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REVIEW

# The unique features of follicular T cell subsets

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**Abstract** The germinal center (GC) reaction is critical for humoral immunity, but also contributes adversely to a variety of autoimmune diseases. While the major protective function of GCs is mediated by plasma cells and memory B cells, follicular helper T (TFH) cells represent a specialized T cell subset that provides essential help to the antigen-specific B cells in the form of membrane-bound ligands and secreted factors such as IL-21. Recent studies have revealed that TFH cells are capable of considerable functional diversity as well as possessing the ability to form memory cells. The molecular basis of this plasticity and heterogeneity is only now emerging. It has also become apparent that several other populations of follicular T cells exist, including natural killer T cells and regulatory T cells. In this review we will discuss the function of follicular T cells and interaction of these populations within the GC response.

**Keywords** Germinal center · Follicular helper T cell · IL-21 · Immune response · Antibody production

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## Introduction to the germinal center reaction

To counteract the fast evolution potential of pathogens, the immune system needs a way to generate rapidly adaptive effectors. During a humoral immune response, after a first and immediate wave of low affinity immunoglobulins (Ig), the response progressively evolves towards antibodies of higher affinity. This focusing of the immune response is permitted by the massive generation of B cells that undergo somatic hypermutation, a process of extremely high rate mutations affecting the variable regions of the B cell receptor (BCR). As the mutation process is random, it is followed by a selection of the lymphocytes bearing receptors of the highest affinity. In parallel to the diversification of the variable region, the antibody repertoire also evolves in its effector functions through Ig class switch recombination (CSR). Depending on extracellular signals, activated B cells, initially IgM, are able to convert to another isotype (IgG, IgA, or IgE) best adapted to the nature of the pathogen or the target tissue.

B cell proliferation, somatic hypermutation, selection, and CSR all occur within a specialized structure termed the germinal center (GC, Fig. 1). GCs emerge from secondary lymphoid organ follicles after encounter with a T cell-dependent antigen. A GC gathers three types of cells: B cells, follicular dendritic cells (FDC), and $CD4^+$ T helper (Th) cells. The FDC are stromal cells that present at their surface the antigens. They also secrete the chemokine CXCL13, which will attract $CXCR5^+$ lymphocytes towards the GC [1]. The Th cells provide the signals to the antigen-specific B cells that guide their survival, expansion or differentiation. The importance of T cells in the process is highlighted by the fact that GC are unable to form in their absence [2]. In this review we will discuss our current understanding of the functions and diversity of follicular T cells during the GC response.

