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Follicular T cell subsets in HIV infection: recent advances in pathogenesis research

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Abstract

Purpose of review—T cells within B cell follicles of secondary lymphoid tissues play key roles in HIV immunopathogenesis. This review highlights recent findings and identifies gaps in current knowledge.

Recent findings—B cell follicles are major sites of virus replication and demonstrate significant impairments in generation of humoral immunity in HIV infection. Follicular T helper cells (T_{fh}), follicular T regulatory cells (T_{fr}) and follicular CD8 T cells (fCD8) play key roles in HIV immunopathogenesis. T_{fh} and more recently T_{fr} are highly permissive to HIV, and may serve as reservoirs of HIV in treated infection. Virus-specific CD8 T cells are less abundant in B cell follicles than extrafollicular regions, but their effector mechanisms remain an area of significant controversy. Impairments in T_{fh} likely contribute to impaired humoral immunity and potential mechanisms include B cell counter-regulatory mechanisms, T_{fr} suppression, and diminished repertoire breadth. A better understanding of the roles of T_{fh}, T_{fr} and fCD8 in HIV immunopathogenesis is critical to development of effective HIV vaccines and cure strategies.

Summary—T_{fh}, T_{fr}, and fCD8 contribute to HIV persistence and impaired humoral immunity. A better understanding of their roles could facilitate vaccine development and HIV cure strategies.

Keywords

HIV; germinal centre; T follicular helper cell; T follicular regulatory cells; follicular CD8+ T cells

Introduction

The majority of HIV-1 (HIV) replication occurs in secondary lymphoid tissues and is concentrated in follicular T helper cells (T_{fh}) within B cell follicles and germinal centers (GC) [1–3]. T_{fh} are critical to humoral immunity as they facilitate maturation of B cells into plasma cells and memory B cells. Paradoxically, although T_{fh} are preferential targets of HIV, they expand in numbers during early infection [4] as do follicular T regulatory cells

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Conflicts of interest

None

(Tfr) [5], a CD4⁺ T cell subset found within follicles and GC that regulates Tfh activity. Infection and replication of HIV in GC Tfh is likely promoted by large quantities of extracellular virus [6] that is primarily trapped in the form of infectious immune complexes on follicular dendritic cells (FDC) within GC and results in efficient infection of neighboring T cells [7, 8]. In treated HIV/SIV infection, concentrations of HIV RNA⁺ cells and virions bound to FDC decline in lymph nodes (LN) overall, but higher concentrations of HIV RNA⁺ cells are still found in B cell follicles of LN compared to extrafollicular regions [9, 10]. Infiltration of CD8⁺ T cells into B cell follicles in LN from individuals with HIV infection was recognized more than 30 years ago [11], but these follicular CD8⁺ T cells (fCD8) are less abundant in the B cell follicles and GC than in extrafollicular regions of LN [12]. Similarly, virus-specific CD8⁺ T cells and cytotoxic CD4⁺ T cells [13] are relatively uncommon in the B cell follicle and GC in HIV/SIV infection, compared to extrafollicular areas of LN and spleen, suggesting that GC and B cell follicles may be relatively immune privileged sites [2, 14]. Despite increased numbers of Tfh and GC during HIV disease, suboptimal humoral responses are common in HIV-infected individuals, even in those who are on suppressive antiretroviral therapy (ART) [15]. Mechanisms that underlie defects in humoral immunity in HIV infection are not fully understood, but impairments of Tfh have increasingly been implicated. New findings regarding the contributions of Tfh, Tfr and fCD8 T cells to HIV/SIV pathogenesis will be highlighted in this review as well as gaps in knowledge and future research directions.

Role of follicular T cells in HIV infection, replication, and persistence

Tfh were once hypothesised to be exempt from HIV infection based on the assumption that they lack CCR5. Although this was observed in uninfected macaques [16, 17], it is not the case in humans [3, 18]. CCR5 is expressed on a substantial proportion of Tfh rendering them readily infected by CCR5-tropic HIV [18]. By spinoculating tonsil cells from children at low risk of HIV infection with R5- and X4-tropic HIV, Miller et al. showed that GC Tfr, defined as CD3⁺CD8⁺CXCR5^{hi}PD-1^{hi}CD25⁺CD127⁻, were even more permissive to R5-tropic HIV (but not X4-tropic HIV) than GC Tfh [19*]. Tfr were shown to support increased R5 viral fusion due to high surface expression of CCR5. Increased levels of HIV RNA were linked to heightened Ki67 expression both *in vitro* and *in vivo*, but not to other markers of immune activation or proliferation [19*]. In another tonsil model, using a BlaM-Vpr HIV system and a CyTOF-compatible reporter virus, Cavrois et al determined that viral fusion preferentially occurred in Treg, Th2-like, and Th17-like cells, but not in Tfh, suggesting that other mechanisms in addition to CCR5 expression may promote HIV replication in Tfh [20**]. However, Tfh and Th17-like cells were shown to support greater replication than other subsets in the tonsil model. They reported that markers of immune activation were not linked to HIV replication *in vitro*, but did not evaluate Ki67 expression [20**].

