Review

The elusive identity of T follicular helper cells

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Follicular helper T (Tfh) cells provide help to B cells and allow formation of long-lived antibody responses. Despite an improved understanding of the molecular program that drives Tfh cell formation, their definition remains elusive: neither follicular homing ability, Bcl-6 expression nor IL-21 secretion are exclusive properties of T cells that help B cells, and not all follicular T cells are B cell helpers. Indeed some follicular T cells appear to be suppressive. Furthermore, Tfh cells evolve during an immune response and B cells that have recently bound antigen, germinal center (GC) B cells and plasmablasts interact with phenotypically distinct Tfh cells. Here we propose that distinction between non-GC Tfh and GC Tfh cells might reconcile emerging controversies on Tfh cytokine secretion and the requirement of T–B cell interactions and SAP expression for Tfh formation.

Follicular helper T (Tfh) cells: the challenge of a definition

Within secondary lymphoid organs, Tfh cells provide help to B cells to allow formation of long-lived antibody responses. Beyond their ability to provide B cell help, Tfh cells are arguably the helper T (Th) cell subset that has been most challenging to define. This is partly because: (i) T cell–B cell interactions occur at several distinct phases of thymus-dependent (TD) antibody responses and T cells evolve in phenotype and function during this response; (ii) CD4 T cells in B cell follicles are heterogeneous [1,2]; and (iii) there is a lack of consensus on whether T cells that provide help to B cells at extrafollicular sites belong to the Tfh lineage and the point in ontogeny where a Th cell becomes a Tfh cell is still not clearly defined. Expression of CXC chemokine receptor 5 (CXCR5) and/or ICOS and/or PD-1 is commonly used to identify Tfh cells. However, there is emerging evidence that it is the combination, and more importantly, the amount of CXCR5 and PD-1 (and ICOS in humans, but not mice) expressed that is useful to identify Tfh cell subsets. With these considerations in mind, it is perhaps not surprising that discrepancies arise regarding the molecular requirements for Tfh formation and function and the cytokine secretion profiles of Tfh. To reconcile these emerging discrepancies, it is crucial to reach an agreement on what Tfh cells are, and are not, and an in-depth characterization of the phenotype of the different types of B Th cells is needed. This review articulates the key problematic questions in the nature, function and development of Tfh cells and attempts to reconcile some of the apparently conflicting results.

Defining Tfh cells: the controversies and dilemmas

As highlighted in Figure 1 and reviewed in [3], during immune responses to TD protein antigens, primed T cells initially interact and provide help to B cells at the T–B border, and at this stage immunoglobulin isotype switching is initiated [4–6]. B cells then begin to divide at the perimeter of the follicle [7,8]. Some B blasts migrate to extrafollicular sites and differentiate into low-affinity (unmutated) plasmablasts that may contact and possibly receive help from rare T cells at these locations [8,9]. Other B blasts move to the center of the follicle to form germinal centers (GCs) [7,8]. GC B and T cells develop in parallel, with mutual support, and GC T cells help select mutated GC B cells to undergo differentiation into high-affinity plasma cells or memory B cells. At least for particulate antigens, memory B cells establish contacts with cognate T cells for reactivation [10,11]. Formation of low-affinity memory B cells along a T cell-dependent but GC-, ICOS-, IL-21- and Bcl-6-independent pathway has also been described [8,12–15]. It therefore emerges that there is more than one type of Tfh cell.

Th lineages have been classically defined by their functional specialization, target cells, transcription factor expression and cytokine production. In this paradigm, one could define Tfh cells as cells that help clear viral and bacterial infections and their products (toxins) through their ability to provide help to B cells for antibody production, express the transcription factor Bcl-6 and secrete IL-21. CXCR5 expression and follicular homing ability also frequently, if not always, accompanies the definition of Tfh cells [1,2,16].

Is CXCR5 expression or follicular homing ability an obligatory or exclusive property of Tfh cells?