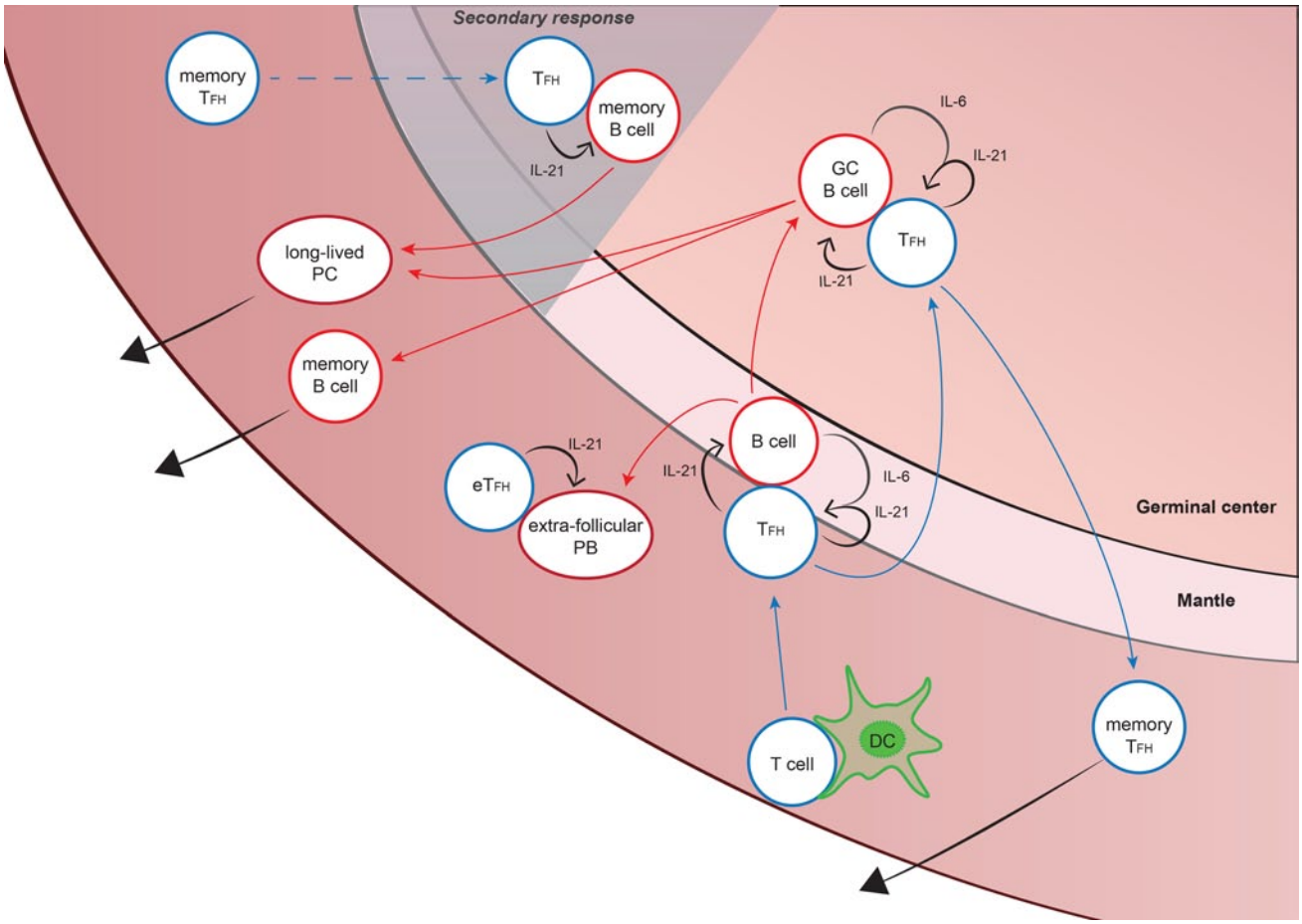


**Fig. 1** TFH-secreted IL-21 coordinates the GC cell fate in multiple ways. DC-primed T cells up-regulate Bcl6, and subsequently CXCR5, and migrate toward the B cell follicle. Upon the encounter with an antigen-activated B cell, they further mature and secrete IL-21 that triggers an autocrine loop promoting the TFH cell commitment. The autocrine function of IL-21 appears to be largely redundant with IL-6 that is derived from activated B cells and myeloid cells. In parallel, the IL-21 fosters in the B cell partner the expression of Bcl6 or its antagonist, Blimp-1. Early in the response, the B cells preferentially leave the follicle to become short-lived extrafollicular plasmablasts (PB). Extrafollicular (e) TFH cells sustain this early phase, notably by secreting IL-21 that promotes the isotype switching and Blimp-1 expression. Alternatively, both T and B cells migrate to the GC where TFH cells reach their full potential as IL-21 producers. IL-21 both maintains the TFH cell phenotype and plays a major role in antibody diversification mechanisms. At the completion of the GC reaction long-lived plasma cells (PC) and memory B cells and potentially memory TFH cells exit the follicular region and enter the circulation. If IL-21 actions on TFH cells can be compensated by IL-6, those on B cells can only be partially undertaken by IL-4

## Follicular helper T cells, a professional B cell helper

Among $CD4^+$ T cells, several subsets can be defined on the basis of their function (e.g., effector versus regulatory), and according to the type of immune response they promote (Th1, Th2, etc.), but besides these pro-inflammatory and regulatory abilities, another major role of the $CD4^+$ T cell population is to provide help to the B cells within the secondary lymphoid organ. The T cell subset dedicated to this function in the GCs is termed follicular helper T (TFH) cells.

### The hallmarks of TFH cells

The term follicular helper T cell emerged in 2000 with the demonstration of human tonsillar $CD4^+$ T cells within the GC that expressed co-stimulatory molecules and were able to provide an efficient help for Ig production by B cells [3, 4]. A key to the initial designation of TFH cells was the expression of CXCR5, the chemokine receptor driving migration to B cell follicles [5–7]. A high-affinity T cell receptor (TCR) is also a characteristic of TFH cells [8], with strong TCR signaling favoring commitment toward the follicular helper fate [9]. Once formed, TFH cells require continuous exposure to antigen for their maintenance, with the antigen dose determining the size and duration of the TFH response [10, 11]. While in normal circumstances GC B cells, which are required for the GC reaction, present the antigen, this requirement can be overcome by very large antigen loads, suggesting that it is the antigen-presenting function of B cells and not unique B

cell-derived signals that promote the maintenance of TFH cells [11].

The dedication of TFH cells to B cell help is clearly displayed by the molecules present at their surface. A key co-stimulatory molecule expressed on TFH cells is CD40 ligand (CD40L), the unique activator of CD40, a central player in B cell activation, proliferation, survival, and differentiation, i.e., all aspects of the help supplied by TFH cells. Deficiency of one of those two partners prevents any GC development and antibody production [12, 13]. It has been shown in vitro that CD40L rescues GC B cells from apoptosis and inhibits plasma cell commitment in favor of the memory B cell fate [14, 15]. In humans, mutations in the *CD40* gene, or its ligand, result in a defective CSR and somatic hypermutation, and a lack of memory B cells, all indicative of the absence of GC [16–20].

TFH cells also express other members of the tumor necrosis factor receptor family, like CD30 or OX-40, that both provide pro-survival signals [21]. CD30-deficient, and even more markedly CD30 and OX-40-doubly deficient mice, showed an impaired capacity to sustain GC responses. OX-40 is also involved in TFH cell migration to B cell follicle as it induces the transcription of CXCR5 [22]. On the B cell side, OX-40L engagement also improves antibody secretion [23].

Programmed cell death 1 (PD-1) is similarly highly expressed by TFH cells [24, 25], while GC B cells exhibit both PD-1 ligands (PD-L1 and PD-L2) at their surface [26]. PD-1 is induced by sustained TCR signaling and acts as a negative regulator of $CD4^+$ T cell proliferation. As a result, the absence of PD-1 signaling triggers a higher frequency of TFH cells [26]. However, the deficiency of PD-1 or PD-L2, due to an enhanced GC B cell apoptosis, leads to decreased long-lived plasma cell formation.

