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Emerging concepts on T_{FH} cell dynamics in HIV infection

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Abstract

Inducing cross-reactive broadly neutralizing antibody (bNAb) responses to HIV through vaccination remains an insurmountable challenge. T follicular helper cells (T_{FH}) are fundamental for the development of antigen-specific antibody responses and therefore critical for anti-HIV vaccine design. Here we review recent studies supporting an intricate involvement of T_{FH} in HIV pathogenesis and bNAb development in HIV infection. We also examine emerging data suggesting that T_{FH} responses may be traceable in peripheral blood, and discuss the implications of these findings in the context of vaccine design and future research in T_{FH} immunobiology.

Keywords

Follicular B helper T cell; T_{FH}; HIV; HIV NAb; bNAb; Neutralizing antibodies; pT_{FH}; Vaccine; T follicular helper cells

Antibody-mediated immunity and HIV

In the face of 2 million new infections per year, the stagnation of progress towards an efficacious HIV vaccine is sobering. In 2009 a double-blind phase III HIV vaccine RV144 “Thai” trial that used a combination of a recombinant canarypox vector (ALVAC-HIV [vCP1521]) and two booster injections of a recombinant glycoprotein 120 subunit vaccine (AIDSVAX B/E) showed marginal, yet significant protection from HIV acquisition[1] raising considerable hope for a protective vaccine. However, the results of follow-up assessments of the correlates of protection were surprising and unexpected[2]: Assessing multiple immunological parameters, the study found that not only HIV Envelope (Env)-specific, non-neutralizing IgG antibody (Ab) responses were correlated with protection, but also that protection was lost in the presence of high levels of anti-Env IgA Ab[2]. These findings raise questions regarding whether other Ab-related effector mechanisms [such as

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Ab-mediated cellular cytotoxicity (ADCC)] may have played a role in the marginal levels of protection observed in RV144, in contrast to most vaccines that commonly –yet not exclusively - induce neutralizing Ab (NAb) responses[3]. These data highlights critical gaps in our understanding of what constitutes a protective vaccine-induced immune response, and more importantly, raises questions as to the mechanisms that shape the Ab response to viral challenges and how these can be tailored by vaccination.

The large genetic diversity of global HIV variants stipulates broad reactivity of NAbs. Thus the ideal Ab response against HIV infection would have broadly neutralizing activity covering the wealth of different worldwide HIV variants. Passive transfer of such broadly cross-reactive neutralizing Abs (bNAbs) has been shown to protect both prophylactically[4, 5] and therapeutically[6, 7] in both non-human primates (NHP) and mice. Yet, no immunization strategy attempted against HIV has succeeded in inducing bNAbs, and only a fraction of HIV-infected individuals naturally develop bNAbs after years of HIV infection[8–15]. The difficulty of generating such a response may, in part, lie in the particular characteristics of bNAbs. Overall, monoclonal bNAbs isolated from infected individuals are characterized by unusual properties such as long antigen-contacting sites [complementarity determining region-3 (CDR-3)] and tremendous levels of somatic hypermutation (SHM), the extent of which is uncommon in other Ab responses[10, 16, 17]. These changes are characteristic of Ab affinity maturation processes, which occur in germinal centers (GC) within lymphoid tissues. T follicular helper cells (T_{FH}) play a cardinal role in Ab affinity maturation by promoting immunoglobulin class switching [class-switch recombination (CSR)], SHM, and B cell differentiation and survival[18]. During this process, termed the GC reaction, T_{FH} cells and antigen-specific B cells undergo a tight regulatory program that drives these mechanisms[19]. Thus, insight into the generation of protective Ab responses requires understanding of these cellular interactions and the underlying mechanisms.

There have been significant advances in our understanding of T_{FH} immunobiology in both natural settings and during HIV infection. While perturbation of the B cell compartment in HIV infection is well established[20], a number of recent studies have shown a link to T_{FH} immunopathology during HIV/SIV infection, including changes in T_{FH} frequency, phenotype and function[21–25]. Recent studies suggest that the rare and infrequent generation of HIV-specific bNAbs may be associated with T_{FH} frequency[12, 26]. This is further supported by mouse studies demonstrating a dynamic exchange of T_{FH} cells between different GCs[27], ensuring the maximal diversification of T cell help. Using two-photon microscopy, it was demonstrated that the ability of T_{FH} cells to enter ongoing GCs may accommodate antigenic variation during the immune response, maximizing the humoral response[27]. Moreover, emerging data suggest that T_{FH} responses may be traceable in peripheral blood[26, 28–31], providing a potential tool to monitor vaccine efficacy. In this article, we therefore focus on the immunobiology of T_{FH} cells in natural HIV infection and their involvement in HIV pathogenesis and bNAb development. We discuss studies that examine whether peripheral blood T_{FH} counterparts (pT_{FH}) may serve as surrogates for T_{FH} cells in lymphoid tissues, and provide an outlook on how these and other recent findings might inform HIV vaccine design.

T follicular helper cells and antibody-mediated immunity

Burnet's proposal for the clonal selection theory of Ab formation[32] launched the modern age of cellular immunology, in which lymphocytes were established as the agents of immunity and divided into distinct humoral and cellular components. Subsequently, in the 1960s, Miller and Claman described for the first time a dependence of Ab production on the presence of T cells[33, 34], termed T-dependent responses. Then in 1986, Velardi and colleagues described a CD4+ T cell subset within the GC[35]. While this subset showed properties distinct from the known T helper 1 (Th1) and Th2 CD4 T cell subsets, these cells were formally recognized as a distinct subset only in early 2000, taking center stage as a key player for T-dependent responses[18]. Since then our understanding of the development and function of T_{FH} cells in both health and disease has grown rapidly in the past decade.

The transcriptional repressor B-cell lymphoma-6 (Bcl6) facilitates transcription of signature T_{FH} markers that mediate T_{FH} migration and function[36–38] while antagonizing other CD4 T cell differentiation programs[37, 38]. Expression of Bcl6 is initiated by DC-mediated priming of naïve CD4 T cells, triggering differentiation into T_{FH} cells in T cell zones of lymphoid follicles[39]. Bcl6 induction promotes CXCR5 expression, which in turn facilitates T_{FH} migration to the T–B cell border, promoting encounters with B cells specific for the same antigen (cognate) that reinforce T_{FH} lineage commitment[40]. Inducible T-cell costimulator (ICOS), a surface protein expressed on activated T cells, is central to all phases of T_{FH} development and GC formation[41, 42]. Additionally, in conjunction with IL-6, ICOS also promotes expression of IL-21, which is the cardinal cytokine for T_{FH} cells[43, 44]. IL-21 further augments Bcl6 expression to drive T_{FH} lineage differentiation[44–46] as shown by the inability of IL-$21^{-/-}$ mice to develop T_{FH} cells with full helper activity and maintain GCs[45, 46]. These various cellular and molecular signals together contribute to B cell responses by initiating the T_{FH} program.