CXCR5 (the receptor for B cell follicle-produced CXCL13) is the most widely used marker for identification of Tfh cells [1,17,18]. Definition of Tfh cells according to CXCR5 expression however, poses several problems. First and foremost, CXCR5 is upregulated to intermediate levels upon T cell priming *in vivo*, regardless of which type of immune response is being elicited – Th1, Th2, Th17 or Tfh [19,20]. Concomitant downregulation of CCR7 (the receptor for T zone-expressed CCL19 and CCL21) directs activated Th

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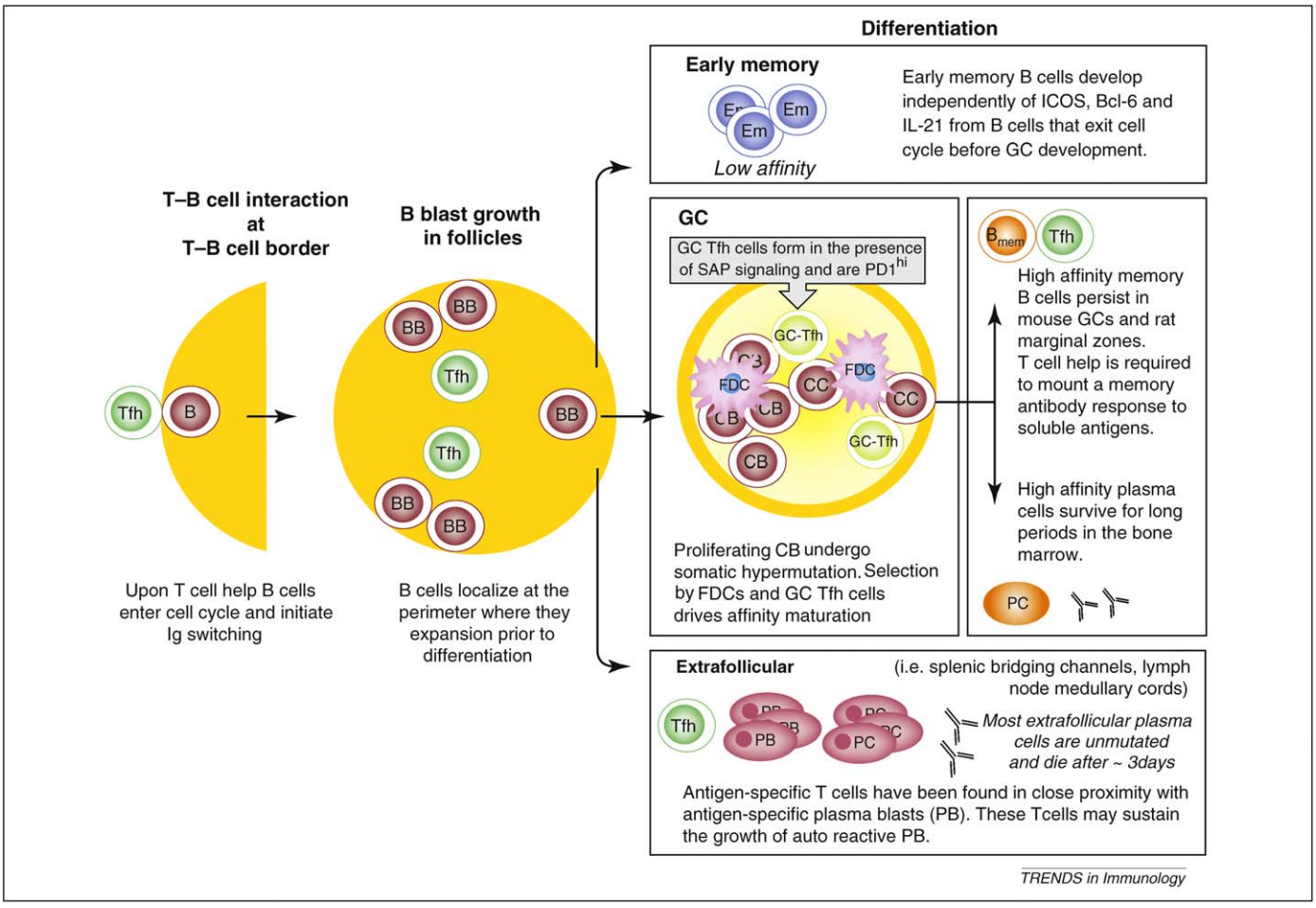

Figure 1. T–B cell cognate interactions during TD antibody responses. Several distinct T–B cell cognate interactions might occur in the context of TD antibody responses in secondary lymphoid organs. The initial T–B cell interaction occurs at the T–B border, and B cells grow in follicles where they might interact with T cells before differentiating along one of three routes: early memory B cells, GC B cells, or extrafollicular plasma cells. GC B cells receive selection signals from T cells. T cells might also provide help to extrafollicular plasmablasts, and appear to be required for reactivation of at least some memory B cells.

cells to the outer T cell zones, positioning them at the T–B border, and facilitating their entry into the follicles [21,22]. Not all activated T cells upregulate CXCR5 to the same extent, and only a proportion of $CXCR5^{+}$ $CCR7^{low}$ cells migrate into follicles after exposure to antigen [19]. Deficiency of CXCR5 in T cells impairs their migration into B cell follicles, but only reduces the frequency of GC B cells by ~2-fold [22,23]. *Cxcr5*$^{-/-}$ T cells localize normally to the T–B border, presumably because of downregulation of CCR7, and are capable of inducing extrafollicular antibody responses in a manner comparable to wild-type T cells [21–23].