Another essential co-stimulatory molecule to GC formation is inducible T cell costimulator (ICOS), required for TFH cell differentiation and maintenance [27, 28]. In humans, ICOS deficiency is characterized by the absence of memory B cells, severe defects in CSR, and GC formation [29]. ICOS is required very early in TFH cell ontogeny, where it induces Bcl6 expression following DC priming. This requirement for ICOS continues into the GC where ICOSL on antigen-specific B cells maintains Bcl6 and TFH cell fate [10, 30]. Whether ICOSL provides any signal to the GC B cell remains unknown. In addition to its co-stimulation role, ICOS also plays a pivotal function in TFH cell recruitment to the follicle [31]. ICOS fosters TFH cell motility by enhancing pseudopod dynamics. The ICOSL engagement is provided by the follicular by-stander B cells and is therefore independent of any antigen specificity.

The ICOS-related molecule CD28 was one of the first co-stimulatory molecules to be identified as essential for GC formation [32], probably due to the inability of T cell activation [33], and thus of differentiation of TFH cells. However, a B cell-specific requirement for its ligands, CD86 and CD80, has also been reported [34–36]. CD86 appears to be the most influential, playing a role in GC initiation. The requirement of CD80 has been more controversial, being reported as either facultative [34, 35] or more involved in GC B cell survival and plasma cell generation [36].

The TFH cell help to the GC B cell cannot be efficiently delivered without the formation of a stable conjugate between the two partners (Fig. 1). Therefore, adhesion molecules play a critical role in the establishment of this synapse, and among them, one family has focused attention, the signaling lymphocyte activation molecule (SLAM) receptors. CD84 and Ly108 that are both expressed by TFH cells and GC B cells represent the two main adhesion-mediating actors. CD84 influences TFH cell late differentiation and GC maintenance [37]. It has functional redundancy with Ly108, as shown by the diminished ability to form conjugates in absence of both molecules. Both CD84 and Ly108 signal via the adaptor SLAM-associated protein (SAP). The deficiency in this molecule in TFH cells leads to an absence of long-lived plasma cells and memory B cells, and a severe impairment in GC formation [38]. In humans, *SH2D1A*, the gene encoding for SAP, is mutated in X-linked lymphoproliferative (XLP) syndrome [39], where the pathology is very similar to that of SAP-deficient mice. Otherwise competent helpers, SAP-deficient TFH cells are unable to stably interact with B cells, and as a result to induce their normal proliferation [40]. This faulty conjugate outcome also impaired the recruitment and retention of TFH cells in forming GC. Recently, Kageyama et al. [41] demonstrated that SLAM receptor function involved more than adhesion. They reported that the double deficiency in SAP and Ly108 overcame the TFH cell differentiation defect arising from the absence of SAP. The underlying mechanism was competition between SAP and the phosphatase SHP1 for binding to Ly108, which resulted in respectively positive and negative signals for TFH cells.

## Transcriptional regulation of TFH cell differentiation

B cell lymphoma 6 (Bcl6) is considered the master regulator of TFH cell identity as its absence prevents any TFH cell commitment and its forced expression is sufficient to induce TFH cells [42–44]. Bcl6 is induced very early in the differentiation of TFH cells by ICOSL expression on DC and is maintained through TFH cell ontogeny by ICOSL signals derived from GC B cells [30, 45–48]. Bcl6 is essential for CXCR5 expression and thus follicular homing and appears to function by repressing expression of a group of microRNAs that target crucial TFH cell genes, such as *Cxcr5* [43]. Bcl6 may also

repress the expression of genes encoding T-bet, RORγt, and GATA3, crucial regulators of other helper T cell lineages, although this regulation cannot be absolute, as at least T-bet and GATA3 can be expressed by TFH cells [49].

Remarkably, Bcl6 also drives the GC B cell differentiation, but through a distinct set of genes [50]. This discrepancy is likely due to the property of Bcl6 to interact with different partners as mutation of the domain of Bcl6 which binds to the corepressors BCOR, NCOR, and SMRT blocked GC B cell formation, but not TFH cell differentiation [51]. Strikingly, Bcl6 expression in GC B cells is thought to be regulated by TFH cell-derived IL-21 (see below, [52]). One common Bcl6 target is *Blimp1*, whose expression is specifically excluded from TFH cells, despite being generally expressed in most other activated T cell subsets. Ectopic Blimp-1 blocks *Bcl6* expression and TFH cell formation, while Blimp-1-deficiency increases TFH cell numbers [44]. Interestingly, Irf4, another key regulator of GC B cells, is also required for TFH cell development [53], where it is again likely to be involved in the STAT3-dependent IL-21 response [54]. How Irf4, which represses *Bcl6* and induces *Blimp1* in B cells, acts differently in TFH cells is unknown. Nevertheless, the striking parallels between the genetic programs, with TFH cells and GC B cells promoting the expression of Bcl6 in each other, as well as their cellular interdependence suggests that the two cell types have co-evolved to fulfill this highly specialized role.

## Interleukin-21 a major effector of TFH cell function

IL-21 is the last identified member of the $\gamma_c$-receptor cytokine family that includes also IL-2, IL-7, or IL-15 [55]. Its expression is restricted to activated $CD4^+$ T [55] and natural killer T (NKT) [56] cells. Interestingly, innate signals can also foster IL-21 expression as NKT cells secrete it in response to *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) vaccination [57] and *Listeria monocytogenes* infection [58]. The strong association of IL-21 with TFH cells was first revealed by microarray profiling of human TFH cells [59]. Among TFH cells, a reporter mouse has shown that about a third of the population express IL-21 in the context of a T cell-dependent immunization [49].