The coalescence of T_{FH} and B cells initiates the GC reaction, during which T_{FH} cells become fully polarized, highly activated GC T_{FH} cells[19]. GC T_{FH} cells are exposed to ongoing stimulation by cognate B cells presenting antigen and co-stimulatory signals resulting in heightened expression of T_{FH} markers, including ICOS and PD-1, in GC T_{FH} compared to T_{FH} cells[27]. ICOS signaling in GC T_{FH} cells induces expression of the transcription factor c-Maf[47], which synergizes with Bcl6 to orchestrate further initiation and maintenance of the T_{FH} program[48]. T_{FH} lineage differentiation is therefore tightly linked to its functional role in B cell differentiation, survival, and longevity of Ab responses.

Upon encountering antigen, B cells can differentiate directly into short-lived plasma cells (PC) or migrate into the lymphoid follicles to receive T_{FH}-derived help. Here B cells compete for a limiting source of T_{FH} signals based on the amount of MHC-II:antigen complexes presented on B cells[49]. The degree of T_{FH} signal is dictated by the quality and duration of B cell interactions with molecules expressed on the surface of T_{FH} cells[50]. These T_{FH} signals are critical for differentiation of GC B cells into long-lived PCs and memory B cells, as well as the ensuing maturation of Ab affinity through CSR and SHM[19]. Thus B cells with the strongest interactions with T_{FH} cells subsequently either become memory B cells or leave the GCs and differentiate into long-lived PCs[51],

colonizing the bone marrow or tissues underlying epithelial surface. Several surface receptors govern this interaction in addition to MHC-II: First, the signaling lymphocytic activation molecule (SLAM) family of receptors and the SLAM-associated protein (SAP) family of intracellular adaptors are responsible for maintaining long-duration contacts with antigen-specific B cells[52]. SLAM-bound SAP triggers positive signaling through Src-family kinases while inhibiting negative signaling through the negative signaling receptor, LY108[50, 52]. Another T_{FH} surface molecule, the inhibitory receptor programmed death-1 (PD-1) is highly expressed on T_{FH} cells and actively involved in sustaining the GC reaction by inhibiting follicular regulatory T cells that suppress the GC response[53, 54]. Among other molecules, the interaction between CD40 on activated B cells and its ligand (CD40L) expressed on T_{FH} cells is one of the most crucial receptors for the development of T-dependent Ab responses and the generation of affinity-matured Abs. CD40 ligation leads to expression of activation-induced cytidine deaminase (AID), which is a critical enzyme regulating CSR, SHM and likely many other additional aspects in Ab affinity maturation[55]. Whether HIV-specific T_{FH} responses –including CD40L expression and cytokine secretion - differ between individuals that develop bNAbs or those that fail to do so remains unknown.

Another prominent factor for Ab affinity maturation is the cardinal T_{FH} cytokine, Interleukin-21 (IL-21). In addition to its central role in T_{FH} development, IL-21 is indispensable for Ab affinity maturation. IL-21 and Interleukin-4 have been shown to cooperatively direct Ab responses in both mice and humans[56], and further instructs the differentiation of B cells by stimulating expression of B cell master transcription factors[57]. Together, data suggest T_{FH} cells provide critical help to GC B cells and promote Ab affinity maturation by providing IL-21 in the presence of CD40 ligation. Additionally, SAP/SLAM-dependent IL-4 production provides further survival signaling[58]. Interestingly, IL-21-secreting CD4 T cells have also been shown to help sustain CD8 T cell effector function during chronic viral infections[59, 60] and are associated with HIV control[61]. However, whether T_{FH} cells, or a distinct IL-21-secreting CD4 subset, mediate this IL-21-dependent CD8 T cell “help” remains to be established.

T_{FH} cells in HIV pathogenesis

A fundamental characteristic of HIV pathogenesis is widespread immune activation and perturbation of the B cell compartment[20]. That CD4 T cells are the primary targets of HIV[21] and the specific induction of T_{FH} cells through vaccination may (temporarily) increase the pool of HIV target cells represents a duality in harnessing T_{FH} cells for HIV vaccinology. Thus understanding the immunobiology of T_{FH} cells in HIV infection and their involvement in the generation of bNAbs during natural HIV infection –in spite of a preferential depletion - is a fundamental question for HIV vaccine development. Currently, several studies have begun to explore T_{FH} cells in the context of HIV/SIV.

All studies of these cells to date have consistently demonstrated an unexpected expansion of both general and virus-specific T_{FH} frequency in both chronic HIV[21, 23, 24] and SIV[22, 25] infection, despite potential preferential depletion of antigen-specific CD4 T cells[62]. While no direct relationship with viral loads was observed[22, 23, 25], T_{FH} frequencies

substantially decreased with ART treatment, suggesting a role for antigen levels in driving T_{FH} differentiation[21, 23]. Similarly, T_{FH} expansion has been previously reported in the lymphocytic choriomeningitis virus (LCMV) mouse model where it has been suggested that viral persistence and prolonged T cell receptor stimulation may progressively direct T cells towards a T_{FH} program[63]. However, another study of LCMV infection demonstrated a biphasic secretion of IL-6, the strongest driver of T_{FH} development, during acute infection and again during the chronic phase. Yet, only the chronic phase of IL-6 secretion seems to be responsible for driving subsequent T_{FH} expansion[64]. Interestingly, a similar biphasic IL-6 response has been demonstrated in acute HIV infection[65], but an association with human T_{FH} cell expansion in HIV remains to be shown. Nevertheless, studies of SIV infection have shown that T_{FH} expansion is strongly linked to a skewing in the IL-6 signaling axis[22]. Likewise, they have also reported elevated T_{FH} levels in chronic, compared to acute, infection suggesting that immune activation, rather than antigen levels may be driving T_{FH} expansion.

Expansion of T_{FH} cells has been associated with perturbation of the B cell compartment. In particular, GC B cell and PC frequency was positively correlated with T_{FH} frequency, which is not surprising given T_{FH} cells' involvement in the development of these cells. Moreover, memory B cells in HIV^+ Lymph Nodes (LN) were negatively correlated to T_{FH} frequency[23, 24]. In addition, aside from a general expansion of T_{FH} frequency, T_{FH} function seems to also be altered in HIV/SIV infection. Cubas et al. demonstrated that T_{FH} cells from HIV+ LNs less-efficiently promoted B cell survival and differentiation, likely due to deficient IL-21 secretion. However, T_{FH} dysfunction was primarily attributed to B cell dysregulation in that excess PD-1–PD-L1 ligation by dysregulated B cells impaired the overall T_{FH} cell response[24].

Despite compromised T_{FH}-derived help, T_{FH} expansion correlates with enhanced circulating SIV-specific IgG in NHP [22, 25] and hypergammaglobulinemia in humans[23]. Petrovas *et al*. described a positive association between T_{FH} frequency, expansion of the B cell compartment and increased circulating high-avidity SIV-specific Abs in chronic SIV infection; this finding suggests that chronic SIV infection may not disrupt T_{FH}-derived Ab affinity maturation and SIV-specific Ab production[22], but Ab affinity maturation may be impaired overall. Indeed, while higher T_{FH} frequency drives B cell proliferation, constant antigenic stimulation may lead to the selection of low affinity B cells[25]. Thus, it is likely that the low-quality Ab production described in HIV/SIV infection may be induced by a combination of factors, including T_{FH} /B cell dysfunction, high levels of antigenemia, and immune activation.