The next point of conflict is the dependence of T–B cell interactions for T cell migration into follicles, which has been reported by some laboratories, but not others. T cell migration into the follicles has been elegantly shown to depend dendritic cell (DC) CD40 and OX40L expression, and migration occurs normally in the absence of B cell CD40 or MHC class II expression [24]. The finding that T cell migration into follicles does not require cognate T cell–B cell interactions is, at face value, at odds with the finding that Tfh cells fail to form in μMT mice (that lack B cells) and CD19-deficient mice (that support early plasma-cell responses but mount defective GC responses) [22,25], and are reduced in mice in which B cells lack ICOSL expression [26]. In the case of μMT mice, B cell deficiency (accompanied by FDC deficiency and therefore reduced CXCL13 production [27]) alters the microenvironment required for normal migration. However, CD19-deficient mice contain normal B and T cell numbers but still fail to form Tfh cells, which suggests that interaction with B cells is likely to be important for complete Tfh differentiation.

Overall, although the initial migration of Tfh into follicles appears to be independent of cognate T–B cell interactions, formation of CG Tfh cells appears to depend on T cells being able to establish stable contact interactions with B cells. Recent studies have shown that GC Tfh cells – that is, $PD\text{-}1^{hi}$ $CXCR5^{hi}$ cells, located in GCs – do not form when B cells lack MHC class II or CD40 expression [28]. However, DCs can substitute B cells as antigen-presenting cells (APCs) when mice receive an antigen boost, which suggests that the dependence on B cells for Tfh formation relates to the responding B cells being the predominant APCs when antigen is not present in excess [28]. Thus, the nature of antigens and adjuvant might determine the type of APCs required for Tfh development and function. SAP expression, which is crucial for forming prolonged and stable T–B cell interactions [29,30], is also important for the formation of GC Tfh cells [28,31,32]. Thus, although

initial T cell migration into follicles might be B cell-independent, formation of GC Tfh cells appears to depend in most cases on the formation of stable cognate T–B interactions.

Given that OX40L is required for follicular migration, and that CD4 T cell memory is defective in the absence of OX40 [33] and in mice deficient for both OX40 and CD30 [34], it has been suggested that follicular homing ability is a requisite for normal memory T cell formation [24]. Thus, migration into follicles might not be an exclusive property of Tfh cells, but might also be important for normal T cell expansion and generation of T cell memory. Furthermore, there is evidence to show that the follicle is the crucial niche for the optimal expansion and survival of activated T cells [35–37].

Another important issue is whether T cells that provide help to B cells at extrafollicular sites should be considered Tfh cells. T cells have been found in close contact with plasmablasts in extrafollicular foci during responses to foreign protein antigen [6,8] and in mice with chronic autoreactive antibody responses [9]. In the latter case, these cells are $CXCR5^{low}$ $CXCR4^{high}$ $ICOS^{high}$ and expression of ICOS and IL-21 is associated with autoreactive antibody production. Although a definition of Tfh cells based on CXCR5 expression and follicular migration excludes these cells, their B cell helper activity suggests that they are closely related to Tfh cells. It is probable that their cytokine expression pattern is different from that of GC Tfh cells, and therefore, improved phenotypic characterization is essential to reconcile some of the emerging conflicting data on Tfh cells.

Some follicle-localizing CD4+ T cells might not be specialized in providing help to B cells. T cells that localize in the follicles include $CD25^+$ $Foxp3^+$ cells that also share with Tfh cells high expression of CXCR5, ICOS and PD-1 [38,39]. However, instead of a helper function, these cells appear to be specialized in the suppression of T cells [38] and probably also B cells [40], at least *in vitro*. It is therefore unlikely that these $Foxp3^+$ Tfh cells are B cell helpers, and their Bcl-6 dependence is unknown to date.