### The transcriptional regulation of *Il21*

The transcriptional controls on *Il21* expression are still emerging (Fig. 2). It has been shown that nuclear factor of activated T cells (NFATc2) [60] and c-Rel [61] bind and activate the *Il21* promoter. In addition to antigen stimulation, IL-21 provides autocrine stimulation, via STAT3 [62]. The same pathway can potentially be triggered by IL-6 [62, 63] or IL-27 [64], and by IL-12 in humans [65]. Patients deficient for STAT3 show a decrease in IL-12-induced secretion of IL-21 and TFH cell generation [66]. STAT3-activating cytokines induce the expression of c-Maf, another transcription factor that transactivates the *Il21* promoter [67, 68]. c-Maf is highly expressed in TFH cells and

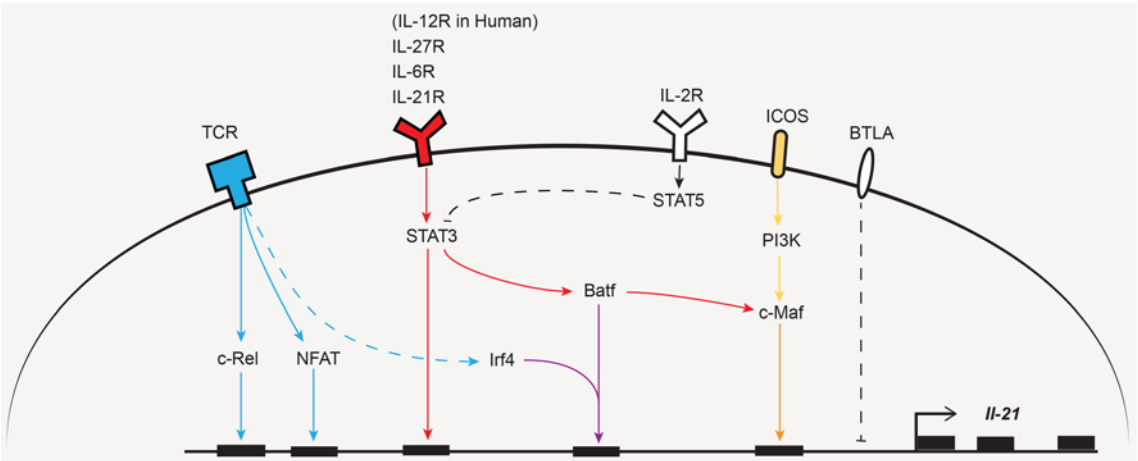


**Fig. 2** The regulation of *Il21* expression in TFH cells. Schematic of the published interactions of the major signaling pathways that are known to synergize in promoting the expression of *Il21* in TFH cells. The *arrow* indicates positive interactions, the *bar* denotes negative regulation, and the *dashed line* indicates putative interactions. Note that the diagram is schematic only and not intended to be an accurate representation of the *Il21* promoter. Antigenic stimulation of the T cell receptor (TCR) leads to the binding of c-Rel and NFAT to the promoter of *Il21*. The binding of IL-21 to its receptor triggers through STAT3 the upregulation of Batf that fosters the expression of c-Maf. Other cytokines, as IL-6, IL-27, or IL-12 in humans, can activate the same pathway. c-Maf induction is, however, predominantly dependent upon the PI3 kinase (PI3 K) pathway downstream of ICOS. Batf, associated with Irf4, c-Maf, and STAT3, are all transactivators of *Il21* transcription. In addition, there are two known inhibitory signals: BTLA and IL-2. The engagement of the IL-2 receptor activates STAT5 that may compete with STAT3, while the mechanism by which BTLA inhibits *Il21* is unclear

its loss leads to fewer TFH cells and a decrease in IL-21 secretion [69]. In this population, c-Maf expression is highly dependent on ICOS signaling [69] through the activity of the PI3 (phosphoinositide) kinase [70]. c-Maf expression is likewise regulated by the AP-1 superfamily member, Batf [71], which is itself required for TFH cell differentiation and IL-21 expression [72].

Irf4 is also involved in the regulation of *Il21* expression and its positive feedback loop in $CD4^+$ T cells [73]. Conversely, the deficiency in an Irf4 inhibitory binding partner Def6 leads to an up-regulation of ICOS and an increase of IL-21 production by $CD4^+$ T cells [74, 75]. The similarity in phenotype of Irf4 and Batf-deficient mice, both being required for GCs and for Th17 cell differentiation, was recently explained by the finding that both factors cooperatively bind together to a novel composite recognition site in T cells [76–78].

Considering the negative regulation of *Il21* expression, the axis IL-2/STAT5/Blimp-1 plays a major role in the limitation of TFH cell differentiation and IL-21 expression by antagonizing Bcl6, and likely STAT3 [79–81]. The inhibitory co-receptor BTLA down-modulates IL-21 expression by a yet-to-be-determined mechanism [82].

### The function of IL-21 in germinal centers

IL-21 can induce its own expression in TFH cells, as well as trigger a TFH cell-like state and Bcl6 expression in vitro [42, 83]. Mice lacking the IL-21R have defects in antibody responses and CSR [84], a phenotype that was initially ascribed to an essential role for IL-21 in TFH cell generation [28, 85]. Subsequent studies using bone marrow chimeras have instead demonstrated that IL-21 functions predominantly on GC B cells with TFH cell development being IL-21-independent [52, 86–88]. The function of IL-21 in TFH cells is likely to be redundant with IL-6 as the combined deficiency in IL-21 and IL-6 leads to a decrease in the TFH cell population [89, 90] (Fig. 1).