Expansion of virus-specific T_{FH} cells seems to conflict with the profound depletion of activated antigen-specific CD4 T cells seen in HIV infection, raising the question of whether T_{FH} cells are somewhat protected from infection/depletion. However, it has been demonstrated that SIV readily infects T_{FH} cells in GCs[22] and the T cell population within the lymph node that corresponds to T_{FH} cells ($CXCR5^+PD\text{-}1^+Bcl6^+$) serves as the major T cell compartment for HIV infection, replication, and production[21]. Interestingly, a study of pigtail macaques found infection of T_{FH} cells in the absence of CCR5 or other HIV/SIV co-receptor expression[66]; however, whether infection of T_{FH} cells occurs through a different

co-receptor or whether T_{FH} cells are infected prior to differentiation and subsequent CCR5 downregulation remains to be seen. Overall, the current data suggest that T_{FH} cells may represent a continually replenishing pool of target cells for SIV[22, 25, 66] and HIV[21]. Taken together, these data indicate a model in which HIV promotes B cell dysfunction while securing a viral reservoir in lymphoid tissues that are relatively devoid of CD8 CTLs[66] (Figure 1).

While significant progress has been made in understanding the immunbiology of T_{FH} cells in HIV infection, several outstanding questions remain regarding how T_{FH} cells are involved in B cell dysfunction and/or the rare generation of bNAbs that occur after years of infection. Of particular interest is the causality of these observations: that is, it is unknown whether T_{FH} expansion directly promotes the perturbation of the B cell compartment or whether these are two simultaneously occurring infection-mediated mechanisms. Moreover, it remains to be shown whether the described alterations are overall beneficial or disadvantageous overall for the generation of bNAbs. As all HIV-infected individuals develop NAbs but only a fraction develops bNAbs, it will be critical to understand whether antigen-specific T_{FH} function, HIV antigenemia, immune activation, other unknown factors, or a combination of these drives the extensive levels of SHM observed in bNAbs. It is likely that multiple factors synergize to dampen or delay bNAb generation; for example, low-affinity Ab responses to linear epitopes may arise from lowered selection and competition threshold for T_{FH} help (from T_{FH} expansion) between B cells (with low-affinity BCR) specific for different viral quasispecies[49]; this may divert the Ab response away from neotopes. Coincidentally, HIV- or Env-derived B cell deregulation may impair T_{FH}-derived B cell help, disrupting affinity maturation, and further promoting low-affinity Ab production. However, this hypothesis is challenged by findings describing associations of T_{FH}-like counterparts circulating in peripheral blood with bNAb development (see below). Overall, dissecting the signals involved in promoting SHM for the generation of bNAbs and understanding which T_{FH} functions and B cell perturbations are unique to HIV (or other chronic infections) will be critical hurdles to overcome for effective vaccine design.

Broadly cross-reactive NAbs and T_{FH} cells

In recent years, there has been substantial progress in the identification and isolation of bNAb-producing B cells from chronically infected individuals. The profound SHM displayed by most bNAbs[67] suggests extended affinity maturation signals, likely mediated by T_{FH} cells, thus implicating a prominent role of T_{FH} cells in bNAb development.

In most patients, bNAbs develop only after years of chronic infection[9, 11, 12] and show remarkable properties including extremely high levels of SHM[10, 16, 68, 69], long CDR3 regions[70], and, in certain cases, polyreactivity[71]. For example, Klein *et al*. showed that monoclonal bNAbs undergo SHM in Ab framework regions that control molecular structure. Furthermore, these mutations were essential for bNAb function, as removing point mutation occurring in these regions ablated neutralizing activity[67]. Similarly, restoring monoclonal bNAbs to their germline sequences removes neutralization potency and Env specificity[72]. These high levels of Ab rearrangements (up to 42%) are not commonly found in other Abs[10, 16, 17], suggesting a constant and prolonged activation of AID. Activation and

phosphorylation processes of AID occur through CD40-CD40L signaling[55] provided by T_{FH} cells suggesting an intimate role for T_{FH} cells in bNAb development as they undergo multiple rounds of selection in response to a continually evolving pathogen[73]. While these observations provide a glimpse into the difficulty of bNAb development, the mechanisms behind this process, the extent of T_{FH} involvement and the specific signals provided by T_{FH} cells remain unknown.

In this regard, optimal antigen recognition and presentation mechanisms would likely optimize T_{FH} B helper activity[51]. Interestingly, a retrospective study conducted on uninfected RV144 vaccine recipients demonstrated that patients carrying HLA-DQA1*05:01 and DQB1*03:01 alleles were less likely to produce NAbs[74] suggesting a direct involvement of antigen presentation to the induction of NAbs in vaccine recipients. No association between bNAb generation and HLA-II expression has yet been described in natural HIV infection, however. The extent to which HLA-II alleles influence B cell selection and whether HLA-DR or HLA-DP plays a more predominant role over HLA-DQ remains unknown. Moreover, it is also unknown whether different epitope-specificities of CD4 T cell responses are involved in NAb development and whether the abundance of Gag-specific responses over Env-specific T_{FH} responses impacts the development of NAbs in natural HIV infection[23].

Recent landmark studies reporting the isolation of potent bNAbs from HIV-infected patients with broad neutralization breadth and advances in deep sequencing of single-cell bNAb-secreting memory B cells and PCs have provided important insights into their maturation pathways[16, 75, 76] as well as bNAb binding mechanisms[10]. While these illustrate the profound intricacies of bNAb development, the hope is that they will inform rational vaccine design to harness T_{FH} cells to mediate targeted affinity maturation, PC differentiation and stimulation of bNAb responses. However, many questions surround this approach, particularly regarding what signals are required for optimized T_{FH} generation, and how to induce them through vaccination (Box 1).

Peripheral T_{FH} cells in infection and vaccination

One inherent problem to studying co-dynamics of T_{FH} and Ab development in infection and vaccines in human patients is the paucity of lymphoid tissue available for experimental analyses. Thus, the ability to define a population of memory T_{FH} cells in peripheral blood (pT_{FH}) would facilitate our understanding of CD4 T cell dynamics within lymphoid tissue during vaccination and infection. Initially, Mikell *et al*. reported higher frequencies of circulating CD4 T cells expressing PD-1 in individuals with broad neutralizing serum activity[12], thus potentially associating the emergence of a T_{FH}-like population in peripheral blood with bNAb development, as PD-1 is highly expressed on T_{FH} cells[53, 54]. While this study was not designed to investigate pT_{FH} cell immunobiology, it was the first study to shine a spotlight on the potential associations between T_{FH} (and pT_{FH}) cells and bNAb development. Given the importance of such findings for the design of potential vaccines to specifically promote bNAb development, several groups have subsequently focused on characterizing the association between GC T_{FH} and peripheral counterparts during HIV-specific and other types of Ab responses[26, 28–31, 77].

