV infection did not impact splenic Tfh/Tfr ratio suggesting that Tfr and Tfh cell subsets expended equally during HIV infection (8). However, Chowdhury et al. have shown a limited expansion of Tfr cells as compared to the one of Tfh cells during SIV infection (49). They explored the transcriptional profile of CXCR5+PD1hiCD127–CD25+ Tfr cells after SIV infection. Overall, genes linked with Tfh differentiation and functions, such as PD-1, IL-6R, SLAMF6, and CD84, were more expressed in Tfr cells, while expression of IL-2RA linked with Treg functions was reduced after SIV infection suggesting that SIV infection might impair expression of genes associated with Treg and thus Tfr regulatory functions (49).

According to their transcription profile, Tfr cells are situated between Tfh and Treg cell subsets. However, foxp3- expression is not taken into account in most Tfh cell studies that de facto include Tfr subset among Tfh cells. Recently, adding to Tfr cells, Treg cells expressing CTLA-4 have been reported as major inhibitors of B cell expression of CD80 and CD86, which are essential to the induction of Tfh cells (50–52).

**Tfh CELL DYNAMICS DURING THE COURSE OF HIV/SIV INFECTION**

Follicular helper T and cTfh cells are targeted by HIV/SIV very early after infection and constitute a major compartment for HIV replication and production of viral particles in LNs and periphery of viremic individuals (7–9, 11). Despite their high susceptibility to HIV/SIV infection, most studies reported an accumulation of tissue-resident or cTfh cell populations (7, 8, 12, 13). Interestingly, the Tfh cell frequency positively correlates with plasma viremia levels (7, 12), and Tfh cell accumulation is reduced in individuals that control SIV infection (53), suggesting that the persistence of viral antigens might drive Tfh cell expansion. Accordingly, cTfh cell expansion has been recently reported in untreated individuals while the frequency of cTfh cells is restored to normal levels under cART suggesting that HIV replication also drives cTfh cell dynamics (10). Most studies report an increase of Tfh cells among memory CD4 T cells during HIV/SIV infection, whereas others conclude with the opposite statement (9, 54). In SIV-infected rhesus macaques, Moukambi et al. recently showed that Tfh dynamics differs from one compartment to another (peripheral blood vs. LNs or spleen) (9). Moreover, the Tfh cell frequency varies according to (i) the stage of HIV/SIV infection (53), (ii) the severity of the disease, and (iii) the ability to develop broadly neutralizing antibodies (bNAbs) (39, 55). In Table 1, we summarized Tfh cell dynamics from various studies taking into account: the type of infection (HIV/SIV), the phase (acute vs. chronic), the disease progression (slow vs. fast), the immune compartment (peripheral blood vs. secondary lymphoid organs), the phenotype, and the antigen specificity of the Tfh cells. Irrespective of the immune compartment (LNs, spleen, or blood), Tfh cells are preserved in HIV/SIV controllers, displaying no Tfh cell accumulation or loss. On the contrary, Tfh cell loss is reported in fast progressors as well as in the late stages of disease.

Follicular helper T cell accumulation is reported during slow progression (SP) or chronic stage of the disease. Indeed, evidences support the pivotal role of persistent viral antigen within the GC in driving Tfh cell expansion. HIV particles are associated with FDC in tonsils and LNs from infected patients (58–60) and Cheynier et al. reported the persistence of high levels of HIV particles in GC of HIV+ spleens from untreated subjects (61). In addition to FDC-bound virions, opsonized HIV particles interact with B cells trough CD21 membrane receptor (62, 63). Remarkably, the accessibility of CTL to GC is reduced, thus limiting the elimination of HIV-infected cells (64). Therefore, B cell follicles locally concentrate cell subsets implicated in HIV replication and viral production, which maintain antigen persistence and GC reactions. It appears that antigen persistence sustains ongoing GC reactions in which Tfh and GC B cell frequencies are highly correlated (8, 12, 65). However, a limited number of fully functional Tfh cells is required for the induction of bNAbs (42).