The primary function of IL-21 in the GC appears to be to promote GC B cell persistence potentially by Bcl6 up-regulation [52, 86]. Moreover it has been long known that IL-21 could foster proliferation or apoptosis of B cells according to the stimulation context [55, 91, 92]. A non-specific signal like TLR (Toll-like receptor) engagement triggers death through the expression of the pro-apoptotic molecule Bim, while activation by the BCR associated with co-stimulation elicits proliferation. The ultimate outcome of IL-21 stimulation on GC B cells is also dependent on the potent plasma cell differentiation capacity of IL-21 in both mice and humans [93–95]. IL-21 induces the expression of *Blimp1* [94] in a STAT3- and Irf4-dependent manner [54]. IL-21 is thus able to foster the expression of both Bcl6 and Blimp-1, two antagonist transcription factors in lymphocyte differentiation.

The main role of the GC is to produce high-affinity antibodies of various isotypes. Both somatic hypermutation and CSR are mediated by the enzyme activation-induced deaminase (AID) in the centrocytes. It has been reported that IL-21 was a switch factor of IgA, IgG1, and IgG3 in human B cells [96, 97], and that IL-21R-deficient mice exhibited a lack of IgG1 combined with an increase of IgE [84]. This last hyperglobulinemia seems to be mediated by an apoptotic mechanism rather than by a bias in the CSR. Indeed, IL-21 signaling results in the formation of a complex between Bcl-2 and Bmf, an $IgE^+$ B cell-specific pro-apoptotic molecule, which results in the selective loss of the IgE secretion [57]. Affinity maturation is also influenced by IL-21 as its deficiency leads B cells to undergo fewer rounds of mutations, potentially through the inability of IL-2R-deficient B cells to sustain the GC [86]. The pathway linking IL-21 to these mechanisms is not yet completely understood, but it has been recently demonstrated that Bcl-6 up-regulated AID expression via the repression of miR (microRNA)-155 [98].

Mice deficient for IL-21, or its receptor, do not fully uncover all the actions of this molecule on GC B cells, as IL-4, that also utilizes the $\gamma_c$-chain receptor, secreted by TFH cells has some overlapping functions with IL-21 [84, 99]. This finding is in keeping with the ability of $IL\text{-}21^+$ TFH cells to simultaneously secrete other cytokines such as IL-4, IFN-γ, or IL-17 [49, 69].

## The diversity of TFH cells

The idea that TFH cells could be considered as a distinct subset of T cells, dedicated to the B cell help, has been substantiated by the identification of Bcl6 as a critical regulator that dictates the expression of a unique transcriptome [43] and inhibited the differentiation of the canonical effector subset programs by binding the promoter of their master regulators (T-bet and GATA-3) or by inhibiting their function (RORγt) [42]. Moreover, TFH cells can differentiate in mice bearing deficiencies that prevent any Th1 or Th2 commitment [28, 49]. Though, depending on the environment, TFH cells are able to secrete IFN-γ [28, 49] or IL-5 and IL-13 [100], whereas the reports about IL-17 remain controversial [49, 69, 85]. Furthermore, in parasitic infection models, TFH cells represent the major source of IL-4 in lymph nodes, while Th2 were the IL-4 producers within the tissues [99]. All these cytokines are CSR factors that contribute to the TFH cell function by conferring on them the capacity to orientate the humoral immune reaction [99]. As a result, there is no absolute separation between Bcl6 expression and that of those other master regulators,

suggesting the possibility that TFH cells are a specialized differentiation state of activated Th cells, akin to the positioning of GC B cells in the B cell lineage [101].

### Extrafollicular TFH cells

TFH-like cells are also involved in the extrafollicular generation of short-lived unmutated plasma blasts. Their different location is mediated by their differential chemokine receptor expression, CXCR5 for GC TFH cells, and CXCR4 for extrafollicular TFH cells, while P-selectin glycoprotein ligand 1 (PSGL-1) is down-regulated on both populations [102]. The extrafollicular foci appear within 2 days after primary encounter with antigens at the borders between the T cell zone and the red pulp or in the lymph node extramedullary cords (Fig. 1). Extrafollicular TFH cells are Bcl6 and ICOS-dependent and express PD-1 and CD40L [102, 103]. In their absence, the extrafollicular response is not abrogated, but greatly reduced. Their secretion of IL-21 is of major importance to the help they deliver [86, 103] and dependent of ICOS expression [102]. During an infection with *Salmonella* that triggers uniquely an extrafollicular response, the absence of TFH cells blocks CSR without modifying the anti-IgM production [103]. So, extrafollicular TFH cells enhance the generation of short-lived plasma blasts and induce the isotype switching of these cells.

### Follicular helper NKT cells

It has emerged recently that conventional $CD4^+$ T cells were not the only lymphocytes that exhibits TFH cell properties. GC-resident NKT cells, termed NKTFH cells, have been described that can be elicited in response to immunization with a lipid antigen [58, 104]. NKTFH cells share with TFH cells their dependency on Bcl6, CD28, SAP, and B cells to develop, the expression of CXCR5 and PD-1, and their ability to secrete IL-21. The help provided by NKTFH cells induces the B cell differentiation program characterized by extrafollicular plasma blasts, GC formation, affinity maturation, and a primary IgG antibody response, but in contrast to the conventional GC response, NKTFH cells do not promote the formation of long-lived plasma cells or memory B cells. The capacity of NKTFH cells to recognize lipids antigens allows broadening the humoral response to this kind of antigens.