While most studies agree that CXCR5$^+$CD4 T cells in peripheral blood represent a counterpart of T_{FH} cells, their relationship to GC T_{FH} cells has been challenged based on differences in surface marker phenotypes and transcription factor expression levels [18]. Indeed, a large heterogeneity of circulating CXCR5$^+$CD4 T cell subsets has been described in terms of gene expression[26, 31, 77], surface marker expression, and function[26, 28, 29, 31, 77], complicating clear conclusions on this issue. Indeed, emerging studies describe biologically relevant B helper activity in various subsets of CXCR5$^+$CD4 pT_{FH} cells; moreover, these subsets have been defined using different groups of cell surface markers, further complicating comparisons between these studies (see below and Table 1). Emerging evidence suggests that T_{FH} memory is generated along with B cell memory, and combinations of phenotypic markers likely represent different layers of T_{FH} differentiation and effector function that is reflected in peripheral blood[28, 29]. Indeed, evidence shows that pT_{FH} cells (CXCR5$^+$CD4$^+$) are central memory cells comprised of Th1-, 2-, and 17-like subsets (based on CXCR3 and CCR6 expression) in healthy adults, each with diverse B helper functions in co-culture and *in vivo*[29, 30]. Of these, the Th1-like, **CXCR3$^+$CCR6$^-$CXCR5$^+$CD4** subset lacks naïve or memory B helper capacity in mice[29]. However, upon activation by trivalent influenza vaccine in humans, activated Th1-like **ICOS$^+$CXCR3$^+$CCR6$^-$**CXCR5$^+$CD4 subset efficiently induced memory, but not naïve, antigen-specific B cells to proliferate, differentiate, and produce Igs[30]. In contrast, the Th2- and −17-like pT_{FH} (CXCR3$^-$CCR6$^{+/-}$) supported naïve B cell differentiation in mice[29] and humans[30]. Supporting these observations, evidence from studies of healthy and HIV-infected human specimens suggests **CCR6^{high}CXCR3$^{+/-}$**CXCR5$^+$CD4 pT_{FH} induce Ab secretion from naïve B cells, although helper capacity is impaired in HIV-infected samples[77]. Indeed, this pT_{FH} subset (CCR7^{high}CXCR5^{high}CCR6^{high}PD-1^{high}) displayed the highest IL-21 production and naïve B cell help, inducing the greatest production of IgG1, IgG3, and IgA compared to other subsets in co-culture with naïve B cells[77]. These data suggest that perhaps CXCR3$^-$CXCR5$^+$CD4 pT_{FH} cells are potent inducers of naïve B cell differentiation while CXCR3$^+$CXCR5$^+$CD4 pT_{FH} primarily induce memory B cell differentiation.

Other studies have explored the relatedness of pT_{FH} with GC T_{FH} in the lymphoid follicle, based on other combinations of surface marker and gene expression profiles. These studies demonstrate that CXCR5$^+$CD4 pT_{FH} cells are either activated (PD-1$^+$ICOS$^+$[30] or PD1$^+$CCR7^{lo}[28]), or quiescent (PD-1$^{+/-}$ICOS$^-$[26, 28]) cells. In this regard, **CXCR3$^-$** (PD-1$^+$CXCR3$^-$CXCR5$^+$CD4$^+$) pT_{FH} were described as memory cells with highly functional B helper activity in co-culture that share a transcriptional profile signature with GC T_{FH} cells, including expression of *MAF* and *SLAMF6*[26]. On the other hand, a slightly different pT_{FH} subset (CXCR5^{high}CCR6^{high}PD-1^{high}) was concluded to more closely resemble a memory, non-T_{FH} CD4 T cell population from the tonsil compared to non-GC and GC T_{FH} cells (based on gene expression analyses), as transcript levels of *MAF*, *BCL6*, *IL-21* were lower in pT_{FH} than tonsillar T_{FH} cells[77]. While Boswell *et al*. suggested that the pT_{FH} subset they identified was predominantly CXCR3$^-$CCR6^{high}, Locci *et al*. did not incorporate CCR6 in their phenotypic panel[26, 77]. Yet a separate study using both mouse and human specimens found high CCR6 expression in the PD-1^{hi}**CCR7^{lo}** subset of CXCR5$^+$CD4 pT_{FH}; this subset, but not PD-1^{lo}CCR7^{hi} reportedly indicates active GC T_{FH}

differentiation[28]. Further studies are required to reconcile the various surface markers used to derive the conclusions described above.

Interestingly, *in vivo* functional relevance was shown for each of the particular pT_{FH} subsets identified. Both subsets of activated pT_{FH} (PD-1^{+}ICOS^{+} and PD-1^{+}CCR7^{lo}) cells were associated with Ab responses to influenza vaccination[28, 30] and autoimmune Ab production[28]. In co-culture assays, PD-1^{hi}CCR7^{lo} pT_{FH} potently induced plasmablast and PC differentiation as well as total antigen-specific IgG production to Influenza[28, 30] and doublestranded DNA[28]. Similarly, the ratio of Th1-, 2-, and 17-like pT_{FH} subsets were reportedly skewed in patients with dermatomyositis, an autoantibody-mediated autoimmune disease, compared to healthy controls. This resulted in increased frequency of naïve B cell helper vs. non-helper pT_{FH} cells that further correlated with disease severity and circulating plasmablasts[29].

Establishing a significant association between HIV-specific NAb and bNAb development, however, is much more convoluted. On one hand, higher frequencies of quiescent pT_{FH} (PD-1^{+}CXCR3^{-}CXCR5^{+}CD4^{+})[26] and PD-1^{+}CD4 T cells[12] were described in HIV-infected donors exhibiting **broad and potent serum neutralization activity**[12, 26], while no association was observed between pT_{FH} frequency (irrespective of phenotype) and HIV Env-specific Ab titers, total IgG levels, or HIV-specific serum neutralizing activity in HIV-infected individuals exhibiting **normal serum neutralization activity**[77]. The difference in breadth and potency between the donors used in these studies may contribute to the discordant results. However, similarly discordant associations exist even in analyses using only samples from HIV-infected donors with broad and potent neutralizing activity, further complicating the situation. Indeed, while Mikell *et al*. found higher frequencies of PD-1^{+}CD4 T cells in these patients[12], subsequent analyses failed to corroborate this association using a slightly further delineated PD-1^{+} pT_{FH} subset (PD-1^{hi}ICOS^{+}CXCR5^{+}CD4^{+})[26]. The complex nature of bNAb development is confounded by classification of “good neutralizers” and subjects with broad and potent neutralizing serum activity[12, 26, 77]. Indeed, while all HIV-infected individuals develop patient-specific NAb, only a minority develops broad and potent neutralization activity[73]. Furthermore, an important caveat to consider is that broad and potent neutralizing activity appears in serum of chronically infected subjects, meaning bNAb development likely takes years to develop under normal conditions; thus, perhaps pT_{FH}:B cell dynamics should be monitored during the acute, rather than chronic, phase of HIV infection. In this regard, Locci *et al*. observed a correlation between the frequency of PD-1^{+}CXCR3^{-}CXCR5^{+} pT_{FH} cells at approximately 4 months post infection and the capacity of individuals to subsequently develop bNAbs during the chronic phase[26]. Although this association remained during the time of bNAb development months later, examining the pT_{FH}:B cell dynamics may elucidate early events that facilitate the ensuing development of bNAbs, and explain why this occurs in only a minority of individuals. While the evidence presented thus far is quite encouraging, the novelty of using pT_{FH} cells to track bNAb responses necessitates further experimentation to reconcile the different conclusions described. It may be possible, however, that all these subsets play varying roles in different facets of Ab responses. Indeed, recent evidence suggests that surface expression of certain T_{FH} markers is downregulated in

memory T_{FH} cells after antigen clearance, while the T_{FH} lineage of these cells remains intact[78].