The role of follicular CD4⁺ T cells in HIV persistence during ART is not well understood. Banga et al reported that in virologically suppressed individuals, LN PD-1⁺CD4⁺ T cells (65% of which were Tfh) were the major sources of HIV transcription and production. Although cell-associated HIV RNA greatly declined after prolonged ART, it remained detectable in LN PD-1⁺CD4⁺ T cells even after 12 years. The observed decline of cell-associated RNA may reflect the relative instability of effector cells, including PD-1⁺CD4⁺ T

cells, which express viral proteins, or a decrease in the number of infectious virions that remain on FDCs within GC, thereby limiting new and preferential infection of Tfh [9]. In peripheral blood, Garcia et al. reported no differences in HIV DNA concentrations between cells with a Tfh phenotype and those without a Tfh phenotype in either elite controllers or virologically suppressed individuals on ART [21]. Whether peripheral blood cells with a Tfh phenotype truly reflect lymphoid tissue Tfh has not been firmly established, although a clonal relationship was recently reported between these subsets [22**]. The findings of Garcia et al are consistent with prior studies in ART-treated rhesus macaques that found no differences in frequencies of SIV DNA+ cells in follicular and extrafollicular regions of secondary lymphoid tissues [10]. McGary et al identified CD4+ T cells that are CTLA4+PD-1- as well as Tfh as important sources of SIV DNA in rhesus macaques [23**]. The fraction of SIV DNA harboured by CTLA4+PD-1- cells increased over time during virologic suppression on ART. They suggested that the CTLA4+ cells are regulatory T cells, although cells were stimulated briefly prior to phenotyping, which may have induced CTLA4 expression on non-Tregs. Using the *in vitro* tonsil model, Cavois et al identified CD127+ cells, which are primarily localized outside of the B cell follicle, as highly susceptible to HIV infection without HIV expression, suggesting that they might be important sources of HIV persistence in the context of ART [20**]. A fundamental challenge in the study of HIV reservoirs during ART is that T cells may alter their phenotype over time such that a cell that initially had a Tfh or Tfr phenotype may not retain that phenotype or location over many years of ART. Further studies are needed to understand the roles of Tfh, Tfr and other lymphoid tissue subsets in HIV persistence in the context of ART.

Lack of efficient HIV control by fCD8 T cells

Virus-specific CD8+ T cells play a major role in controlling HIV infection by killing infected cells that are transcribing viral genes (reviewed in [24, 25]). An infiltrate of CD8+ T cells within the B cell follicle occurs in HIV infection that is not seen in HIV seronegative individuals. This was first described in the 1980s [11], although their specificity and function was not known. Virus-specific cytotoxic CD8+ T cells were shown subsequently to be present at significantly lower concentrations within the B cell follicle as compared to extrafollicular tissues in both HIV and SIV infection [2, 12, 14, 26, 27], and this has been confirmed in more recent studies [28–31]. fCD8 share the CXCR5+CCR7- phenotype with GC Tfh and GC Tfr, which is required for their localization into the GC, and their differentiation may be partially dependent on Bcl6 [27]. PD-1 expression is also higher on fCD8 compared to CXCR5-CCR7+ CD8+ T cells, possibly inhibiting their cytokine production [29**].

The effector functions of LN CD8+ T cells are controversial. Using tetramer staining to label HIV-specific CD8+ T cells from both lymphoid tissue and blood, Reuter et al. found that HIV-specific CD8+ T cells co-expressing granzyme B and perforin were generally absent from lymphoid tissue. They further demonstrated that fCD8 expressed lower levels of the transcription factor T-bet and higher levels of Eomes, a phenotype generally associated with decreased effector function, whereas HIV-specific blood CD8+ T cells expressed the phenotype T-bet^{hi}Eomes^{dim} associated with greater cytolytic potential [32*]. Based on these data they concluded that lymphoid tissue CD8+ T cells have reduced cytolytic function.