The corollary from these studies is that initial T cell migration into follicles is not sufficient for Tfh cell formation, and CXCR5 expression alone is inadequate to define Tfh cells.

Is Bcl-6 expression an exclusive property of Tfh cells?

Three groups have recently reported that the transcriptional repressor Bcl-6 drives Tfh formation. Bcl-6 expression in T cells is essential for CXCR5 expression, T cell migration into follicles, and GC formation [25,41,42].

Bcl-6 overexpression is sufficient to upregulate CXCR5, CXCR4, PD-1, ICOS, IL-21R and IL-6R, and downregulate CCR7 *in vitro* and *in vivo* [25,41,42]. Many of these effects can be antagonized by forced expression of Blimp-1, which represses Bcl-6 and is downregulated in Tfh cells (Figure 2) [25]. Bcl-6 is a transcriptional repressor of gene expression [43]. How a transcriptional repressor enhances expression of multiple genes and positively directs differentiation might be explained, at least in part, by our discovery that Bcl-6 downregulates a vast number of microRNAs (miRNAs); many of which are predicted to target the 3′ untranslated region (3′UTR) of the Tfh signature transcripts, such as CXCR5 (Figure 2) [42,44]. This miRNA-repressive activity of Bcl-6 has subsequently also been also reported in T regulatory cells (Tregs), with a threefold increase in miR-21 in Bcl-6-deficient Tregs [45].

Bcl-6 probably acts in a dose-dependent manner. Most lymphocytes including $CD4^+$ T cells express some amount of Bcl-6, and T cell activation induces Bcl-6 expression as early as 2 h after stimulation [46]. This low amount of Bcl-6 probably contributes to CXCR5 expression by primed T cells; the most striking phenotype of Bcl-6-deficient T cells is their complete failure to upregulate CXCR5. Although it