Collectively, most recent studies reinforce the notion that a circulating pool of memory T_{FH} cells exists in both humans and mice. Indeed, in an elegant study using both mice and human specimens, He et al. demonstrated that PD-1^{hi}($ICOS^{+}CCR7^{lo}$) pT_{FH} are activated cells that identify active GC T_{FH} differentiation[28]. Furthermore, these authors demonstrated that pT_{FH} cells are antigen-experienced precursors of GC T_{FH} cells, as pT_{FH} generation occurred independent of GC-forming factors (SAP) but still required T_{FH} differentiating factors (ICOS and Bcl6)[28]. While the relatedness of pT_{FH} and T_{FH} cells in lymphoid follicles was challenged by another study based on naïve B cell helper capacity, CCR6 expression, and gene expression profiles[77], it did not directly address the relationship between GC T_{FH} in LN and pT_{FH} from matched donors as was done by He *et al*. It may be possible that T_{FH} marker expression and B helper function is affected by phase of infection or nature of immunogen. Indeed, Bentebibel *et al*. suggested that different vaccination modalities and/or adjuvants might stimulate divergent subsets of pT_{FH}, skewing the Ab response to the vaccine[30]. Thus future studies investigating the pT_{FH} profiles induced by various previously tested vaccines would be informative in this regard. Importantly, by showing that $CCR7^{lo}$PD-1^{hi} pT_{FH} are precursor T_{FH} cells with prominent helper capacity that circulate through nondraining LN, He *et al*. proposed that pT_{FH} cells can rapidly differentiate into mature T_{FH} cells upon antigen re-exposure to support GC formation and mediate B cell help, thus implying a mechanism by which T_{FH} cells exit the secondary LN and are poised to accelerate Ab responses in non-draining LN, likely to contain a spreading infectious agent[28]. These studies provide a better-defined phenotype with which to monitor the status and specificities of T_{FH} cells and provide critical support to using pT_{FH} to study the development of affinity matured Abs, such as bNAbs.

A key question that remains is the role of antigen persistence in vaccine responses and whether and how this contributes to divergent pT_{FH} phenotypes, function, Ab potency and duration. Moreover, it may be possible that all identified potential pT_{FH} characteristics are correct in different settings of diseases, vaccination or populations. Indeed, while antigen specificity of T_{FH} cells in bNAb development remains to be established[26], a possible explanation for the divergent results between *in vivo* HIV bNAb and influenza-specific Ab development is that the $CXCR3^{+}CXCR5^{+}CD4^{+}$ T cell population induced by influenza vaccination may provision suboptimal B cell help by only promoting memory responses[29, 30], thus potentially explaining the low efficiency and duration of seasonal influenza vaccine campaigns. Future studies exploring the interplay between pT_{FH} cells, B cells and Ab production will likely provide insight into how to best design more effective vaccines.

Concluding remarks

Current research highlights the tight involvement of T_{FH} cells in the development of bNAbs during natural HIV/SIV infection despite evidence of dysregulated T_{FH}-mediated B cell help. Exploring pT_{FH} dynamics may facilitate the dissection of T_{FH}:B cell interactions that culminate in bNAb development during chronic infection, providing insight into how T_{FH} responses can be manipulated to optimize AID expression, SHM, and CSR and long-lived B

cell responses. Knowledge of how to tailor T_{FH} responses through vaccination will inform vaccine development for HIV and others diseases.

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References

1. Rerks-Ngarm S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. 2009; 361:2209–2220. [PubMed: 19843557]
2. Haynes BF, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med. 2012; 366:1275–1286. [PubMed: 22475592]
3. Plotkin SA. Vaccines: correlates of vaccine-induced immunity. Clin Infect Dis. 2008; 47:401–409. [PubMed: 18558875]
4. Moldt B, et al. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci U S A. 2012; 109:18921–18925. [PubMed: 23100539]
5. Balazs AB, et al. Antibody-based protection against HIV infection by vectored immunoprophylaxis. Nature. 2012; 481:81–84. [PubMed: 22139420]
6. Shingai M, et al. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. Nature. 2013; 503:277–280. [PubMed: 24172896]
7. Barouch DH, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. Nature. 2013; 503:224–228. [PubMed: 24172905]
8. Huang J, et al. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. Nature. 2012; 491:406–412. [PubMed: 23151583]
9. Walker LM, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science. 2009; 326:285–289. [PubMed: 19729618]
10. Scheid JF, et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science. 2011; 333:1633–1637. [PubMed: 21764753]
11. Sather DN, et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J Virol. 2009; 83:757–769. [PubMed: 18987148]
12. Mikell I, et al. Characteristics of the earliest cross-neutralizing antibody response to HIV-1. PLoS Pathog. 2011; 7:e1001251–100. [PubMed: 21249232]
13. Gray ES, et al. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. J Virol. 2011; 85:4828–4840. [PubMed: 21389135]
14. Simek MD, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J Virol. 2009; 83:7337–7348. [PubMed: 19439467]
15. Doria-Rose NA, et al. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J Virol. 2010; 84:1631–1636. [PubMed: 19923174]
16. Wu X, et al. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science. 2011; 333:1593–1602. [PubMed: 21835983]
17. Wu X, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science. 2010; 329:856–861. [PubMed: 20616233]
18. Tangye SG, et al. The good, the bad and the ugly - TFH cells in human health and disease. Nat Rev Immunol. 2013; 13:412–426. [PubMed: 23681096]