Another clue supporting the pivotal role of GC in driving Tfh cell expansion is that the disruption of GC organization coincides with the loss of Tfh cells and the onset of AIDS in terminal stages of SIV infection (53). PD1/PD-L2 axis contributes to the survival of Tfh and B cells. Interestingly, the expression of PD-L2 on B cells is severely impacted in the late stages of SIV infection potentially contributing to a decreased survival of T and B cells and the termination of GC reaction (53).

In sum, increased proportions of Tfh cells do not necessarily result in a better immune control of HIV infection, and Tfh cell proportions must be tightly regulated to allow efficient maturation and selection of B cells displaying high B cell receptor (BCR)
impacted by chronic HIV infection (implicated in splenic Tfh and GC Tfh cell functions are deeply 
Fluidigm BioMark HD), we showed that expression of genes TH
not impact the Ab responses (to decreased proportion of circulating memory B cells but do 
implication in HIV/SIV infection (+869) while Moukambi et al. reported a similar level of 
implication in HIV/SIV infection (+54) and our unpublished data. The phenotype and the immune compartment (spleen, blood, or lymph nodes (LNs)) are mentioned.

The key role of Tfh cells is to provide B cell helper signals and to promote their differentiation into memory B cell displaying high affinity for pathogens. These signals consist of production of cytokines, such as IL-4 and IL-21, and the expression of cell surface molecules, such as OX40, ICOS, and CD40L, by Tfh cells (1). As other groups, we analyzed the transcriptome profiles of Tfh as a mean to assess potential Tfh dysfunctions (see references in Table 2). Using single-cell sorting and high-throughput PCR (Fluidigm BioMark HD), we showed that expression of genes implicated in splenic Tfh and GC Tfh cell functions are deeply impacted by chronic HIV infection (8). In this section, we intend to review the impact of HIV/SIV infection on the main signals implicated in Tfh cell functions.

CD40L–CD40 (expressed by Tfh and B cell, respectively) interactions are required for the induction and maintenance of GC reaction. Blocking this molecular axis leads to GC disruption (67). In line with this, mutation in the CD40L gene is responsible for the X-linked hyper-IgM syndrome in humans characterized by a markedly decreased serum concentrations of IgA, IgE, and IgG (68). Our transcriptional data showed that the expression of CD40L gene is severely impacted in Tfh and GC Tfh from HIV-infected spleens (8). Of note, CD40L down modulation has been reported in global CD4+ T cell population during the late stages of HIV infection (69) and our unpublished data.

OX40–OX40L interaction is required for B cell differentiation into plasma cells (70). In humans, mutations in OX40 gene lead to decreased proportion of circulating memory B cells but do not impact the Ab responses (71). HIV-infected spleens exhibit defective expression of gene encoding OX40 in Tfh and GC Tfh cells (8). Intriguingly, we reported a reduction of memory B cell compartment in chronically HIV-infected individuals. Whether OX40 defective expression by Tfh is involved in this decrease in memory B cell should be further investigated.

During GC reactions, the expression ICOS by Tfh cells plays a major role in the process of selection of high affinity B cells. ICOS ligation leads to the overexpression of CD40L by Tfh cells that, in turn, promote the expression of ICOSL by GC B cells (72). In mice, recent findings emphasize the crucial role of T and B cell interactions through ICOS–ICOSL and CD40L–CD40 molecular axis in the maintenance of GC reactions and the production of high affinity bone-marrow plasma cells. ICOSL has been identified as a key regulator of positive selection of high affinity B cells during T–B cell interaction. Noteworthy, in comparison with uninfected donors, ICOS expression is enhanced in cTfh of ART-treated HIV-infected individuals (54) suggesting an overall immune activation of cTfh in those patients.