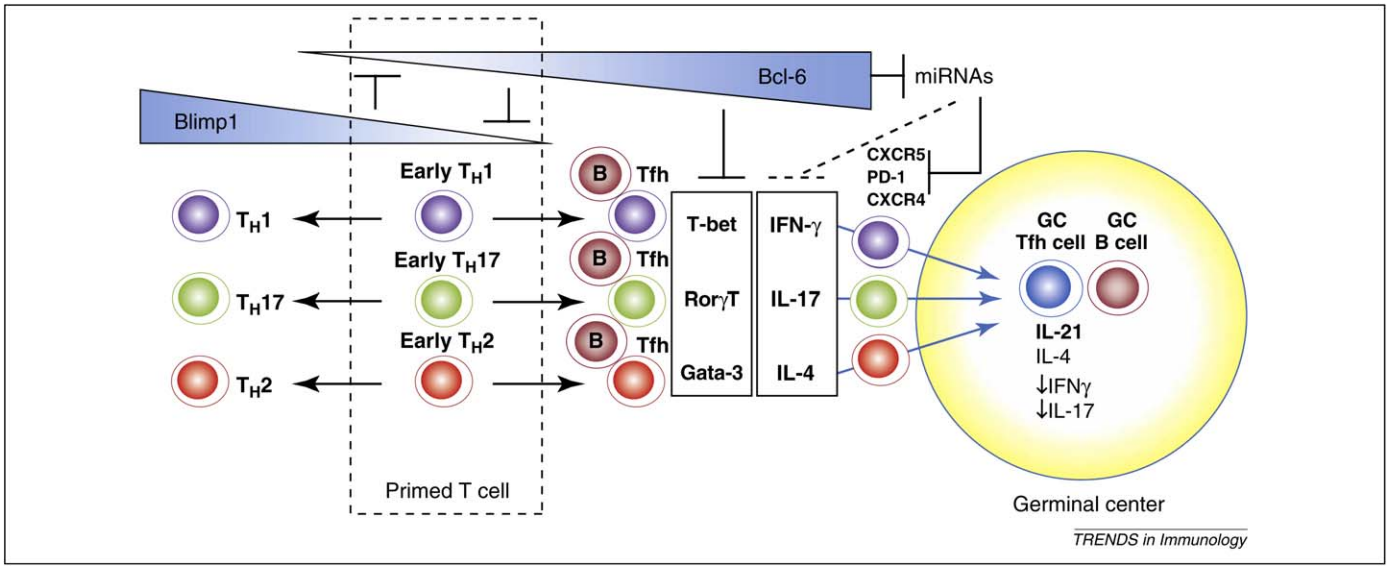

Figure 2. Possible model of Tfh cell formation. Depending on the pathogen and/or adjuvants, T cell priming by APCs leads to early polarization into cells that are capable of secreting Th1, Th2 and/or Th17 cytokines. Depending on the balance between Bcl-6 and Blimp-1 expression, which are mutually repressive, and interaction with B cells, some T cells increase the levels of Bcl-6. This would induce upregulation of CXCR5, at least in part through repression of targeting miRNAs, together with increased expression of PD-1, ICOS, and other Tfh signature molecules. As Tfh cells express the highest levels of Bcl-6, these cells lower their production of cytokines typical of Th1, Th2 and Th17 cells, at least in part through the ability of Bcl-6 to repress their transcriptional regulators.

is clear that Th1, Th2 and Th17 cells form normally in the absence of Bcl-6 [41], whether Bcl-6 plays a role in Th1, Th2 and Th17 cell biology, such as enhancing their initial proliferation, remains to be elucidated. Bcl-6 is also expressed in Tregs, and plays a role in Treg-mediated control of Th2-driven inflammation [45].

T cells that differentiate along the Tfh pathway continue to increase their level of Bcl-6, whereas T cells that polarize to other lineages maintain low amounts of Bcl-6, probably as a consequence of Blimp-1 upregulation [47]. Further increases in Bcl-6 expression have been reported under prolonged stimulation in Th1- or Th17- (but not Th2)-polarizing conditions, compared to non-polarizing conditions [46,48,49]. CXCR5^{hi} PD-1^{hi} Tfh cells express the highest amounts of Bcl-6 (Figure 3a) [50,51]. Expression of high levels of Bcl-6 reduces the production of Th1, Th2 and Th17 cytokines, represses T-bet expression [41,42], and reduces *Gata3* mRNA and *Gata3* protein [52] (Figure 2). Bcl-6 binds to the *TBX21* and *Tbx21* promoter, to the *RORc* promoter (in humans) and possibly to the promoter of *Il17a* [41,42] (Figure 2).

Thus, while Bcl-6 expression is not an exclusive property of Tfh cells, high amounts in cells that express low amounts of T-bet, Rorγt and Gata-3 is to date the best defining feature of the transcriptional profile of Tfh cells.

Is IL-21 expression a selective signature of Tfh cells?

Tfh cells have been shown by different groups to produce vast amounts of *Il21* mRNA and IL-21 protein, with variable amounts of other cytokines [26,50,53]. IL-21 can also be produced by NKT cells [54]. Among CD4+ T cells, IL-21 can be detected in cells that also express IFN-γ, IL-4 or IL-17. Indeed, Th17 cells can produce large amounts of this cytokine [54]. IL-21 is generally, if not always, highly expressed in CXCR5^{high}PD-1^{high}ICOS^{high} Tfh cells from human tonsil (Figure 3a) and mouse secondary lymphoid organs [26,42,48,53].

IL-21 promotes Tfh survival in an autocrine manner [26,53] and is important for GC B cell survival and differentiation through direct effects on B cells [14,15,55]. Association of a CXCR5^{high} phenotype with IL-21 production is not surprising, given that IL-21 enhances Bcl-6 upregulation in T and B cells [26,42,48,53] and Bcl-6 enhances CXCR5 expression [41,42]. Although IL-21 alone cannot identify Tfh cells, production of high levels of IL-21 in combination with moderate levels of IL-4, and low levels of IFN-γ and IL-17 might be typical of the subset of Tfh cells that become GC Tfh cells.

In conclusion, Tfh cells are best defined by high amounts of Bcl-6, low amounts of T-bet, Gata-3, Rorγt and Foxp3, accompanied by expression of CXCR5, ICOS, PD-1 and IL-21. The amounts of these molecules can be variable in early Tfh cells (i.e. those that precede GC formation), or those B cell Th cells located outside follicles, but appear to be consistently high as Tfh cells differentiate to become GC Tfh cells.

The evolving phenotype of Tfh cells

Until recently, it has been difficult to distinguish the different types of Tfh cells on the basis of surface phenotype. Nevertheless, combined evidence from several groups suggests that GC Tfh cells are phenotypically distinct from Tfh cells at other locations or developmental stages.