19. Victora GD, Nussenzweig MC. Germinal centers. Annu Rev Immunol. 2012; 30:429–457. [PubMed: 22224772]
20. Moir S, Fauci AS. Insights into B cells and HIV-specific B-cell responses in HIV-infected individuals. Immunol Rev. 2013; 254:207–224. [PubMed: 23772622]
21. Perreau M, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J Exp Med. 2013; 210:143–156. [PubMed: 23254284]
22. Petrovas C, et al. CD4 T follicular helper cell dynamics during SIV infection. J Clin Invest. 2012; 122:3281–3294. [PubMed: 22922258]
23. Lindqvist M, et al. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. J Clin Invest. 2012; 122:3271–3280. [PubMed: 22922259]
24. Cubas RA, et al. Inadequate T follicular cell help impairs B cell immunity during HIV infection. Nat Med. 2013; 19:494–499. [PubMed: 23475201]
25. Hong JJ, et al. Spatial alterations between CD4(+) T follicular helper, B, and CD8(+) T cells during simian immunodeficiency virus infection: T/B cell homeostasis, activation, and potential mechanism for viral escape. J Immunol. 2012; 188:3247–3256. [PubMed: 22387550]
26. Locci M, et al. Human Circulating PD-1CXCR3CXCR5 Memory Tfh Cells Are Highly Functional and Correlate with Broadly Neutralizing HIV Antibody Responses. Immunity. 2013
27. Shulman Z, et al. T follicular helper cell dynamics in germinal centers. Science. 2013; 341:673–677. [PubMed: 23887872]
28. He J, et al. Circulating Precursor CCR7(lo)PD-1(hi) CXCR5(+) CD4(+) T Cells Indicate Tfh Cell Activity and Promote Antibody Responses upon Antigen Reexposure. Immunity. 2013; 39:770–781. [PubMed: 24138884]
29. Morita R, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. Immunity. 2011; 34:108–121. [PubMed: 21215658]
30. Bentebibel SE, et al. Induction of ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination. Sci Transl Med. 2013; 5:176ra132.
31. Chevalier N, et al. CXCR5 expressing human central memory CD4 T cells and their relevance for humoral immune responses. J Immunol. 2011; 186:5556–5568. [PubMed: 21471443]
32. Burnet FM. A modification of Jerne's theory of antibody production using the concept of clonal selection. Australian Journal of Science. 1957:67–69.
33. Miller JF, et al. Thymus and the production of antibody-plaque-forming cells. Nature. 1965; 208:1332–1334. [PubMed: 5870196]
34. Claman HN, et al. Thymus-marrow cell combinations. Synergism in antibody production. Proc Soc Exp Biol Med. 1966; 122:1167–1171. [PubMed: 5918139]
35. Velardi A, et al. Functional analysis of cloned germinal center CD4+ cells with natural killer cell-related features. Divergence from typical T helper cells. J Immunol. 1986; 137:2808–2813. [PubMed: 2944964]
36. Johnston RJ, et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science. 2009; 325:1006–1010. [PubMed: 19608860]
37. Nurieva RI, et al. Bcl6 mediates the development of T follicular helper cells. Science. 2009; 325:1001–1005. [PubMed: 19628815]
38. Yu D, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity. 2009; 31:457–468. [PubMed: 19631565]
39. Kerfoot SM, et al. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. Immunity. 2011; 34:947–960. [PubMed: 21636295]
40. Crotty S. Follicular helper CD4 T cells (TFH). Annu Rev Immunol. 2011; 29:621–663. [PubMed: 21314428]
41. Choi YS, et al. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. Immunity. 2011; 34:932–946. [PubMed: 21636296]
42. Xu H, et al. Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. Nature. 2013; 496:523–527. [PubMed: 23619696]

43. Bauquet AT, et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. Nat Immunol. 2009; 10:167–175. [PubMed: 19098919]
44. Nurieva RI, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity. 2008; 29:138–149. [PubMed: 18599325]
45. Eto D, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. PLoS One. 2011; 6:e17739. [PubMed: 21423809]
46. Rasheed MA, et al. Interleukin-21 is a critical cytokine for the generation of virus-specific long-lived plasma cells. J Virol. 2013; 87:7737–7746. [PubMed: 23637417]
47. Hiramatsu Y, et al. c-Maf activates the promoter and enhancer of the IL-21 gene, and TGF-beta inhibits c-Maf-induced IL-21 production in CD4+ T cells. J Leukoc Biol. 2010; 87:703–712. [PubMed: 20042469]
48. Kroenke MA, et al. Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. J Immunol. 2012; 188:3734–3744. [PubMed: 22427637]
49. Schwickert TA, et al. Germinal center reutilization by newly activated B cells. J Exp Med. 2009; 206:2907–2914. [PubMed: 19934021]
50. Kageyama R, et al. The receptor Ly108 functions as a SAP adaptor-dependent on-off switch for T cell help to B cells and NKT cell development. Immunity. 2012; 36:986–1002. [PubMed: 22683125]
51. Schwickert TA, et al. A dynamic T cell-limited checkpoint regulates affinity-dependent B cell entry into the germinal center. J Exp Med. 2011; 208:1243–1252. [PubMed: 21576382]
52. Cannons JL, et al. SLAM family receptors and SAP adaptors in immunity. Annu Rev Immunol. 2011; 29:665–705. [PubMed: 21219180]
53. Sage PT, et al. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. Nat Immunol. 2013; 14:152–161. [PubMed: 23242415]
54. Good-Jacobson KL, et al. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. Nat Immunol. 2010; 11:535–542. [PubMed: 20453843]
55. Pavri R, Nussenzweig MC. AID targeting in antibody diversity. Adv Immunol. 2011; 110:1–26. [PubMed: 21762814]
56. Zotos D, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010; 207:365–378. [PubMed: 20142430]
57. Linterman MA, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010; 207:353–363. [PubMed: 20142429]
58. Cannons JL, et al. Biochemical and genetic evidence for a SAP-PKC-theta interaction contributing to IL-4 regulation. J Immunol. 2010; 185:2819–2827. [PubMed: 20668219]
59. Frohlich A, et al. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science. 2009; 324:1576–1580. [PubMed: 19478140]
60. Elsaesser H, et al. IL-21 is required to control chronic viral infection. Science. 2009; 324:1569–1572. [PubMed: 19423777]
61. Chevalier MF, et al. HIV-1-specific interleukin-21+ CD4+ T cell responses contribute to durable viral control through the modulation of HIV-specific CD8+ T cell function. J Virol. 2011; 85:733–741. [PubMed: 21047960]
62. Douek DC, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature. 2002; 417:95–98. [PubMed: 11986671]
63. Fahey LM, et al. Viral persistence redirects CD4 T cell differentiation toward T follicular helper cells. J Exp Med. 2011; 208:987–999. [PubMed: 21536743]
64. Harker JA, et al. Late interleukin-6 escalates T follicular helper cell responses and controls a chronic viral infection. Science. 2011; 334:825–829. [PubMed: 21960530]
65. Slike B, et al. Early immune events during acute HIV infection. Retrovirology. 2012; 9:181.

66. Xu Y, et al. Simian immunodeficiency virus infects follicular helper CD4 T cells in lymphoid tissues during pathogenic infection of pigtail macaques. J Virol. 2013; 87:3760–3773. [PubMed: 23325697]

67. Klein F, et al. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. Cell. 2013; 153:126–138. [PubMed: 23540694]

68. Scheid JF, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature. 2009; 458:636–640. [PubMed: 19287373]

69. Corti D, et al. Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. PLoS One. 2010; 5:e8805. [PubMed: 20098712]

70. McLellan JS, et al. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature. 2011; 480:336–343. [PubMed: 22113616]

71. Mouquet H, et al. Memory B cell antibodies to HIV-1 gp140 cloned from individuals infected with clade A and B viruses. PLoS One. 2011; 6:e24078. [PubMed: 21931643]

72. McGuire AT, et al. Engineering HIV envelope protein to activate germline B cell receptors of broadly neutralizing anti-CD4 binding site antibodies. J Exp Med. 2013; 210:655–663. [PubMed: 23530120]

73. Klein F, et al. Antibodies in HIV-1 vaccine development and therapy. Science. 2013; 341:1199–1204. [PubMed: 24031012]

74. Paris R, et al. HLA class II restriction of HIV-1 clade-specific neutralizing antibody responses in ethnic Thai recipients of the RV144 prime-boost vaccine combination of ALVAC-HIV and AIDSVAX((R)) B/E. Vaccine. 2012; 30:832–836. [PubMed: 22085554]

75. Liao HX, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature. 2013; 496:469–476. [PubMed: 23552890]

76. Zhou T, et al. Multidonor analysis reveals structural elements, genetic determinants, and maturation pathway for HIV-1 neutralization by VRC01-class antibodies. Immunity. 2013; 39:245–258. [PubMed: 23911655]

77. Boswell KL, et al. Loss of circulating CD4 T cells with B cell helper function during chronic HIV infection. PLoS Pathog. in press.