### Follicular T regulatory (TFR) cells

In addition to TFH cells, another $CD4^+$ T cell population can be found within the GC, the follicular regulatory T (TFR) cells. Regulatory T ($T_{reg}$) cells were described in human tonsils in 2004 [105], but their origin and features have until recently remained elusive. It is now apparent that TFR are not just $T_{reg}$ cells with a follicular location but a distinct subset that co-opts characteristics from both Treg and TFH cell populations [106, 107]. This hybrid phenotype is not a unique case amid $T_{reg}$ cells as other subpopulations that acquire features of conventional $CD4^+$ Th cells subsets have been recently described [108]. Thus, TFR cells, like TFH cells, express CXCR5 and PD-1, concomitantly with $T_{reg}$ cell features as CTLA-4 or GITR. Of importance, these cells co-express the transcription factors Bcl6, Foxp3, and Blimp-1. Blimp-1 expression, described as characteristic of the effector $T_{reg}$ cells [109], restrains the number of TFR cells, as the population doubled in its absence, while Bcl6 is essential for the differentiation of TFR cells. Although TFR cells share several features with TFH cells, they are not B cell helpers as they lack the expression of CD40L, IL-4 or IL-21, and instead appear to play a regulatory role, as TFR cell-deficient mice exhibit increased TFH cell and GC B cell populations. While the evidence to date favors a thymic origin of TFR cells [106], it remains possible that some cells in this population are induced in the periphery from conventional $CD4^+$ T cells.

## TFH cell plasticity

The transcriptional and functional distinctiveness of TFH cells clearly distinguishes them from conventional $CD4^+$ T cells; however the relationship of the various subsets has been less easily deciphered. It has been shown by several groups that transferred $CD4^+$ T cells, lacking TFH cell markers, can acquire these characteristics after in vivo stimulation [49, 100, 110]. In particular, $CXCR5^-PD\text{-}1^-$ or $CXCR5^+PD\text{-}1^+IL\text{-}21^-$ $CD4^+$ T cells can become $IL\text{-}21^+$ TFH cells [49]. Nevertheless, these studies did not elucidate the frequency of these contributions, or the actual efficiency of these cells as B cell helpers. Besides the relationship between TFH cell and effector compartments, it has been proposed that in the Peyer's patches, TFH cells arose from both Treg and Th17 cells. The transfer of $Foxp3^+$ Treg cells into T cell-deficient mice allowed the formation of GC in the Peyer's patches more efficiently than the transfer of total $CD4^+$ T cells [111]. Moreover, $FoxP3^-$ cells were unable to sustain the constitution of GC, despite their ability to acquire a TFH cell–like phenotype. The differentiation of Treg cells into TFH cells accompanied the down-regulation of FoxP3 and the up-regulation of Bcl6, CXCR5, and IL-21. Very recently, lineage-tracing experiments using a reporter for IL-17 expression have revealed that Th17 cells preferably home to the gastrointestinal tract, and in Peyer's patches acquired a TFH cell phenotype. Importantly, these ex-Th17 cells supported GC formation and IgA CSR and secretion, a process that depended on the Th17 master regulator RORγT [112].

The relationship between TFH cells and effector Th cells has also been assessed after infection with the helminth, *Nippostrongylus brasiliensis* [99]. In this model, the dominant response is IL-4 mediated. It has been shown that lymph node TFH cells were the major IL-4 producers, whereas Th2 cells dominate in the lungs, where they recruited eosinophils. The transfer of IL-4$^+$ Th2 or TFH cells from an infected animal to an IL-4-deficient host, subsequently infected, showed that TFH cells were far less efficient at playing the role of a Th2 cell [99]. This work elegantly demonstrates that TFH cells are specialized helper cells, functionally different from their effector counterpart.

A number of studies have also reported the induction of a TFH-like cellular state from naive CD4$^+$ T cells in vitro, using the cytokines IL-6 and IL-21 [28, 89, 113]. These TFH-like cells express some endogenous IL-21 and Bcl6 and, when transferred in vivo, can provide help to GC B cells in a SAP-dependent manner [83]. TFH-like cells could also be produced from already polarized Th1, Th2, and Th17 cells. ChIPseq analysis of histone modifications revealed that the genes encoding the major regulators of Th1 (*Tbx21*), Th2 (*Gata3*), and Th17 (*Rorc*) cells maintained a signature of active chromatin (H3K4me3), a finding supportive of their plasticity between TFH- and Th-cell populations. Repression of the TFH cell program in Th1 cells in vitro required high IL-2 concentrations, which inhibited Bcl6 expression through direct repression by Blimp-1 [81]. Further evidence that Th cells may pass through a TFH-like state was provided by Nakayamada and colleagues who found that IL-12 acting via STAT4 transiently induced both IL-21 and Bcl6 that was subsequently repressed by T-bet. A function for T-bet in restraining TFH cell expansion and GC formation in response to *Toxoplasma gondii* infection was described, a finding that contrasts with the normal TFH cell function reported from influenza infected T-bet-deficient mice [49, 114]. Clearly, more work is required to resolve the relationship between in vivo TFH cells and in vitro derived TFH-like cells.