GC Tfh cells have a unique CXCR5^{high} PD-1^{high} phenotype

The first piece of evidence to suggest that the phenotype of Tfh cells has important functional consequences came from studies that showed that human effector (CD45RA) tonsillar T cells could be divided into three clearly de-

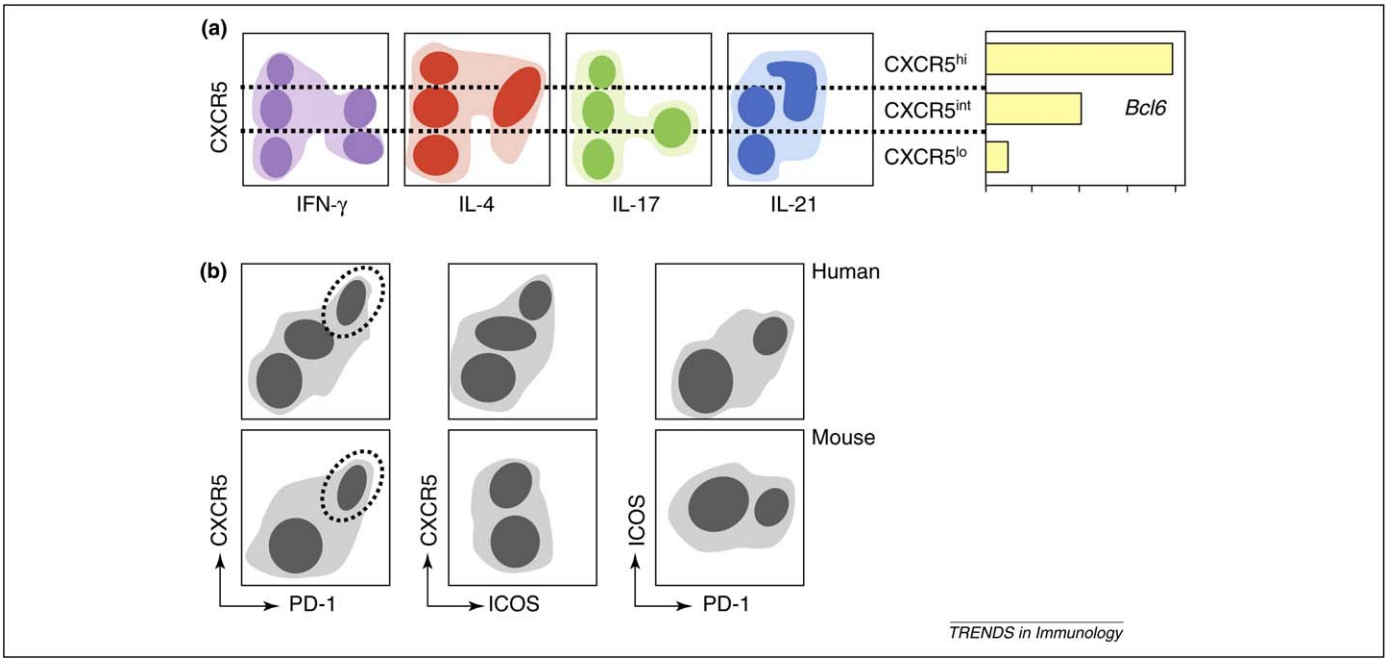

Figure 3. Differences in phenotype and cytokine secretion pattern between GC Tfh and non-GC Tfh cells. **(a)** Diagramatic representation of cytokine production and *BCL6* mRNA expression by tonsil human CD4$^+$ T cells, which highlights the differences between CXCR5^{high} (GC Tfh cells) CXCR5^{int} (non-GC Tfh cells) and CXCR5^{low} populations, as recently reported [28,42,48]. **(b)** Diagramatic representation of CXCR5, PD-1 and ICOS expression by human tonsillar CD4$^+$ T cells (top panel) [42,70] and mouse OT-II cells after transfer into C57BL/6 recipients and OVA-alum immunization (bottom panel) [42,28]. The gates are drawn around GC Tfh cells.

marcated subsets: CXCR5^{low} ICOS^{low}; CXCR5^{int} ICOS^{int}; and CXCR5^{high}ICOS^{high} [51]. In the absence of stimulation, only the CXCR5^{high} ICOS^{high} subset had potent B cell helper function, capable of inducing robust IgG secretion by autologous tonsillar B cells [23]. Gene expression profiling of these subsets also revealed a strong correlation between high amounts of ICOS and CXCR5 expression, and a high amount of PD-1, SAP, IL-21, OX40, CD30L and BTLA [23]. The positive correlation between CXCR5, ICOS and PD-1 expression is also evident at the protein level (Figure 3b). Bcl-6 is also more strongly expressed in CXCR5^{high} cells compared with CXCR5^{int} cells (Figure 3a).