These findings are not consistent with one prior study that reported similar or greater HIV-specific cytotoxicity in LN cells compared to PBMC of 2 untreated HIV-infected individuals using ⁵¹chromium release assays [2]. Furthermore, CD8 depletion studies in SIV-infected rhesus macaque demonstrated increased virus replication particularly in the extrafollicular regions of LN, but also in the B cell follicles [33], suggesting indeed that CD8 cells in LN mediate potent effector function. Whether this is due to cytolytic activity or other CD8 T cell effector functions is unclear. Therefore, co-expression of perforin and granzyme B may not accurately reflect antiviral effector function of CD8 T cells in lymphoid tissues.

Within the LN, controversy further exists about the relative effector functions of fCD8 as compared to extrafollicular CD8+ T cells. The frequency of virus-specific fCD8 was observed to be inversely correlated with pVL [28, 31, 32*]. Petrovas et al reported no correlation between the frequency of fCD8 and viral transcription in Tfh [29**], but an earlier study found a strong inverse correlation between SIV-specific tetramer staining cells in both follicular and extrafollicular compartments and frequencies of SIV RNA+ cells in those compartments [14]. Miles et al reported that fCD8 largely had a regulatory phenotype, and these cells exhibited lower expression of perforin and higher expression of IL-10 compared to other CD8+ T cells in *ex vivo* infected tonsil cells and SIV-infected LN cells [34]. Mylvaganam et al. showed that fCD8 from SIV-infected rhesus macaques were able to limit Tfh proliferation in a peptide-pulsed, stimulated expansion assay, although the mechanism was not determined [28]. In a cytotoxicity assay performed by Rahman et al. using CD8+ T cells and autologous SIV peptide-pulsed CD4+ cells from pre-vaccinated SIV-infected macaques, fCD8 outperformed non-fCD8 T cells. However, their report also showed that SIV-specific cells were more abundant in fCD8 than non-fCD8, which may have accounted for this difference [31*]. Rahman et al. reported that in animals with low pVL, the frequency of fCD8 was positively correlated with the frequency of Tfr and negatively correlated with pVL, but in animals with high pVL, the frequencies of fCD8 and Tfr was inversely correlated. Although they concluded that fCD8 activity may be regulated by Tfr in a dose dependent manner, they used relative percentages of these cells and not absolute frequencies of these cells *in vivo*, which is likely the more relevant measure [31*]. Further study is needed to determine the relative capacity and mechanisms by which virus-specific fCD8 suppress HIV replication in follicular CD4+ T cells.

In light of the relative paucity of fCD8, strategies are currently being developed to improve CD8 T cell migration into B cell follicles and GC. One approach has been to transduce CD8+ T cells to induce expression of the follicular homing molecule CXCR5. This resulted in a 120-fold increase in CXCR5-expressing CD8+ T compared to untransduced cells in the B cell follicles of LN and spleen in rhesus macaques *in vivo* [35**]. Introduction of CXCR5 into SIV-specific chimeric antigen receptor (CAR) T cells is a similar approach that has been shown to result in preferential migration of CAR T cells to B cell follicles in an *ex vivo* model using rhesus macaque LN [36*]. The IL-15 superagonist ALT-803 was shown to induce CXCR5 expression on CD8+ T cells *in vitro* and to induce migration of virus-specific CTL into B cell follicles *in vivo* [37**], although this resulted in only modest reductions in SIV RNA+ cells in the B cell follicles. Treatment with heterodimeric IL-15 also increased cytotoxic effector cells in B cell follicles and reduced virus replication in SHIV-infected macaques [38**]. Nevertheless, as the effects of these IL-15 based therapies

are only transient [39*], it is unclear that they will lead to an effective long-term strategy to clear virus-producing cells from B cell follicles.

Role of Tfh in Impaired Humoral Immunity during HIV infection

Multiple impairments in humoral immunity in HIV infection have been described including decreased responses to vaccines as well as failure to produce broadly neutralizing antibodies (bnAbs) to HIV [15, 40]. Mounting evidence suggests that some of these defects are related to Tfh dysfunction. Tfh numbers and/or function have been linked to the development of bnAbs in humans [41, 42] and SHIV-infected rhesus macaques [43], as well as responses to influenza vaccine [44]. Interestingly, in chronically HIV-infected individuals on ART, immunization with seasonal influenza vaccine reduced the frequencies and numbers of Tfh cells in the draining LN by 10 to 14 days post vaccination, but had no effect on controls, suggesting altered Tfh kinetics in HIV-infected individuals [45*].