## TFH cell memory

The fate of TFH cells after GC involution has until recently remained unclear. A first study has reported persistent TFH cells within the lymph nodes several weeks after immunization [115]. Importantly, as antigen persisted in this model, it was not clear that those cells were true memory cells. Conversely, in a Th1-biased *L. monocytogenes* infection model, CD4$^+$ effector memory T cells (Tem) and follicular helper-like central memory T cells (Tcm) cells were found to derive from the CXCR5$^-$CCR7$^-$ effector compartment and a Bcl6-dependent, CXCR5$^+$CCR7$^+$ population, respectively [116]. TFH cells themselves seemed not to persist as memory cells in this model. Following transfer and immediate challenge, the Tem compartment generated overwhelmingly effector Th1 cells while the Tcm compartment was more multipotent, giving rise to effector Th1 cells, Tcm and TFH cells [116]. More recently, the ability of TFH cells to contribute to the memory compartment has been more directly addressed using transfer experiments that allowed a contraction phase in absence of antigen [49, 117]. One of these studies performed the transfer of influenza- experienced TFH cells to naive mice that were their turn infected a month later [49]. Persistent TFH and non-TFH cells both proliferated in the spleen and mediastinal lymph nodes, and infiltrated the lungs. The lung TFH cells lost CXCR5 and IL-21 expression, whereas splenic TFH cells secreted IFN-γ and IL-2. This suggests that memory TFH cells are able to persist and become conventional Th1 effector cells under recall. However, memory TFH cells preferentially entered the GC reaction, compared to conventional memory cells, and memory IL-21$^+$ TFH cells arose primarily from already IL-21-expressing TFH cells. Similar findings were presented by another study that used immunization of mice harboring transgenic CD4$^+$ T cells [117]. These authors also found that memory TFH cells down-regulated canonical TFH cells markers and were predisposed to re-express IL-21 upon re-challenge.

In humans, CXCR5$^+$ circulating CD4$^+$ T cells can be found in the blood [3]. These cells express CCR7 that allows them to re-enter the secondary lymphoid organs, which they down-regulate after stimulation, but express a similar amount of Bcl6 or IL-21 as their CXCR5$^-$ counterpart [118]. Morita et al. [119] proposed that human blood TFH cells could be divided into three subsets on the basis of their expression of chemokine receptors and cytokines: a Th1 (CXCR3$^+$ IFN-γ$^+$), a Th2 (CXCR3$^-$ CCR6$^-$ IL-4$^+$ IL-5$^+$ IL-13$^+$) and a Th17 (CXCR3$^-$ CCR6$^+$ IL-17$^+$) subset. Only the Th2 and Th17-like subsets were able to deliver help to B cell via the secretion of IL-21. While other studies have not found the same polarization or a significant role for IL-21, they did demonstrate a helper capacity specific to the CXCR5$^+$ compartment, which instead relied on IL-10 secretion [118]. Interestingly, these putative TFH-Th2 and TFH-Th17 cell subsets were found increased in the blood of patients suffering a chronic autoimmune disease, the juvenile dermatomycosis, compared to healthy donors or to psoriasis patients [119]. This skewing is correlated with an increase of circulating plasma blasts and more pronounced in patients displaying active symptoms. These data suggest a connection between TFH cell deregulation and autoimmunity.

## TFH cell-associated pathologies

### Autoimmunity

Similarly to the juvenile dermatomycosis, increased proportions of circulating TFH-like CXCR5$^+$ CD4$^+$ T cells are observed in some patients with systemic lupus erythematosus (SLE), Sjögren's syndrome, rheumatoid arthritis, or autoimmune thyroiditis, all autoimmune pathologies partially mediated by autoantibodies [120–126]. The involvement of TFH cells in SLE is also supported by the correlated decrease of proliferating B cells, plasma cells, and autoantibodies in the blood of patients treated with an anti-CD40L antibody [127]. Intriguingly, increased frequency of CXCR5$^+$ CD4$^+$ T cells could be correlated with high titers of autoantibodies and tissue damage [122–125], as with IL-21 levels and/or Bcl6 expression [121–126] in all of these pathologies.

Several mouse models have been enlightening about the role of TFH cells in autoimmunity. The BXSB.Yaa mouse exhibits spontaneous development of GC with expansion of the TFH cell compartment and increase of IL-21 secretion [94, 128]. In this mouse, the *Tlr7* gene is duplicated [129], resulting in excessive signal triggering by self-RNA. IL-21 is a key player in this model as IL-21R deficiency totally abrogates the symptoms [130]. Similarly, in the lupus-prone MLR-Fas$^{lpr/lpr}$ mouse, the deficiency in IL-21 receptor prevents the spontaneous formation of GC, the accumulation of plasma cells, and leads to a significant decrease of nephritis severity and autoantibody titers [131]. Extrafollicular TFH cells strongly accumulate in MLR-Fas$^{lpr/lpr}$ and are likely to act as the primary inducers of autoantibody production, as this accumulation does not happen in the absence of IL-21 signaling. Another group has also demonstrated that the neutralization of IL-21 by an IL-21R.Fc fusion protein decreased the circulating autoantibody levels and the tissue damage [132]. However, as the treatment was administrated before the appearance of the first symptoms, it is not known whether this kind of neutralization could have a curative action.

Another murine model, the Sanroque mouse, shows a spontaneous deregulation of the GC reaction and circulating autoantibodies production in relation with an increased TFH cell population and secretion of IL-21 [133]. The causative mutation affects the gene *Roquin-1 (Rc3h1)*, a repressor of ICOS expression, resulting in TFH cell hyperactivity. Abrogation of TFH cell function by removing SAP ameliorated the pathology, demonstrating that the TFH cell function was an essential component of the disease. The increased IL-21 seems to be a by-stander effect, and to not contribute to the SLE-like disease [134]. While Roquin-1 appears to direct the degradation of *Icos* mRNA in a dicer-independent manner, the mechanism underlying aberrant function of the *Sanroque* allele was initially unclear, as the deletion of the gene does not recapitulate the Sanroque phenotype [135]. This paradox has been recently resolved with the finding that the ablation of Roquin-1 and its close paralog Roquin-2 results in deregulated ICOS expression and systemic inflammation [136, 137].