IL-21 is considered as the cardinal cytokine of the Tfh cell population. Tfh-secreted IL-21 induces B cell affinity maturation (73). A defective production of IL-21 by Tfh cells as well as a defective expression of its receptor by B cells severely impacts B cell proliferation and their differentiation into plasma cells (74). In the context of HIV infection, circulating CD4+ T cells secreting IL-21 are defined as the closest relative of tissue-resident (from secondary lymphoid organs) Tfh cells, both phenotypically and transcriptionally (40). cTfh cells from chronically HIV-infected individuals present altered expression of IL-21 gene (54) suggesting defective helper function. Conversely, we showed higher level of IL-21 transcripts in Tfh cells from chronically HIV-infected spleens (8) while Moukambi et al. reported a similar level of expression between Tfh from uninfected macaques and SIV-infected macaques during the early and the chronic phases of SIV infection (9). Discrepancies concerning the stage of the disease as well as the immune compartment (blood, spleen, and LNs) might explain these conflicting observations. Higher levels of IL-21 transcripts in cTfh cells are associated with HIV-controller

| Phase | Disease outcome | Compartment | Antigen specificity | Dynamics | Reference |
|-------|----------------|-------------|--------------------|----------|-----------|
| HIV Acute | P | Spleen | CXCR5+PD-1+ | Total | Loss | (9) |
| HIV Acute | Slow P | LN | CXCR5+PD-1+ | Total | Accumulation | (14) |
| HIV Acute | Fast P | LN | CXCR5+PD-1+ | Total | Accumulation | (58) |
| HIV Chronic | Slow P | LN | CXCR5+PD-1+ | Total | Accumulation | (9) |
| HIV Chronic | Fast P | LN | CXCR5+PD-1+ | Total | Loss | (9) |
| HIV Late | P | LN | CXCR5+PD-1+ | Total | Loss | (53) |
| HIV Chronic | C | LN | CXCR5+PD-1+ | Total | Preservation | (53) |
| HIV Chronic | P | LN | CXCR5+PD-1+ | Total | Accumulation | (57) |
| HIV Chronic | ND | LN | CD28hiCD95hi | Total | Accumulation | (13) |
| HIV Acute | C | Blood | CXCR5+PD-1+ | Total | Preservation | (55) |
| HIV Chronic | P | Blood | CXCR7+CXCR5+CXR6+PD-1+(7CXCR3) | Total | Loss | (54) |
| HIV Chronic | High neutralizers | Blood | CXCR7+OX40+PD-1+ | Total | Preservation | (59) |
| HIV Chronic | ND | Blood | CXCR7+OX40+PD-1+ | Total | Accumulation | (13) |
| HIV Chronic | ND | LN | CXCR5+PD-1+Bcl-6+ | Total | HIV-specific | (7) |
| HIV Chronic | ND | LN | CXCR5+PD-1+Bcl-6+(CR7–CD45RA–) | Total | HIV-specific | (12) |
| HIV Chronic | ND | Spleen | CCR7–CD45RA–CXCR5+PD-1+ | Total | Accumulation | (8) |


### Table 2 | Transcriptional profiles of Tfh cells in HIV/SIV infection.

| Compartment | Population | Method | Helper functions | Differentiation | Regulation | Reference |
|-------------|------------|--------|------------------|-----------------|------------|-----------|
| HIV/SIV− vs. HIV/SIV+ | Lymphoid organs | Tfh CXCR5+PD1+ bright | OR | IL-21 = IL-4 | OX40 CD40L ICOS | BCL6 CCR7 CXCR5 MAF CXCL13 STAT3 | BLIMP1 PD-1 IL-10 CTLA4 | (9) |
| HIV/SIV− vs. HIV/SIV+ | Lymphoid organs | CD28hiCD95hiCXCL10− | OR | CD28hiCD45RA− | CXCR5 CXCL13 | + | + ns + | = + − − − | (13) |
| HIV/SIV− vs. HIV/SIV+ | Lymphoid organs | CD154+ | OR | CD28hiCD95hiCXCR5 | + | + + | + − + − − | | (65) |
| HIV/SIV− vs. HIV/SIV+ | Blood | CD45RA−CXCR5+CD28+ | SC | + | + | + + | + | | (66) |

Gene expression profiles presented by functions: helper, differentiation, migration, or regulation. Top of the table: differential expression of genes in HIV− or SIV− infected individuals as compared to HIV− or SIV− negative individuals. Bottom of the table: differential expression of genes depending on disease outcome. SP: slow progression, individuals presenting slow progressions (SP), high neutralization titers (Neutra), or HIV-control (C) are compared to individuals with fast progression, poor neutralization, and absence of HIV control, respectively.