Several mechanisms have been proposed to contribute to Tfh dysfunction in HIV infection. Cubas et al. showed that PD-L1 was upregulated on *ex vivo* GC B cells from infected individuals, and ligating PD-1 on Tfh cells resulted in their decreased proliferation, ICOS expression, and cytokine secretion *in vitro* [46]. Tfr were implicated in Tfh dysfunction by Miles et al., who showed using a tonsil cell model that Tfr inhibit Tfh proliferation, cytokine production, and ICOS expression, and demonstrated that these effects were more pronounced after *in vitro* spinoculation of tonsil cells with HIV [5]. The relationship between Tfr and Tfh, however, is somewhat controversial. Although Miles and colleagues showed a decreased Tfh:Tfr ratio during chronic SIV infection [5], Chowdury et al reported an increased Tfh:Tfr ratio, and hypothesized that this contributed to increased Tfh expansion in SIV and HIV infection [47]. More recently, Fan and colleagues also reported an increased Tfh:Tfr ratio during acute to early chronic SIV infection (4–25 weeks), which they linked to autoantibody production [48*]. In addition, Hey-Nguyen et al showed that the ratios of Tfh to extrafollicular Tregs increased in untreated HIV-infected patients [49]. Miles et al demonstrated that regulatory CD8 cells, which are the predominant phenotype of fCD8, inhibited production of IgG by B cells in HIV-infected tonsil cells even in the absence of Tfh, suggesting direct effects of fCD8 on B cells [34]. Further investigation of the impact of both Tfr and fCD8 on humoral immune responses are critical to dissect out these mechanisms.

A novel theory of Tfh-mediated immunodominance was recently proposed to explain humoral immune impairments in HIV infection. In vaccine studies, animals that have successfully generated tier 2 bnAbs also show strong responses to non-neutralizing epitopes such as the V3 loop [41, 50]. These vaccine studies suggest that immunodominance potentially hinders the generation of HIV bnAbs. Switched HIV-specific antibodies are detectable throughout the course of disease but the generation of bnAbs are rare and delayed [51]. Wendel and colleagues analyzed the TCR repertoire in GC Tfh cells and Gag-specific lymph node CD4+ T cells from untreated chronic HIV infected individuals [52**]. They found clonally expanded TCRs in GC Tfh that overlap with Gag-specific TCRs and provided evidence for intact responses of Tfh to HIV antigens and reduced Tfh repertoire diversity. They also showed that driven by HIV antigen, Tfh acquired an activation-related

signature and loss of polyfunctionality, skewing B cell differentiation towards plasma cells and away from switched memory cells. Mathematical modelling suggested that the breadth of the Tfh cell repertoire was crucial to broadly neutralizing selection [53]. Thus, the loss of the repertoire favours B cell responses to high affinity, non-neutralizing epitopes and may explain the impaired humoral responses to vaccination against other pathogens in untreated chronic HIV-infected patients. Further confirmation that the breadth of the Tfh repertoire is related to bnAb development is important, as this has major implications for HIV vaccine strategies.

Conclusion

T cells within B cell follicles play key roles in HIV immunopathogenesis. Tfh and Tfr are highly permissive to HIV; their roles in HIV persistence remain to be defined. fCD8⁺ T cells are relatively less abundant than extrafollicular CD8 T cells; fCD8 antiviral functions are controversial and need to be established in future studies. Tfh, Tfr, and fCD8 all likely contribute to impaired humoral immunity in HIV infection; dissecting out their roles is an important area for future research. Modulating Tfh, Tfr, and fCD8 during HIV infection could lead to development of effective cure strategies and protective vaccines.

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Key Points

1. Tfh and Tfr play key roles in HIV replication in untreated disease, and may serve as important HIV reservoirs in treated disease.
2. HIV-specific CD8⁺ T cells are less abundant in B cell follicles compared to extrafollicular regions, but the effector functions of these cells remain controversial.
3. Multiple factors likely contribute to impaired Tfh function and diminished humoral immunity in HIV infection including B cell counter-regulatory mechanisms, Tfr, and diminished Tfh repertoire.
4. Modulation of Tfh, Tfr, and fCD8 could lead to innovative strategies to optimize protective vaccines and develop functional cures for HIV infection.