### TFH cell malignancy

Notably, half of heterozygous Sanroque mice develop a lymphadenopathy that is evocative of a peripheral T cell lymphoma, the AITL (angioimmunoblastic T cell lymphoma) [138]. Instead of displaying the systemic pathology that characterizes the homozygous Sanroque mouse, only some lymph nodes were hyperplasic in the heterozygotes. Histological examination of enlarged lymph nodes showed features reminiscent of the AITL, including follicular neoplastic T cells, deregulated B cell growth, hyperglobulinemia, and expansion of the FDC population [139]. The tumoral T cells express CXCL13, as human TFH cells do, a chemokine that promotes induction and proliferation of FDC. The AITL transcriptome is further related to TFH cells with an overexpression of Bcl6, PD-1, or CD40L [140]. The molecular hits causative of AITL remain poorly understood. Recently, it was reported that AITL is characterized by recurrent mutations in the *IDH2* (Isocitrate dehydrogenase) and *TET2* (Ten-eleven translocation) genes [141, 142]. These enzymes are functionally related, as methylcytosine oxidation by TET2 is dependent of a metabolite of IDH2 activity. The accumulation of both mutations in AITL may potentially be responsible for chromatin remodeling alterations. Thus, AITL is the first lymphoma described resultant from the transformation of TFH cells.

### HIV infection

As a key player in the adaptive humoral response, the TFH cell is also a strategic target for infectious agent escape mechanisms. In HIV infection, it is well established that germinal centers represent the largest reservoir of virions [143]. While B cells themselves are not infected, the antibody response is deeply impacted with a skewing of populations towards transitional and plasmablast compartments at the expense of the memory population that is associated to hyperglobulinemia, but of low affinity antibodies [144]. In addition, a fraction of virus-specific memory B cells exhibit an exhausted phenotype, high levels of inhibitory receptors, and decreased effector capacity [145]. It has been hypothesized that the loss of the CD4$^+$ T cells was at least partially contributing to this deregulation; however, all those dysfunctions are unexpectedly reminiscent of SLE [144], pathology with an active TFH cell

population. Studies in humans and rhesus macaques have recently reported that the TFH cell population was significantly increased in lymph nodes upon infection [146–149]. Moreover, not only are TFH cells similarly susceptible to infection as other $CD4^+$ T cells, but the virus replicates more actively within them. This TFH cell expansion may be driven by the high levels of IL-6 induced by infection, accordingly with the elevated expression by the helper population of IL-6 receptor and STAT3 [146]. This accumulation of antigen and TFH cells may contribute to decreased selectivity of GC B cells, and therefore to the abundant production of low-affinity antibodies. IL-21 could also potentiate the differentiation into plasma blasts, as infected TFH cells were high producers [147, 148]. Conflicting data have been published concerning IL-21 production by circulating TFH cells in HIV [150, 151]. One study reported that IL-21 was reduced in sera of HIV-infected patients in correlation with the lymphopenia [151]. They proposed that this decrease moreover originated from a per-cell basis secretion defect as the HIV infection down-modulated c-Maf expression, while a second study described an elevation of circulating virus-specific IL-21 producers [150]. Both studies do agree that such increase is correlated with a relative control of viremia. A third study compared the IL-21 production by TFH cells isolated from the LN of infected or healthy patients, and found it decreased upon HIV infection [149]. This defect was correlated with a reduction of TFH cell activation and ICOS expression. Interestingly, it was demonstrated that an up-regulation of PD-L1 on the HIV patient GC B cells, and that the ligation of PD-1 could recapitulate the defective TFH cell phenotype in vitro. The authors suggested then the chronic infection fostered (potentially through high levels of Interferon) the upregulation of GC B cell PD-L1 expression that in turn triggered excessive PD-1 stimulation on TFH cells, which impaired the helper functions. Thus, the diversion of TFH cell function appears as an important mechanism of HIV escape from humoral immunity.

## Conclusions

Research over the past decade has highlighted the distinct role that TFH cells play in humoral immunity. While the exact relationship between TFH cells and other Th cells is still emerging, it seems likely that TFH cells represent a specialized state of effector $CD4^+$ T cells that are analogous to the status of GC B cells in the B cell lineage in that they depend on Bcl6 and are capable of further differentiation. Such a positioning agrees well with the documented examples of plasticity between TFH and Th cells and with the finding that TFH cells have the capacity to form memory cells and be recalled into both the TFH cell and conventional $CD4^+$ effector T cell compartments. The molecular mechanism by which TFH cell differentiation is initially induced and subsequently maintained is currently a very active area of research that may ultimately allow the development of approaches to therapeutically manipulate TFH cell numbers or function to either enhance or suppress the immune responses as desired.

Recent studies have also revealed that the GC contains at least two other T cell populations, NKTFH and TFR cells. NKTFH cells represent another population of potential therapeutic value as polyclonal activation of NKT cells using lipid antigens such as α-galactosylceramide has been shown to have powerful adjuvant activity. TFR cells in contrast may act to limited GC responses and dampen autoimmunity and thus enhancing TFR activity may be an immune suppressive mechanism by which autoimmune GC could be controlled. Developing a fuller understanding of the functions of these follicular T cell populations and their interactions are important goals for future research.

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