Several lines of evidence suggest that the CXCR5^{high} ICOS^{high} PD-1^{high} cells are GC Tfh cells, whereas CXCR5^{int} ICOS^{int} PD1^{int} Tfh cells are not (Figure 3b). First, staining of human tonsil for ICOS predominantly identifies GC T cells [56], indicating that these are the cells with the highest expression of ICOS. It is important to note that, although high ICOS expression is a distinguishing feature of human Tfh cells (Figure 3b), in mice, Tfh and non-Tfh activated cells can express comparable amounts of ICOS (Figure 3b) [28,42]. This could be because of the sensitivity of the reagents available or intrinsic differences between species.

Next, high PD-1 expression on GC Tfh cells has been consistently found in both humans and mice [22,57,58]. Staining of mouse spleen sections with PD-1 selectively identifies T cells in GCs, but is not sensitive enough to stain T cells in primary follicles, follicular mantles or outer T zones [22,58], which suggests that GC Tfh cells express the highest amount of PD-1. PD-1 expression on GC Tfh cells, presumably through the interaction with the ligand PD-L2 expressed on GC B cells, regulates selection and survival of GC B cells [59]. Finally, it has been recently shown that after lymphocytic choriomeningitis virus (LCMV) infection, T cells that express high amounts of the GC marker GL-7 are also the cells that express the highest levels of PD-1, ICOS and CXCR5 [32].

GC Tfh cells have a different cytokine profile from non-GC Tfh cells

After identification of the phenotypic differences between GC Tfh and non-GC Tfh cells, it becomes possible to review the reported cytokine expression of Tfh cells. CXCR5^{+} cells can secrete many cytokines and localize to the follicles. Indeed, during helminth infection, CXCR5^{+} cells express high amounts of IL-4 [60–62] and localize to the follicles; during *Leishmania* infection, CXCR5^{+} cells form conjugates with AID^{+} B cells, express substantial amounts of IFN-γ, and localize to the follicles [61]; following immunization with myelin oligodendrocyte glycoprotein in an animal model for experimental autoimmune encephalomyelitis, CXCR5^{+} cells express IL-17 [63]; and in mice immunized in the presence of monophosphoryl-lipid-A-based adjuvant Ribi, CXCR5^{+} cells produce high amounts of IFN-γ and IL-4 [64]. Also, in human tonsil, CXCR5^{int} cells can be found producing very high amounts of the cytokines IL-17, IFN-γ and IL-4 (Figure 3a) [42,48].

Three separate groups have quantitatively compared cytokine expression between CXCR5^{hi} cells (which are PD1^{high} and located in GCs) and CXCR5^{int} cells (PD1^{int}), and all reported a reduction in IL-17 and IFN-γ with variable amounts of IL-4 in the CXCR5^{high} cells [32,42,48] (Figure 3a). Consistently, CXCR5^{high} cells have the highest expression of IL-21 [42,48]. Although IFN-γ appears to be virtually absent among CXCR5^{high} cells from human tonsil, it is still detected in GC T cells of LCMV-infected mice, although at lower levels than GL7^{low} non-GC Tfh cells [32], and in GC T cells of *Leishmania*-infected mice [61]. In the latter work, IFN-γ expression within GCs was lower than outside GCs, and IFN-γ-producing cells also appeared to be more abundant outside GCs; nevertheless, this needs to be quantified for firm conclusions to be drawn [61]. It is possible that the chronic inflammatory milieu of human tonsils modulates Tfh cytokine expression, however, it has recently been reported that IL-17 mRNA was undetectable in GC Tfh cells [59]. Thus, it appears safe to conclude that GC Tfh cells express lower IFN-γ and IL-17 than do non-GC Tfh cells (Figure 3a).

IL-21 and IL-4 are produced at high levels by non-GC and GC Tfh cells

Several groups have reported that IL-21 and IL-4 are produced at substantial levels by both CXCR5^{high} PD-1^{high} GC Tfh cells and CXCR5^{int} cells [42,48] (Figure 3a,b). PD-1 expression itself has been reported to be important for the production of maximal amounts of these cytokines [59]. There is recent evidence that IL-21 and IL-4 exert important functions in GCs beyond promotion of isotype switching.