78. Hale JS, et al. Distinct memory CD4+ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. Immunity. 2013; 38:805–817. [PubMed: 23583644]

Highlights

- T follicular helper cells present a latent HIV viral reservoir
- Tfh cells may be intricately involved in the generation of HIV-specific broadly neutralizing Abs
- Insights into Tfh immunobiology may provide inroads into effective vaccine design
- Peripheral Tfh cell counterparts may facilitate studies of Tfh in lymphoid tissues

Box 1: Critical questions regarding T_{FH} cells in HIV vaccine design

Translating lessons from natural HIV infection to vaccination-induced NAb development is a hurdle that over 30 years of active research has yet to overcome. The resolution of several key issues, particularly regarding the duration of NAb development, is imperative to achieve this feat: (1) What is the relationship between T_{FH} cells, B cell dysfunction, and NAb development in natural infection; (2) Does viral quasispecies evolution promote continuous priming of Ab responses to linear epitopes, leading to constant production of low affinity antibodies rather than affinity maturation to conserved regions (or neotopes), and is this a result of B cell competition; (3) Does the number and location of SHM that are required to convey bNAb activity solely explain the delay in NAb development or is the SHM/AID machinery impaired by HIV pathogenesis, thus affecting the efficiency of T_{FH}:B cell help and the ensuing B cell proliferation, differentiation, and Ab affinity maturation; (4) Is the trimeric, highly glycosylated nature of HIV Env detrimental to affinity maturation and SHM by activating T-independent Ab responses or short-lived plasma cells; (5) Are certain Env variants more elusive than others at inducing beneficial T_{FH} responses; (6) Is this an issue of epitope miscommunication between T_{FH} cell and B cells or is it an issue of immunogenic silence; (7) Does antigenemia promote SHM in an individual with a healthier B cell compartment and how can this be achieved through vaccination; (8) Are T_{FH} cells a major latent reservoir during HIV infection; (9) Is T_{FH} cell dysfunction/expansion influencing the lack of bNAb development in the majority of patients and can T_{FH} functionality/dynamics account for the rapid bNAb development in certain subjects; (10) Can the induction of specific T_{FH} functions drive bNAb development; and (11) Can Gag-specific T_{FH} responses provide help for Env-specific Abs.

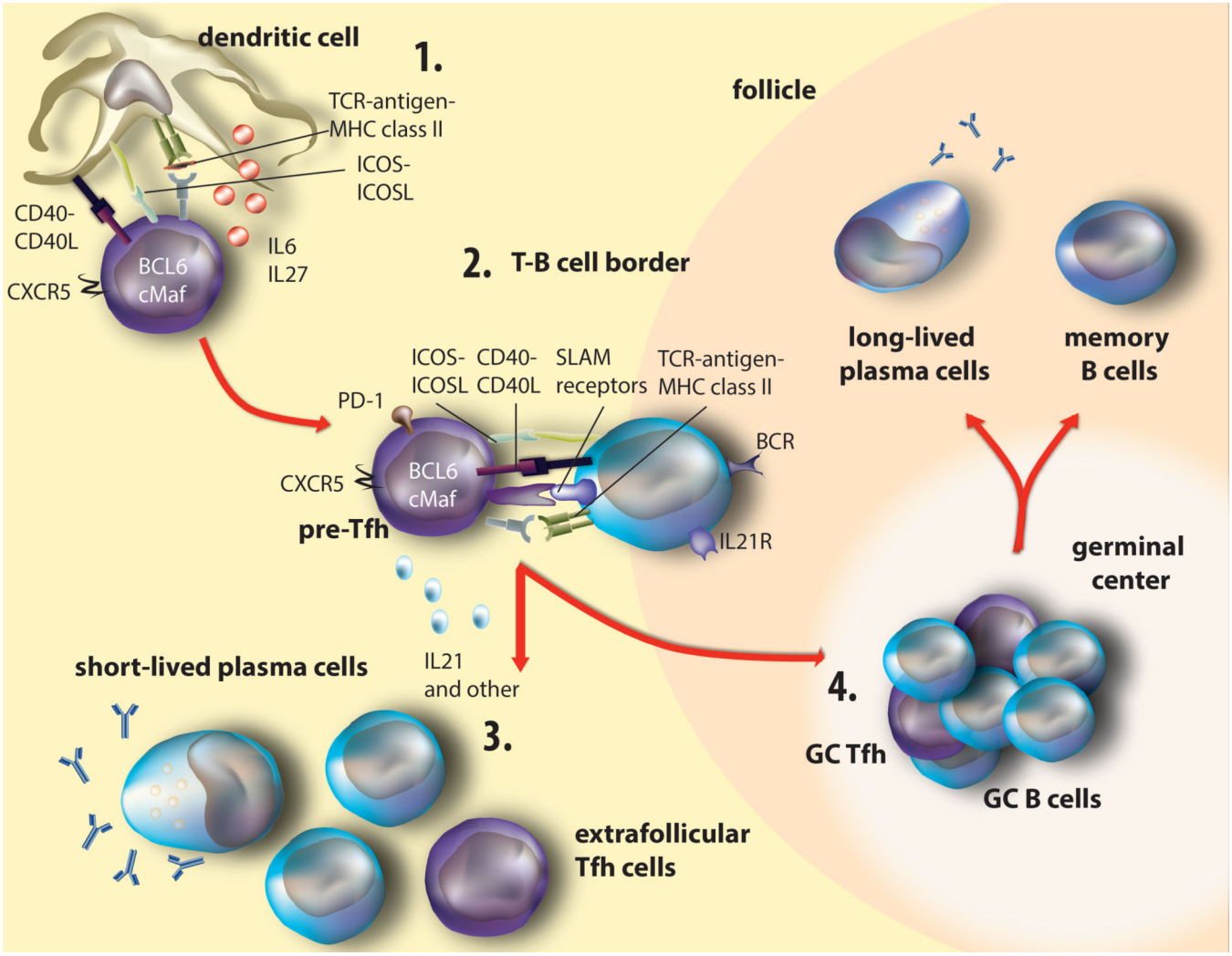

Figure 1. Development of T_{FH} cells and the germinal center reaction

(1) Dendritic cells present antigen and activate naïve CD4 T cells via TCR:MHC-II, ICOS-ICOS-L, and CD40:CD40L interactions to initiate the T_{FH} program. DC- and B cell-derived IL-6 and IL-27 cytokines maintain and reinforce this lineage, leading to upregulation of the T_{FH} transcription factors Bcl6, cMaf, and CXCR5. (2) Expression of CXCR5 facilitates the migration of pre-T_{FH} cells toward the T—B cell interphase. Here, cognate pre-T_{FH} and B cells exchange molecular signals including ICOS:ICOS-L, CD40:CD40L, TCR:MHC-II, and several SLAM receptor family members, as well as several cytokines including the cardinal cytokine IL-21, that reinforce expression of Bcl6 and c-Maf that further increase expression of T_{FH} surface receptors. B cells simultaneously undergo diverse signals that instruct their maturation pathway: either extrafollicular or GC. (3) Extrafollicular activated B cells then convert to short-lived plasma cells and secrete Ab with or without extrafollicular T_{FH} help. (4) In the GC, prolonged interactions between GC T_{FH} and GC B cells further confirm the T_{FH} lineage commitment, resulting in mutual exchange of survival signals that result in GC maintenance. GC T_{FH} cells provide a limiting source of antigen for which B cells compete for survival signals based on BCR affinity. Furthermore, here GC

T_{FH} cells instruct GC B cells to phosphorylate and express Activation-induced Deaminase (AID) that mediates SHM, CSR, memory cell and long-lived plasma cell formation.