Method: OR, overall reaction; SC, single-cell analysis.

+, higher expression; −, lower expression; =, similar expression; ns, non-significant.

### Requirement for Early Art Initiation and Optimal Accessibility to Lymphoid Tissues

HIV/SIV infection results in antigen persistence that limits CD4 T cell recovery, production of B memory cells, and antiviral responses. These events contribute to Tfh cell exhaustion and dysfunction. In untreated patients, Tfh cell population is reduced, and IL-21 production is not fully restored, likely due to a lack of functional CD4 T cells (8). Tfh cell function in HIV/SIV infection is associated with disease progression, and treatment with ART is crucial for restoring Tfh cell function.

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|-------------|------------|--------|------------------|-----------------|------------|-----------|
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| HIV/SIV− vs. HIV/SIV+ | Lymphoid organs | CD28hiCD95hiCXCL10− | OR | CD28hiCD45RA− | CXCR5 CXCL13 | + | + ns + | = + − − − | (13) |
| HIV/SIV− vs. HIV/SIV+ | Lymphoid organs | CD154+ | OR | CD28hiCD95hiCXCR5 | + | + + | + − + − − | | (65) |
| HIV/SIV− vs. HIV/SIV+ | Blood | CD45RA−CXCR5+CD28+ | SC | + | + | + + | + | | (66) |

Gene expression profiles presented by functions: helper, differentiation, migration, or regulation. Top of the table: differential expression of genes in HIV− or SIV− infected individuals as compared to HIV− or SIV− negative individuals. Bottom of the table: differential expression of genes depending on disease outcome. SP: slow progression, individuals presenting slow progressions (SP), high neutralization titers (Neutra), or HIV-control (C) are compared to individuals with fast progression, poor neutralization, and absence of HIV control, respectively.

Method: OR, overall reaction; SC, single-cell analysis.

+, higher expression; −, lower expression; =, similar expression; ns, non-significant.
Early cART initiation during primo infection allows a dramatic decrease of cell-associated HIV DNA and thus limits establishment of the reservoir (82, 83). However, the poor penetration of cART into lymphoid tissues (84) could limit the efficiency of drugs even if cART was initiated early. To address these limitations, several approaches, mainly based on the molecular formulation of drugs, are actually envisaged. Injectable cART could overcome the limited access to lymphoid tissues. In the LATTE clinical trial, injection of combined cabotegravir and rilpivirine was well tolerated and efficient (85). However, the relevance of this approach on the persistence of the HIV reservoir has not been reported yet. Very recently, the efficient elimination of latently HIV-infected cells using HIV protease-sensitive toxin nanocapsules has been reported (86). This strategy presents the advantage to specifically eliminate HIV-infected cells without impacting healthy cells, allowing a less invasive approach. Combining new ART formulations with innovative route of administration could contribute to eradicate HIV reservoir from lymphoid organs.

In conclusion, HIV/SIV infections target Tfh cell subsets and severely affect Tfh cell frequency and functions, with dramatic impact on GC homeostasis. While limited number of fully functional Tfh cells is required for the induction of bNAb, it is now well established that viral antigen persistence drives increased Tfh cell differentiation. To this regard, blocking HIV replication in lymphoid tissues might be a prerequisite to the induction of potent bNAb. Preventing virus from entering lymphoid tissues should give another benefit to early ART initiation as well as the development of new strategies to optimize the access of ART to lymphoid tissues.

**AUTHOR CONTRIBUTIONS**

SG-D, AR, and AM wrote and approved the version to be published.

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