IL-21 is required for optimal GC formation, affinity maturation [14,15] and proliferation of GC B cells [15]. This cytokine also promotes Tfh cell survival in an autocrine fashion [14,26,53]; however, the reported effects on Tfh cells vary from being profound [26,53], to subtle [14], to none at all [15,55]. A point of conflict has been attribution of the effect of IL-21 in GC formation and maintenance to either direct signaling to B cells [14,15,55], or indirect signaling through its effect on Tfh cells [26,53]. The independent conclusions from the three different groups that have used mixed bone marrow chimeras – the most stringent way to test whether a defect is B cell or T cell autonomous – is that IL-21R is required in B cells for normal GC development, survival and Bcl-6 expression [14,15,55]. Most of these effects are likely to be a consequence of reduced Bcl-6 expression in GC B cells that lack IL-21R [14,15]. Nevertheless, it is still possible that the effect of IL-21 on Tfh cell development or maintenance compounds the GC B cell phenotype. Also, it is possible that antigen dose explains some of the discrepant results: the group that has reported a lack of GC formation in the absence of IL-21 used a 10 times lower dose of intraperitoneal SRBC [53] than the dose used by our group, which has reported GC formation in the same mice [14].

Tfh cells in human tonsil GCs produce substantial amounts of IL-4 [42,48], as do Tfh cells within GCs of mice infected with helminths or immunized with T cell-dependent antigens [22,60–62,64]. Studies *in vitro* have long supported a prominent role for IL-4 in driving switching to IgG1 in mice [65]. *In vivo*, B cells that form conjugates with IL-4-producing T cells express more IgG1 post-switch

and germline transcripts than do B cells conjugated with non-IL-4-producing T cells [61]. However, extrafollicular switching to IgG1 is still seen in mice that lack IL-4 and IL-13 [66]. It appears that IL-4 plays a more prominent role during the GC response, particularly in coordination with IL-21: GCs were reduced in size in mice that lacked IL-4Rα, possibly because fewer B cells were selected by T cells [67], and GCs were severely impaired in *Il4*$^{-/-}$ *Il21r*$^{-/-}$ double knockout mice [68]. Taken together, these results indicate an important role for IL-4 in GC B cell survival and selection beyond its isotype switching–enhancing function.

SAP is selectively required for GC Tfh cell formation

A final point of controversy has been the requirement of SAP for Tfh formation. A role for SAP in supporting the helper function of T cells has been known for some time [69]. In the absence of SAP, T cells exhibit a selective defect in their ability to form stable interactions with B cells [29] and SAP expression in T cells is crucial during the late stages of T–B interaction [30]. Without SAP, T cells are recruited to GCs less efficiently and fail to be retained [29]. CXCR5^{+} ICOS^{+} Tfh precursor cells form normally in the absence of SAP, but *Sap*$^{-/-}$ T cells are less prone to move into or stay in the GC. When subsequently, three independent groups enumerated CXCR5^{high} PD-1^{high} cells – which are likely GC Tfh cells – in SAP-deficient mice, all reported impaired Tfh formation in the absence of SAP [28,31,32]. Taken together, these studies highlight that SAP plays a major role in terminal differentiation of Tfh precursors into GC Tfh cells. The observed upregulation of CXCR5 in the absence of SAP might account for the reported ability of SAP-deficient T cells to interact with B cells and support extrafollicular antibody responses [31].

Concluding remarks

Tfh cells have emerged as a subset of Th cells with a unique transcriptional profile and functional capabilities. As we have begun to understand Tfh development, numerous controversies have arisen as a result of the described discrepancies in phenotype, cytokine secretion pattern, and the requirement for interaction with different APCs for Tfh formation. Here, we have reviewed evidence that suggests that Tfh cells that interact with GC B cells – GC Tfh cells – are different in phenotype and function than Tfh cells that interact with other B cell subsets and at other locations. Uncovering the differences between the different subsets of Tfh cells is not only important to reconcile conflicting data, but will also be the basis to explore many unanswered or only partially answered questions about Tfh development and function. For example: (i) by which signals, and at what precise location in secondary lymphoid tissues, does commitment to the Tfh pathway occur? (ii) How is the differentiation of Tfh subsets determined? (iii) Do Tfh phenotype and differentiation requirements vary in response to different infections? (4) What is the relationship between Tfh cells and other Th cell lineages? Answering these questions will be crucial to understand the development of high-affinity B cell memory, which is the basis for effective vaccination, and will help to dissect the pathogenic versus protective roles of GCs in autoimmune diseases.

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