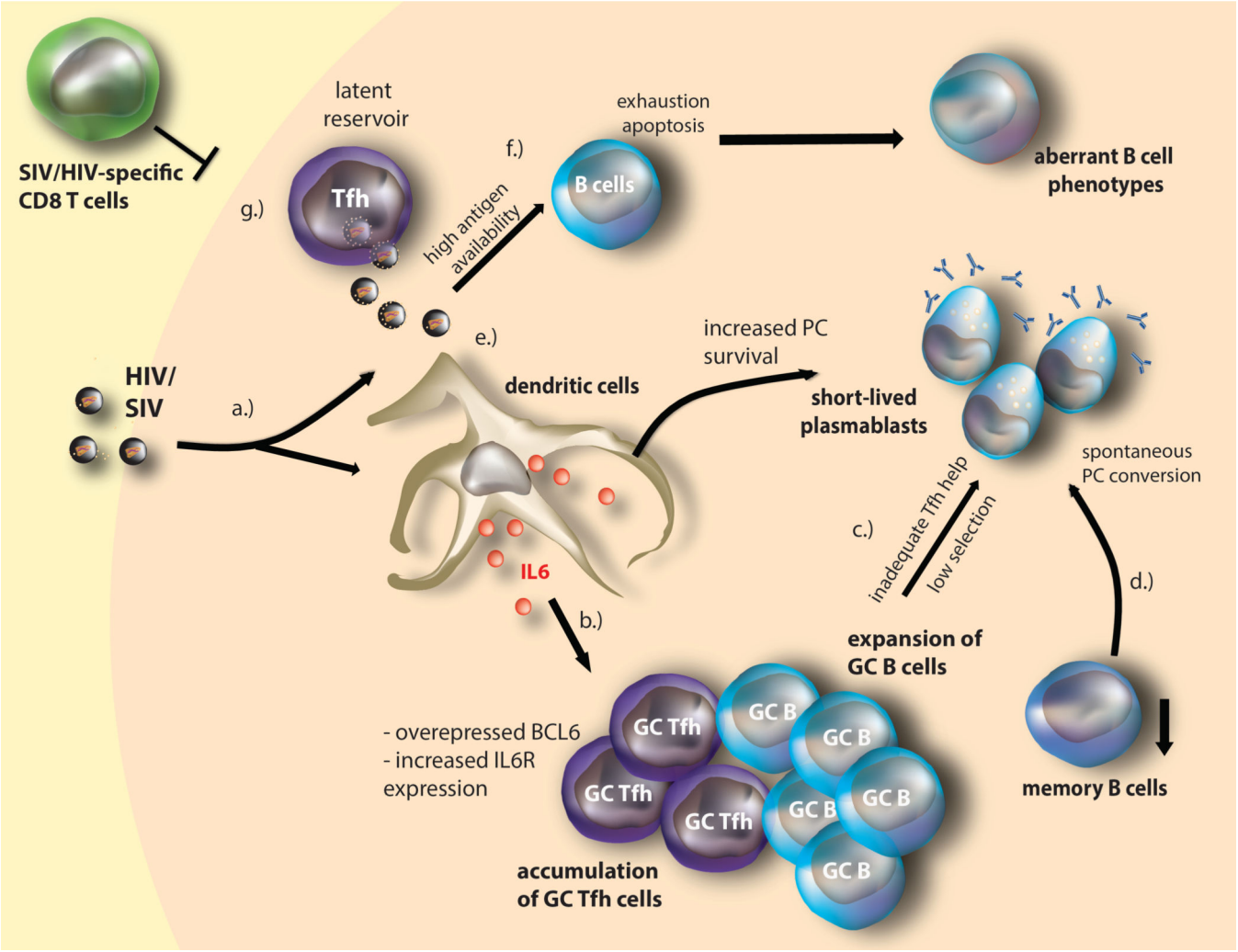

Figure 2. HIV/SIV-mediated T_{FH} dysfunction and immunopathogenesis

(a.) HIV/SIV mediated immune activation induces high IL-6 production found within infected lymph nodes. (b.) IL-6 induces expansion of (potentially dysfunctional) T_{FH} cells expressing high levels of Bcl6 and IL-6Rα. T_{FH} expansion is further associated with increased numbers of GC B cells. (c.) GC T_{FH} expansion increases contact with PD-L1-expressing GC B cells, resulting in deregulated GC T_{FH} cells and inadequate help provisioned to GC B cells, likely by lowering the selection threshold and decreasing IL-21 signaling (and other T_{FH} cytokines such as IFN γ and IL-10) that lead B cell differentiation into short-lived PC formation and increased polyclonal and HIV/SIV-specific (primarily targeting Gag) IgG production. (d.) Similarly, direct IL-6 signaling may also mediate spontaneous terminal differentiation of memory B cell into plasma cells, resulting in observed decrease of memory B cells in HIV/SIV infection. (e.) High antigenic availability within the lymph node likely also enhances plasma cell survival. (f.) High antigenemia likely also contributes to B cell exhaustion, apoptosis, and the subsequent aberrant B cell phenotypes. (g.) HIV/SIV infection of T_{FH} cells (enhanced by IL-6) likely maintains the viral reservoir, as infected T_{FH} cells, may be resistant to apoptosis; this likely constitutes a

latent reservoir within a privileged tissue, as HIV/SIV-specific CD8 CTL relatively seldom enters the lymphoid tissue.

Table 1

Divergent surface marker profile of pT_{FH} cells with B helper activity.

	Morita (2011) pTfh	Mikell (2011)* -	Chevalier (2011) pTfh	Bentebibel (2013) pTfh	Locci (2013) pTfh	He (2013) pTfh & GC Tfh	Boswell (2014) pTfh & GC Tfh
CXCR5	+		+	+	+	+	hi**#
CXCR3	−			+	−		±/−
PD-1	+	+			+	hi	hi
ICOS				+			
CCR6	+					hi	hi
CCR7					low	low	hi

* study was not designed to interogate Tfh phenotype

** $CCR7^{high}$ $CXCR5^{low}$ cells had limited naïve B helper activity

$CXCRS^{low}$ cells in HIV viremic subjects displayed limited naïve B helper activity

Expression levels were described but not incorporated in final phenotype